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Subscription information:
Institute of Technology, EMU
Fr.R. Kreutzwaldi 56,
51006 Tartu,
ESTONIA
e-mail: timo.kikas@emu.ee

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Whey permeate-derived milk acidifier for dairy calves

U. Antone[1,*], I. Eihvalde[2,3], L. Liepa[4], A. Ilgaza[5], M. Zolovs[6,7] and J. Liepins[8]

[1]Latvia University of Life Sciences and Technologies (LLU), Faculty of Food Technology, Department of Food Technology, 22 Rigas Str., LV-3004 Jelgava, Latvia
[2]LLU, Faculty of Agriculture, Institute of Animal Sciences of LLU, 2 Liela Str., LV-3001 Jelgava, Latvia
[3]Training and Research Farm SIA 'Vecauce' of LLU, 11A Akademijas Str., LV-3708 Auce, Auces lauku teritorija, Latvia
[4]LLU, Faculty of Veterinary Medicine, Clinical Institute, 8 Kristapa Helmana Str., LV-3004 Jelgava, Latvia
[5]LLU, Faculty of Veterinary Medicine, Preclinical Institute, 8 Kristapa Helmana Str., LV-3004 Jelgava, Latvia
[6]Daugavpils University, Department of Biosystematics, Institute of Life Sciences and Technology, 1a Parades Str., LV-5401 Daugavpils, Latvia
[7]Riga Stradins University, Statistics Unit, 14 Balozu Str., LV-1007 Riga, Latvia
[8]University of Latvia (LU), Institute of Microbiology and Biotechnology, 1 Jelgavas Str., LV-1050 Riga, Latvia
[*]Correspondence: u.antone@gmail.com

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Abstract. A milk acidifier obtained from whey permeate fermenting it with dairy propionic acid bacteria was tested in this study to evaluate the effects of milk acidification on the health and growth performance of pre-weaned dairy calves. The study consisted of 30 neonatal Holstein female calves, allocated to three treatments fed unacidified (Control group) or acidified (EG-1 and EG-2 groups) pasteurised milk during the 7–75 day age. Control and EG-1 were fed milk by divided method three times daily till one month of age, then twice daily until weaning; EG-2 was basically fed by the undivided method - one week three times daily (7–14 day age), then once daily. Results demonstrate that animal general health status and faecal scores (FS) were good and the tested acidifier can be used for pre-weaned calf milk acidification. Biochemical and haematological indices of blood at the 30 and 60 day age were within normal reference values with both - divided and undivided - milk feeding methods. Mean live weight (LW; 106.6 ± 9.40 kg on average) and live weight gain (LWG; 911.33 ± 109.04 g day^{-1} on average) at weaning did not differ between treatments ($P > 0.05$). Lower intake of starter feed associated with a larger amount of milk consumed was observed in EG-2 animals ($P < 0.05$). As the results observed regarding growth performance and health indices of all dietary treatment groups of calves were similar, we could anticipate that the acidification benefits would be greater when providing unpasteurised milk, or during the hottest weather when the risks of milk spoilage are greater.

Key words: blood; growth performance; heifer calves; propionic acid bacteria.

INTRODUCTION

Digestive problems, e.g., diarrhoea, is the most frequently encountered health problem in young dairy calves prior to weaning (Breen et al., 2012; House et al., 2015, Zhang et al., 2019). In recent years there is growing interest in promoting animal health by natural means of disease prevention to reduce the need for antibiotics and to stabilise gut health (Cardo, 2016; Celi et al., 2017; EC, 2017; Dai et al., 2021). The microbiological quality of the milk fed to the young cattle is of great importance because milk is the main food for calves until weaning, which is usually done at 2–3 months of age. Milk acidification is a preventive method to diminish the use of antibiotics, whose overuse to treat or prevent diarrhoea has led to the development of antibiotic resistance endangering animal and human health. Acidification is a fast and technically simple method, often recommended when pasteurisation and cooling are not practicable; it saves the time and labour required for feeding as well (Coelho et al., 2020). Materials used to acidify animal feed vary - most often these are concentrated organic acids - formic (Li et al., 2019; Chen et al., 2020), hydrochloric (Zhang et al., 2017), lactic (Coelho et al., 2020) and other acids. Propionic acid can also be used for milk acidification purposes; however, when used alone, the high corrosivity and low palatability may limit its usefulness (Jones & Heinrichs, 2014). It is a 3 carbon organic acid, possessing valuable antimicrobial properties (Ranaei et al., 2020), obtained mainly by petrochemical routes (Gupta & Srivastava, 2001; Ali et al., 2021). The availability of fossil resources is declining, while for a food by-product - whey - it is often difficult to find a use. Whey is an abundant and bulky dairy by-product arising from cheese and curd manufacture. Due to high biochemical oxygen demand, the disposal of whey in water streams is not allowed as such poses a high environmental threat (Audic et al., 2003, Bargeman, 2003; El-Tanboly et al., 2017). Meanwhile, due to high sugar lactose content, whey is a good substrate for organic acid production (Audic et al., 2003). Sometimes, in relatively small quantities, whey is used in animal nutrition including ruminant and calf feed (Manurung, 2012; El-Shewy, 2016; El-Tanboly et al., 2017; EWPA, 2020). Therefore, we were looking for new uses for this by-product in animal feed. We fermented whey permeate with dairy propionic acid bacteria and obtained an acidifier for calf's milk. This microorganism species is often used in the production of cheese; some its strains have QPS (Qualified presumption of safety) status (European Food Safety Authority, 2012; Rabah et al., 2017). It is also added to silage as a technological additive, or used as probiotics (Adams et al., 2008; Rabah et al., 2017) and protective microorganisms in a variety of foods due to the antimicrobial properties of their metabolites (Gupta & Srivastava, 2001; Zarate et al., 2011; Poonam et al., 2012; Bai & Rai, 2015; Azzaz et al., 2019). Although the idea of animal feed acidification is not new (Partanen & Mroz, 1999; Diebold & Eidelsburger, 2006; Zou et al., 2017; Long et al., 2018; Coelho et al., 2020), the use of whey to produce acidifier for calves' milk would improve the recycling of dairy industry by-products and lessen the overall environmental burden. The fermented whey products can also serve as functional feed components - a source of B group vitamins, microbial protein, and energy (Widyastuti & Febrisiantosa, 2014; Poonam et al., 2012). Thus, the aim of the present study was to investigate the effects of the new whey-derived milk acidifier on the health and growth performance of dairy calves in a divided and undivided milk feeding system.

MATERIALS AND METHODS

Animals, Management and Feeding

The experiment was conducted at the dairy farm 'Līgotnes' of the Training and Research farm SIA 'Vecauce' of LLU (Dobele district, Latvia); it was approved by the Animal Welfare and Protection Ethics Council of the LLU (permit LLU DZLAP No. 20/3). Test groups were established gradually, subdividing 30 neonatal Holstein female calves, born during the August and September months, into three dietary treatment groups with 10 replicates in each - EG-1, EG-2 and Control. The inclusion of animals in the test groups was decided based on birth weight and health status. This study lasted until December of the same year. Schematic description of milk provided to the calves and its feeding methods are presented in Table 1.

Table 1. Schematic description of milk provided to the calves and its feeding methods

Age of calves (days)	Dietary treatment groups		
	EG-1 (n = 10)	EG-2 (n = 10)	Control (n = 10)
1–4	1. Colostrum and transition milk feeding, similar to all calves Warm (35–38 °C) colostrum or cow transition milk fed 3 times daily (6 ± 1.3 L day^{-1} on average) ↓		
5–6	2. Milk feeding, similar to all calves Pasteurised (72–76 °C, 15–20 s) warm (35–38 °C) bulk milk fed 3 times daily (8 ±0.8 L day^{-1} on average) ↓		
	3. Milk feeding, diversified (8 ± 0.3 L day^{-1} from day 7 till 66; 6 ± 1.4 L day^{-1} from day 67 till 72; 3 ± 1.1 L day^{-1} from day 73 till 75 on average):		
	acidified pasteurised bulk milk; pH 4.6–5.2; t = 20–25 °C	**acidified** pasteurised bulk milk pH 4.2–4.6; t = 20–25 °C	**un-acidified** pasteurised bulk milk pH 6.5; t = 35–38 °C
7–14	fed three times daily till 1 month of age (divided method)	fed three times daily till 14 days of age (divided method)	fed three times daily till 1 month of age (divided method)
15–30		fed once a day (undivided method)	
31–75	fed twice daily (divided method)		fed twice daily (divided method)

After birth, each newborn animal was placed in a special pen with a heating lamp for drying, and on the first time of feeding was fed on colostrum within two hours after birth. After drying, the calves were transferred to the outdoor shed and housed under equal conditions in welfare-sized, individual wooden pens bedded with straw. Before inserting the calves, the cages were cleaned and bleached; the base was disinfected and supplemented with sand and straw. Later, the cages were cleaned and topped up with straw as needed. Starting from 24 to 48 hours after birth for 7 consecutive days, the calves were given a prophylactic oral solution containing the active substance halofuginone (Kriptazen, Virbac, France, administered PO according to the manufacturer's instructions) to prevent *cryptosporidium*-induced diarrhoea. All calves on the first feeding, within 2 hours after birth, received colostrum (only once), the volume of which was 10–12% of the body weight. Calves received colostrum from their respective dams if the quality was adequate (solids content ≥ 22% Brix as determined by hand refractometer). In the case when milking staff was not present, e.g., at night, and therefore no mother milk was available, or there was no mother milk available of adequate quality, thawed colostrum of confirmed quality (previously stored at −20 °C) was provided. Later, up to the 4th day of life (inclusive), calves were fed transition milk. On days 5 and 6 calves were fed pasteurised bulk milk from the same farm. Milk was pasteurised and cooled to the necessary temperature in a water bath. Colostrum and milk was provided to calves from a bucket with a nipple. Until the 6 day age, the temperature of milk fed to all calves was 35–38 °C. From the first day of life, drinking water was available in a separate bucket. From the 4th day, commercial calf starter meal (concentrates), containing vitamins A, D_3, E and microelements, including iron, copper, zinc, and hay was provided *ad libitum*.

From day 7, feeding was diversified by feeding acidified or unacidified whole bulk milk. Control group calves were fed unacidified warm (35–38 °C) milk 3 times daily till one month of age, then twice daily. Group EG-1 calves were fed cool acidified milk (20–25 °C) 3 times daily till one month of age, then twice daily by split or divided method. Group EG-2 calves were fed cool (20–25 °C), stronger acidified milk 3 times a day from the 7th to the 14th day, then once a day (by undivided method) from the 15 day age till weaning. When the undivided/unrestricted method for the EG-2 group animals was applied, the daily milk intake in the morning (4:00), i.e., 8 L day^{-1} per calf was offered. If the calf had consumed the required amount of milk by 16:00, during the 1st month of life it was offered an additional portion of milk (1–3 litres). At the end of the day, the amount of milk consumed by each calf on that day was recorded by measuring the quantity of unconsumed milk. Milk acidification was performed by adding acidifier to cool milk (20–25 °C) in doses sufficient to achieve the desired acidity of the final product and to coagulate it. The target pH of the milk fed in the restricted feeding group (EG-1) was higher (pH 4.6–5.2) because the feeding time of the milk was relatively short (20 min.) and the risk of microbial damage was lower, while the pH of the milk fed in the EG-2 group was lower (target pH 4.2–4.6) - stronger acidification was needed due to the longer feeding time of the milk (approximated method to unlimited milk feeding). For the calves to be gradually accustomed to acidified milk, during the first 5 days the dose of acidifier was gradually increased from 15 mL to 33 mL (EG-1) or to 41 mL (EG-2) per 1 L of milk, at the same time decreasing the temperature of the milk from 35–38 °C to 20–25 °C. Over the 7–66 day age, calves received 8 L milk day^{-1} on average. Starting from 67 up to 75 days of age, calves were gradually weaned, reducing the milk dose. The weaning was finished considering the following praxis: 1) when doubling of calves' live weight according to calf breeding experts was reached, and 2) when starter intake according to starter meal producers' instruction was at least 1 kg for 3 consecutive days.

Main feeding materials of calves are listed in the Table 2. The main raw material of the acidifier tested in the study was milk whey permeate (> 90%), obtained in a milk processing plant, and fermented under laboratory pilot scale conditions at the Faculty of Food Technology of the LLU, using appropriate equipment to ensure sterility and other work safety measures.

Cultures of propionic acid bacterium (DSM 20273, 20535, 16859, and 4902) were obtained from the Leibniz Institute DSMZ culture collection (German Collection of Microorganisms and Cell Cultures, GmbH). Bacterial maintenance and propagation of pure cultures was carried as described by Antone et al. (2021). For product manufacture and stability, yeast extract, salt additives and feed grade formic acid was used. The product (pH 2.3) was ready for adding to milk and did not need to be diluted.

Table 2. Main feeding materials

Items	Fat, %	Protein, %	Lactose, %	DM, %	Crude fibre, %	Crude ash, %	NDF, %
Milk (Control group)	4.41	3.51	4.53	13.40	-	-	-
Milk (EG-1 group)	4.27	3.42	4.68	13.36	-	-	-
Milk (EG-2 group)	4.24	3.40	4.72	13.34	-	-	-
Starter meal for 0–1 month old calves*	4.00	19.40	-	-	5.30	7.50	-
Starter meal for 30–75 day old calves**	2.68	20.00	-	-	4.06	7.15	-
Hay	-	13.64	-	85.04	36.14	6.05	61.64
Milk acidifier	0.03	0.63	9.30	12.00	-	-	-

DM – dry matter; NDF – neutral detergent fiber; *'Danish Calves Crunch' (Vilomix Baltic; mineral content: Ca 1.07%, P 0.41%, Na 0.31%, Mg 0.21%); ** 'Supplementary feed for calves with linseed' (Dobeles Dzirnavnieks; mineral content: Ca 1.00%, P 0.60%, Na 0.30%, Mg 0.29%).

General Health Status and Growth Performance Assessment of Animals

The general health status and clinical signs of disease of the calves were monitored daily by a veterinarian, checking the general appearance, animal activity, appetite, and appearance (colour and consistency) of faeces. Calf live weight (LW) was recorded after birth and later at 15 day intervals: on day 15, 30, 45, 60, 75, and 90. LW of experimental animals at birth was similar in all groups ($P > 0.05$) (Table 6). Calves were weighed on an electronic calibrated scale. All weights were taken approximately 3 h after the morning milk supply. Average live weight gain (LWG) was calculated by subtracting the initial LW from the LW at the end of the period and divided by the number of days. Faecal score (FS) data were recorded daily 2 h after the morning milk feeding. Faeces were scored on a 0 to 4 point scale according to the method used by Teixeira et al. (2015) based on visual consistency and colour evaluation, where: 0 = normally formed faeces with normal colour; 1 = pasty with normal colour; 2 = liquid with normal colour; 3 = watery with normal colour; 4 = watery with abnormal colour. According to the methodology, if the FS was more than or equal to 3, the animal was considered to have diarrhoea.

Feed Intake and Feed Quality Measurements

Milk and starter feed intake was recorded daily. Milk volume was measured with the help of a measuring cup. Milk was sampled daily and frozen at −20 °C for fat, crude protein, lactose, and total solids content analyses by a MilkoScan™ Mars analyser (Foss Electric, Denmark) and detection of pH (pH meter Jenway, Model 3510, Cole-Parmer, Staffordshire, UK). The starter was weighed on a scale.

Blood Biochemical and Haematological Parameters

For the determination of general blood biochemical parameters, blood samples were taken at 6, 30 and 60 days of age. Collection was carried out in the morning, two hours after feeding. Samples were taken from the jugular vein by venipuncture. For analysis of blood serum biochemical indices (aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, total serum protein (TP), albumin, and glucose) samples were taken in non-EDTA vacuum tubes, and transported under refrigeration (2–4 °C) to the Laboratory within three hours after collection. Biochemical analyses were made using automated methods with ABBOTT ARCHITECT analytical equipment (Abbott, Illinois, U.S.A.). For the haematological analyses (erythrocyte count (E), haemoglobin (Hb), and haematocrit (Hct)) samples were collected into EDTA vacuum tubes. Blood samples were tested with the veterinary haematological analyser Exigo EOS Vet (Boule Medical AB, Sweden) having built-in electrical resistance or impedance method for E, and cyanide-free spectrophotometrical method (at 535 ± 5 nm) for Hb. Hct was calculated arithmetically.

Statistical Analysis

The assumption of normal data distribution was assessed by Shapiro-Wilk's test and a visual inspection of their histograms and normal Q-Q plots. The assumption of homogeneity of variances was tested by Levene's test. To determine whether there were any statistically significant differences between the three independent groups, we used a one-way ANOVA with Tukey post hoc comparison or Kruskal-Wallis h test with pair-wise comparisons using Dunn's procedure (1964) with a Bonferroni adjustment. Statistical data analysis was carried out using SPSS Statistics version 23 (IBM Corporation, Chicago, Illinois). When the shape of data distribution was not similar between independent groups the mean ranks were calculated to present the obtained results (Vargha & Delaney, 1998). For the calculation of statistically significant differences of LW and LWG between test groups, the MS Excel program was used. To test the difference between two independent groups the independent samples *T-test* was used. Values of less than 0.05 ($P < 0.05$) were considered significant. Data are presented as *means* ± *SD* unless otherwise stated.

RESULTS AND DISCUSSION

Health Status and Growth Performance of Animals

The overall animal health status was good - the general appearance of calves was normal and alert; appetite was good and satisfactory. No serious health problems were observed during the study. Results of blood biochemical parameters and their reference values of some other studies are listed in Table 3. Most biochemical indicators - serum AST and ALP activity, also TP, albumin, creatinine, and glucose concentrations - at each individual sampling time - at the first sampling before the start of milk acidification, as well as later - at the age of 30 and 60 days, were similar ($P > 0.05$) in all dietary treatment groups. The only difference was significantly lower ($P < 0.05$) relative to the Control, still normal compared to literature data, urea concentration at 2 months of age in EG-2 serum. **Serum AST** activity in all dietary treatment groups was similar to reference values as observed by Ježek (2007) and Yu et al. (2019). Significant increase in AST in the blood of all groups of animals at 1 month and 2 months of age compared to the 6 day age was observed. Since this indices was within the normal reference range, it can be concluded that the animals were not significantly affected by muscle or liver damage (Stämpfli & Espinosa, 2015).

Table 3. Blood serum biochemical parameters of differently fed calves groups

Indices	Groups; *p*-values; Reference values	Median values and intervals (Q1–Q3) in the respective age of the animals (days)		
		6	30	60
AST, U L^{-1}	EG-1	**33.5** (31.0–37.3)	**33.5** (30.0–34.8)	**47.5** (40.3–53.8)
	EG-2	**31.5** (29.3–34.8)	**32.5** (31.3–36.5)	**41.5** (40.0–47.8)
	Control	**34.0** (31.5–37.0)	**33.5** (29.5–38.8)	**46.5** (41.3–49.0)
	p-values	0.725	0.966	0.278
	Reference values	38.7 ± 16.1* 36.6 ± 9.3 ^	33.3 ± 11.0** 42.2 ± 15.0^^	40.3 ± 10.2*** 37.5 ± 10.8^^^
ALP, U L^{-1}	EG-1	**377.5** (276.8–401.8)	**480.5** (362.8–572.0)	**615.5** (524.3–733.3)
	EG-2	**323.0** (276.8 –483.0)	**477.5** (399.0–601.3)	**512.0** (434.0–558.5)
	Control	**324.0** (281.8–405.0)	**421.5** (331.0–792.3)	**439.5** (350.0–560.3)
	p-values	0.961	0.889	0.064
	Reference values	262.2 ± 185* ~780§	157.8 ± 79.7** ~260§§	216.2 ± 97.7*** ~410, 580§§§
Total protein, g L^{-1}	EG-1	**61.5** (58.3–64.8)	**57.0** (56.0–59.0)	**60.5** (59.0–61.8)
	EG-2	**64.5** (60.8–65.8)	**57.5** (55.3–60.8)	**59.0** (57.3–60.5)
	Control	**63.0** (60.0–70.0)	**59.0** (58.0–62.3)	**61.0** (60.0–61.8)
	p-values	0.810	0.180	0.094
	Reference values	52.09 ± 7.09* 51.1 ± 6.3^	52.41 ± 3.91** 49.9 ± 5.0^^	56.94 ± 5.19*** 48.0 ± 5.4^^^
Albumin, g L^{-1}	EG-1	**22.0** (21.0–22.0)	**27.0** (26.3–28.0)	**29.0** (29.0–30.0)
	EG-2	**22.0** (20.0–23.0)	**27.5** (27.0–29.0)	**29.5** (29.0–30.0)
	Control	**22.0** (21.3–23.0)	**28.0** (26.3–28.0)	**29.0** (28.0–30.0)
	p-values	0.668	0.647	0.840
	Reference values	26.35 ± 2.10* ~28§	30.99 ± 2.53** ~35§§	31.76 ± 2.68*** ~37, 36§§§
Urea, mmol L^{-1}	EG-1	**3.65** (3.23–4.10)	**3.80** (3.50–4.35)	**2.60**^{ab} (2.42–2.85)
	EG-2	**3.25** (2.70–4.50)	**3.35** (2.92–3.75)	**2.35**^{b} (2.02–2.60)
	Control	**3.30** (3.13–3.90)	**3.05** (2.55–3.58)	**2.80**^{a} (2.50–3.18)
	p-values	0.845	0.083	0.036
	Reference values	3.64 ± 1.33* 2.53 ± 1.32^	4.01 ± 1.21** 2.31 ± 0.65^^	3.90 ± 1.19*** 1.86 ± 0.48^^^
Creati-nine, µmol L^{-1}	EG-1	**71.5** (65.3–77.0)	**63.0** (60.5–64.0)	**58.5** (57.0–62.0)
	EG-2	**64.5** (62.0–69.8)	**61.0** (58.75–62.5)	**59.5** (56.0–64.5)
	Control	**70.5** (66.5–86.8)	**68.5** (64.8–71.8)	**58.0** (53.0–62.5)
	p-values	0.178	0.089	0.790
	Reference values	113.76 ± 36.20* 88.42 ± 17.68^	93.55 ± 16.66** 73.39 ± 14.14^^	82.34 ± 15.10*** 59.24 ± 10.61^^^
Glucose, mmol L^{-1}	EG-1	**6.61** (5.95–7.66)	**6.21** (6.04–6.76)	**6.25** (6.18–6.64)
	EG-2	**6.85** (6.12–7.37)	**6.28** (5.97–6.94)	**6.25** (6.03–6.70)
	Control	**6.35** (6.15–6.52)	**6.96** (6.33–7.03)	**6.07** (5.55–6.31)
	p-values	0.218	0.624	0.365
	Reference values	6.14 ± 1.23^ 6.2 ± 1.3 (treatment group) and 6.2 ± 1.7 (control)#	5.66 ± 1.02^^	5.17 ± 0.85^^^

$^{a,\ b}$ values with different superscript letters on the same column at each blood indicator are different according to the Kruskal-Wallis h *test* with pair wise comparisons using Dunn's procedure ($P < 0.05$); 1*, 4**, and 8*** week old Holstein-Friesian calves (Ježek, 2007); 2^, 5^^ and 7^^^ week old male Holstein calves, *mean* ± *SD* (Yu et al., 2019); 24–48h§, 28d§§, 56 and 70d§§§ old Holstein calves (Mohri et al., 2007); # 0, 4, 8, and 12-week old Holstein-Friesian bull calves; *mean* ± *SD* (Adams et al., 2008).

Serum ALP activity at the 6 day age in calves' blood of the present study was within normal reference values as observed by Ježek (2007), but lower than Mohri et al. (2007). Later, there was a statistically significant ($P < 0.05$) increase in ALP activity in the blood of all groups of animals at 2 months of age compared to the 6 day and 1 month age. At the age of 1 month the ALP activity in all test groups, but at the 2 month age, especially in experimental groups animals, was higher than in the above-mentioned research. At the age of 1 and 2 months the ALP activity of our experimental groups was similar to Knowles et al. (2000), whose calves' blood serum ALP concentration was around 500–620 U L^{-1}. The ALP activity in young animals can be up to 3 times higher than in adult animals because of the osteoblastic activity during rapid bone growth Bain (2011); up to the age of 6 months it can reach 1,800 U L^{-1} (Kraft, 1999). ALP enzyme comes from the placenta, bone, macrophages, intestinal epithelium, and liver. In very young calves, ALP is increased, probably because of the placental or bone source. In young calves, ALP levels up to 1,000 IU L^{-1} at birth and 500 IU L^{-1} at several weeks of age should be considered normal (Aiello & Moses, 2016). **TP concentration** in calves' blood at each individual sampling time in all the 3 dietary treatment groups was similar ($P > 0.05$). Compared to literature data, at the 6 day and 1 month age it was higher than the data of Ježek (2007) of 1 and 4 week old calves (52.09 ± 7.09 and 52.41 ± 3.91 g L^{-1}, respectively), but similar to Knowles et al. (2000) values (~62–68 and ~56–59 g L^{-1}). TP concentration in calves' blood serum may be within 50–70 g L^{-1} (Kraft & Dürr, 1999). At the 2 month age TP was within normal reference values as observed by Knowles et al. (2000) - ~58–61 g L^{-1} and Ježek (2007) - 56.94 ± 5.19 g L^{-1}. **Serum albumin** concentration at 6 days of age was relatively low - near the bottom line of the 22–36 g L^{-1} reference range of Kessell (2015), and lower compared to Ježek (2007), and Mohri et al. (2007) (Table 3). Over time, the levels of albumin increased ($P < 0.05$), similarly to that observed in the research of Knowles et al. (2000), Mohri et al. (2007) and Ježek (2007). Attention should be paid to the fact that blood levels of albumin in neonatal calves of the present study were relatively low. Decreased concentration of albumin could be associated with different causes, e.g., mild malnutrition, reduced absorption of amino acids in gastrointestinal tract, reduced synthesis of albumin in the liver or due to the invasion of *Cryptosporidium parvum* (Eckersall & Proteins, 2008). **Serum urea** concentration in all group calves' blood of the present study was similar to the concentrations assessed by Yu et al. (2019) and Ježek (2007) (Table 3). Urea and creatinine are waste products of protein catabolism (Kessel, 2015). **Creatinine** concentration in calves' blood serum of the present study was within normal reference values, similar as assessed in the study of Yu et al. (2019), but lower compared to Ježek (2007). **Serum glucose** concentration of all dietary treatment groups was within normal reference values as observed by Ježek (2007) and Adams et al. (2008). This shows the good energy intake status of animals throughout the study (Stämpfli & Espinosa, 2015). **Haematological parameter (E, Hb, and Hct)** results and their reference values are listed in Table 4. At both blood samplings no differences were found between groups ($P > 0.05$) and were within normal reference values as assessed in the study of Ježek (2007), except the Hct level at 2 month age. Yet it was similar to Li et al. (2019) study values (23–26%) of 70 day old female Holstein calves fed reconstituted, acidified reconstituted or acidified fresh milk. Regarding changes with age during the experiment there was a significant increase in E in the blood of all groups of animals ($P < 0.05$) that is also consistent with previous studies of Ježek (2007), and Mohri et al. (2007).

Table 4. Blood serum haematological parameters of differently fed calf groups

Indices	Groups; *p*-Values; Reference values	Median values and intervals (Q1–Q3) in the respective age of the animals (days)	
		6	60
Erythrocytes, $10e^6$ µL^{-1}	EG-1	6.330 (6.20–6.76)	8.880 (8.69–9.59)
	EG-2	6.320 (5.45–7.04)	8.830 (8.31–9.28)
	Control	6.620 (5.95–7.63)	9.090 (8.46–9.44)
	p-Values	0.663	0.842
	Reference values	7.63 ± 1.49*	8.53 ± 1.01**
Haemoglobin, g dL^{-1}	EG-1	8.90 (8.35–9.40)	9.25 (8.88–9.88)
	EG-2	9.00 (7.78–10.10)	9.25 (8.45–9.50)
	Control	9.85 (8.72–11.12)	9.35 (9.30–10.07)
	p-Values	0.220	0.620
	Reference values	10.41 ± 2.21*	9.83 ± 1.29**
Haematocrit, %	EG-1	27.60 (26.85–29.63)	25.10 (24.00–26.35)
	EG-2	26.85 (22.90–29.53)	24.45 (22.68–25.28)
	Control	27.80 (25.00–35.02)	25.20 (24.55–26.82)
	p-Values	0.673	0.561
	Reference values	30 ± 7*	30 ± 5**

1* and 8** week old Holstein-Friesian calves (Ježek, 2007).

In summary, normal compared to reference values, indices of blood show that the health status of animals throughout the study was good. No differences regarding biochemical and haematological indicators at each individual sampling time were observed between groups, except for significantly lower urea concentration at 2 months of age in the EG-2 serum ($P < 0.05$) compared to the Control, still normal compared to literature data (Ježek 2007; Yu et al. 2019). Hence, the animal blood parameters were not largely affected by the feeding of acidified milk or its feeding method.

Faecal score (FS) test showed no cases of diarrhoea - no animals had watery faecal output with FS above 2 points. As most commonly the faecal mass of animals was normally formed and coloured, consequently, calculated median values of all test groups resulted in the score of 0 for all time periods; to show the differences more clearly, results are presented in Table 5 as mean ranks. During the initial 1–15 day period after birth the mean rank of the EG-2 score was slightly but significantly higher ($P < 0.05$) - the consistency of the faecal masses was more liquid compared to the Control, but did not differ from the EG-1 ($P > 0.05$). In the course of the next 16–30 and 31–45 day periods FS in all groups were similar ($P > 0.05$). Later - around the 2 month age - FS significantly differed ($P < 0.05$) between both experimental groups, over the 46–60 day period being slightly higher in EG-1 and over the 61–75 day period in EG-2, yet at the same time being similar ($P > 0.05$) to the Control. In some animals there were short-term

Table 5. Faecal score test results of dairy calves fed different types of milk

Age, days	Dietary treatments			*p*-value
	EG-1	EG-2	Control	
1–15	222.25^{ab}	240.17^{b}	214.07^{a}	0.030
16–30	223.99	223.98	223.89	0.093
31–45	224.78	225.01	224.99	0.072
46–60	229.99^{a}	222.50^{b}	224.01^{ab}	0.030
61–75	222.50^{a}	229.98^{b}	224.02^{ab}	0.030

$^{a, b}$ values with different superscript letters on the same line are different according to the Kruskal-Wallis h test with pair wise comparisons using Dunn's procedure ($P < 0.05$); data are presented as mean ranks.

(up to 2 days) faecal consistency changes to liquid (FS 1–2 points); thus, the possibility of subclinical inflammation of the gut cannot be ruled out.

A looser faecal consistency can also often be associated with increased starter intake causing minor intestinal dysfunction because the microflora of the intestinal tract has not yet matured, and habituation to solid feeds occurs gradually (Adams et al., 2008). This slightly higher FS of individual animals normalised rapidly over time thanks to immediate oral rehydration therapy or habituation to solid feeds without showing significant aggravation of the health status. Higher FS can also be associated with the consumption of greater volumes of milk due to the undivided *(ad libitum)* feeding method (Anderson, 2013; Geiger et al., 2016). The results show that the acidified milk feeding did not strongly affect FS.

Growth Performance

The animal LW and LWG changes until 3 months of age are presented in Table 6.

Growth rate is one of the most important indices during the pre-weaning period as it reflects the overall outcome of management and husbandry (Breen et al., 2014; Van Amburgh, 2017). Regarding calf body LW changes before weaning, the only difference between the test groups was at 1 month of age when the average LW of experimental groups was significantly lower ($P < 0.05$) than the Control. All test group animals' LW at 1 month of age was similar to Schwarzkopf et al. (2019) who had LW results of late-weaned Holstein calves of around 60–68 kg at the 28 day age. Later - at the 60, 75, and 90 day age the average LW in all treatment groups of the present study was similar ($P > 0.05$). At weaning (75 day age), the LW of calves reached 107 ± 9.4 kg on average, which was sufficient to stop milk feeding.

Table 6. Growth performance of dairy calves fed different types of milk

Items	Age (days)	Growth performance						*p*-value
		EG-1		EG-2		Control		
		Mean	*SD*	Mean	*SD*	Mean	*SD*	
LW, kg	0	36.90	3.446	39.25	2.176	38.65	4.217	0.289
	30	60.79^{a}	6.845	60.43^{a}	6.901	66.69^{b}	3.649	0.047
	60	87.44	9.791	88.22	6.892	89.95	8.523	0.797
	75 (weaning)	105.03	11.901	108.02	8.241	106.80	8.374	0.786
	90	127.34	11.474	130.32	11.317	133.04	12.124	0.556
LWG, g day^{-1}	0–30	796.3^{ab}	216.7	706.2^{a}	211.1	934.6^{b}	75.0	0.028
	31–60	888.3^{ab}	149.7	926.3^{a}	104.9	775.3^{b}	198.7	0.098
	61–75	1,172.7	345.6	1,320.0	462.0	1,123.3	369.5	0.520
	0–75 (pre-weaning)	908.4	147.1	916.9	103.3	908.7	77.1	0.982
	76–90 (post-weaning)	1,487.3	413	1,486.7	504.7	1,749.3	350.2	0.301

LW – body live weight; LWG – average daily live weight gain; $^{a,\,b}$ values with different superscript letters on the same line are different according to the independent samples *T-test* ($P < 0.05$).

At the 0–30 day period the LWG of EG-2 was lower than the Control group's ($P < 0.05$), but during the next 31–60 day period the results were opposite - the LWG of EG-2 was higher than the Control group's ($P < 0.05$). The initially lower LWG of EG-2 at 1 month of life may be explained by the lower intake of concentrates during the 15–28 day age (Table 7). The fluctuations of LWG are common and can also be caused by various other causes: infections and parasitism, nutrient deficiencies, environmental factors, as well as combinations thereof (Stratton-Phelps & Maas, 2015). Later - approaching the 2 month age (days 31–60), as well as 2 weeks before and after weaning (days 61–75, 76–90), the mean LWG of all animal groups was similar ($P > 0.05$). Overall, the LWG of the present study animals was typical to the high plane of milk feeding programmes with milk allowances ≥ 20% of the calf's birth weight, i.e., ≥ 750–1,000 g day^{-1} (Appleby et al., 2001; Jasper & Weary, 2002; Miller-Cushon, 2015). The LWG of the 2nd month of life of acidified milk-fed groups was similar to the Li et al. (2019) results of LWG 880–960 g day^{-1} of Holstein female calves (30–70 day age) fed reconstituted, reconstituted + acidified or acidified fresh milk for acidification using formic acid. Interestingly, Li et al. (2019) also observed positive effects of calves fed acidified liquid milk feed - animals exhibited greater withers height gain, as well as lower diarrhoea incidence, white blood cell and lymphocytes counts than calves fed reconstituted milk without acidification.

After weaning (76–90 day age) the LWG of all groups of the present study continued to increase, remaining similar ($P > 0.05$). The LWG of both acidified milk-fed groups was similar to the previously mentioned Sardoabi et al. (2021) study with calf LWG around 1,400–1,500 g day^{-1} at the 93 day age.

Regarding the effects of milk acidification on calves' growth and health, a good general animal health background of all treatment group animals in some respects did not allow the effectiveness of the milk acidification to be fully verified (e.g., the pasteurisation of milk and the cryptosporidial infection prophylaxis may have most likely helped to prevent diarrhoea). Coelho et al. (2020) concluded that acidification does not negatively affect animal growth in comparison to the refrigerated milk feeding; both diets resulted in calves with similar weaning weight; moreover acidification adjourns first diarrhoea and is a benefit itself in hot weather conditions when no refrigeration of milk is needed to maintain milk microbiological quality. A review carried out by Jones & Heinrichs (2014) on acidified milk feeding studies came to the conclusion that the health of calves is often neither improved nor deteriorated, and the results are not always consistent; they also concluded that there is little evidence that acidification itself affects the nutrients in milk or milk replacer or their utilisation by calves, calf performance, and LWG. A noteworthy discovery was made by Zhang et al. (2017) that calf health can also be influenced by the acidity level of milk. Decreasing the pH of milk replacer to a pH of 5.0–5.5 improved the digestive tract of pre-weaned calves (acidifier reduced the pH of digesta in the rumen, reticulum, and omasum, and was beneficial for the development of ruminal epithelium); in this study, hydrochloric acid was used to acidify the milk. However, a reduction to a pH of 4.5 had adverse effects on intestinal epithelium growth. Thus, it could indicate that the pH of the milk may also have affected the growth performance results of the present study - the lower LWG of EG-2 at 1 month of age could not only be related to the lower intake of concentrates, but also to the lower pH of the acidified milk, which could have a greater impact on young animals, as this trend later disappeared.

Feed Intake: Milk Intake - the total amounts of milk provided, consumed by calves and residual milk during the milk feeding diversification period (day 7–75) are shown in Fig. 1.

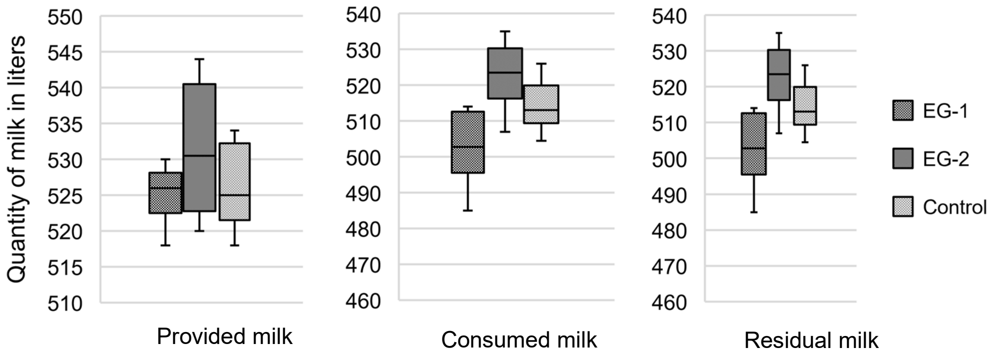

Figure 1. Summed up amounts of provided, eaten and uneaten milk of dairy calves fed different types of milk during the 7–75 day period until weaning.

In this study, none of the calves rejected the acidified milk. On the first days, some calves needed to get used to this taste of milk, but then willingly ate it. Regarding the provided milk during the 7–75 day period the total amount was similar in all treatment groups ($P > 0.05$): EG-1 (526 L; 522.0–528.0 L), EG-2 (530 L; 522.0–540.0 L), and the Control (525 L; 521.0–532.0 L) (medians, and minimum values-maximum values). The amounts of milk consumed by both experimental groups did not differ from the Control (513 L; 509.0–519.0 L) ($P > 0.05$). At the same time, there were significant differences between both experimental groups regarding the amounts of consumed and residual milk. The total consumed milk quantity during the 7–75 day period was significantly ($P < 0.05$) higher in the EG-2 group (523 L; 516.0–530.0 L) compared to EG-1 (502 L; 495.0–512 L) confirming that the method of feeding affects the quantity of the acidified milk consumed. It was already expected, because the milk feeding method of EG-2 was closer to the unrestricted method, and the time allowed for consuming it was longer. The largest amount of unconsumed milk was in group EG-1, being significantly higher than in both other groups ($P < 0.05$). The residual milk quantity for EG-1, EG-2, and the Control was 4.2% (22 L; 13.0–29.0 L), 1.3% (7 L; 5.0–11.0 L), and 1.9% (10 L; 8.0–13.0 L), respectively. Also regarding differences among individual animals, the biggest differences of leftover milk amounts were in the acidified milk-fed EG-1group. The milk was left over most particularly in the first month of life. Differences in appetite, satiety, attitude of individual animals towards the offered milk, its temperature or pH value can influence the milk intake. The results were most probably affected by the lower temperature of acidified milk fed to both experimental groups - 20–25 °C vs. 35–38 °C compared to the Control. Traditionally the milk is fed cool to slow the speed of intake and reduce the chance of gorge feeding (Anderson, 2013). Offering too cold acidified milk tends to reduce intake. There is also a practice to warm acidified milk to 38–40 °C (Coelho et al., 2020), yet milk must be fed immediately at this temperature because holding acidified milk at temperatures above 24 °C will often cause curdling (Anderson, 2013; Jones & Heinrichs, 2014). Another reason for the differences of milk consumed among individuals can be related to milk acidity. Hill et al. (2013) observed that when feeding acidified milk replacer (MR) for *ad libitum* to Holstein calves, some animals completely rejected the MR at pH 4.2, and in general calves consumed more of the MR at pH 5.2 than pH 4.2. Choosing the right amount of acidifier is also important because not adding sufficient acid may affect the keeping quality of the product and lead to high bacteria counts or unpalatable spoiled milk, but reducing the pH by too much may limit palatability and cause calves to drink less milk. To promote the transition, it is advised to increase the acidity of the milk gradually (Jones & Heinrichs, 2014). Since milk is the main food for calves during the first month of life and summarising the above, it would be recommendable to extend the milk feeding time and increase its temperature to reduce the unconsumed milk quantity by younger calves in the case that the restricted (divided) feeding method is applied.

Feeding higher amounts of milk during later periods is not always considered positive regarding development of the rumen. Restricted feeding was introduced to encourage calves to eat concentrates as early as possible and thus to minimise costs for relatively expensive liquid feeds (Kertz et al., 1979). Which amount of milk should be fed is a complex issue that depends on the goals and the management capabilities of each individual farm (Lorenz et al., 2011).

Some aspects should also be noted regarding liquid feed composition changes via the addition of the acidifier being rich in lactose. Acidifier addition increased the milk lactose content by 0.15–0.19%. Thus, acidified milk-fed animals received 13–16 g of lactose per day more than the Control animals. As lactose is thought to promote the assimilation of calcium in the body and accelerate the process of ossification, it could have positive effects on young animal health. However, detailed research should be performed to confirm this assumption. The positive effect of lactose is explained by the fact that unhydrolysed lactose appears to be utilised as a prebiotic to support the growth of health-promoting gut flora, which is recognised as an enhancer of calcium absorption. The stimulating effect of lactose on calcium absorption was apparent in animal studies - both hydrolysed and unhydrolysed forms of lactose appeared to be positively involved in enhancing calcium absorption in mammals, but in humans such an effect is still controversial (Atkinson et al., 1957; Kwak et al., 2012). Lactose is the most important source of glucose for young calves and well metabolised in the body up to the age of 3–4 weeks (Latvietis, 2013). At a later age adult animals metabolise lactose relatively poorly - almost all of it is broken down by the microflora of the digestive tract, resulting in low pH. As the pH of the rumen decreases, systematic, prolonged feeding of lactose affects the intestinal microflora thereby impairing the digestion of other components of feed (Atkinson et al., 1957; Latvietis, 2013; Aschenbach et al., 2019). Thus, lactose ingestion has different effects depending on the age of the calves. Yet, as the increase in lactose content of the liquid feed due to the addition of acidifier in the present study can be considered as relatively small, its effects could be quite negligible and are unlikely to impair calf digestive tract health as evidenced by good FC, blood test, and LWG results of calves at 2 months of age.

Starter Intake - the summed up starter feed amounts eaten during different pre-weaning periods are presented in Fig. 2.

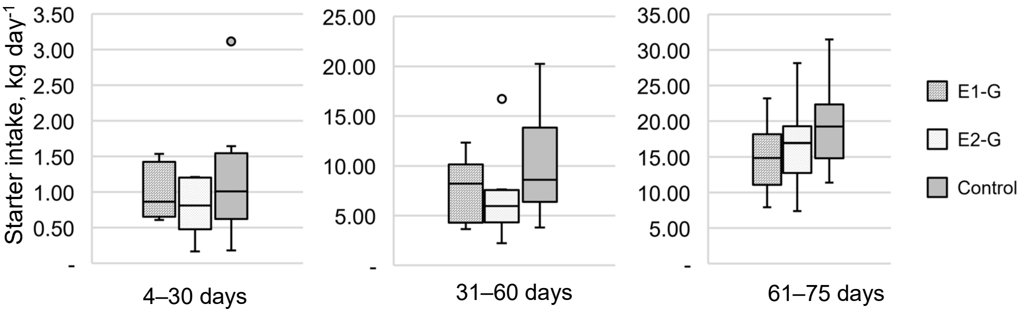

Figure 2. Summed up amounts of starter consumed by differently fed calves until weaning (data shown as median, Q1–Q3).

The differences between the groups on the summed up amounts of consumed starter feed during the 4–30, 31–60, and 61–75 day periods were not statistically significant ($P > 0.05$); however, when splitting the pre-weaning period into shorter periods, significant differences can be seen between the groups (Table 7).

Table 7. Starter intake of differently fed calf groups

Time period (days)	Starter intake, g day^{-1}						*p*-value
	EG-1		EG-2		Control		
	Median	Q1–Q3	Median	Q1–Q3	Median	Q1–Q3	
1–6	0	0–0	0	0–0	0	0–0	NA
7–14	0^{a}	0–23.3	15.0^{b}	0–43.3	3^{ab}	0–27.0	0.009
15–21	37.0^{a}	15.8–57.8	26.5^{b}	7.0–45.0	35.0^{ab}	11.0–73.0	0.044
22–28	64.0^{b}	50.5–94.5	25.0^{a}	11.0–57.8	42.5^{b}	21.0–124.0	0.001
29–35	89.5^{a}	47.3–130.0	58.5^{b}	27.5–103.0	83.5^{ab}	36.5–155.3	0.030
36–42	121.5^{a}	82.5–160.0	87.5^{a}	33.0–172.8	180.0^{b}	97.0–275.3	0.001
43–49	153.0^{a}	123.0–251.8	144.0^{a}	64.0–248.5	219.5^{b}	145.0–402.8	0.001
50–56	354.5^{b}	189.0–500.0	231.5^{a}	130.5–373.3	402.0^{b}	204.0–666.0	0.004
57–63	512.0^{ab}	368.7–882.0	514.5^{a}	357.3–746.3	719.0^{b}	445.3–1,031.0	0.016
64–74	1,046.0^{a}	733.5–1,384.8	1,113.5^{ab}	797.3–1,758.3	1,315.5^{b}	979.0–1,718.8	0.001

$^{a, b}$ Superscript values with different letters on the same line are different according to the Kruskal-Wallis h test with pair wise comparisons using Dunn's procedure ($P < 0.05$); NA – not applicable.

Results suggest that the starter quantity consumed by acidified milk-fed groups, especially EG-2, in quite a few periods was lower ($P < 0.05$) than by the calves of the Control group (36–49 and the 64–75 day periods for EG-1 and 15–63 day period for EG-2). Lower consumption of the starter by EG-2 animals can be associated with a larger amount of milk consumed by the undivided method allied to *ad libitum* feeding ($P < 0.05$) (Anderson, 2013; Akins, 2016; Geiger et al., 2016). Starter intake of the Leal et al. (2021) study calves receiving elevated milk replacer supply (8 L day^{-1}) was similar to our results: ~100 g day^{-1} at the 28 day age, and ~300–600 g day^{-1} at the 49–56 day age. Starter intake of 4 and 8 week old calves in the Coelho et al. (2020) study was higher - around 200–350 g day^{-1} and 750–900 g day^{-1}, respectively. In the present study, consumption of concentrates increased noticeably after 1 month of age when the calf starts transition from a monogastric animal into a ruminant. Results confirm that starter intake is negligible in the first weeks of life, but after the first 3 weeks of life it increases and the calves start to grow rapidly (Lorenz et al., 2011; Schwarzkopf et al., 2019). Proper balancing of the quantities of milk provided with an amount of other kinds of feed is essential for the transition of calves from liquid to solid diets, because dry feeds are the stimulus for the development of the ruminal epithelium (Anderson, 2013; Akins, 2016; Diao et al., 2019; Sardoabi et al., 2021).

It should be mentioned that the starter for calves throughout the study was available *ad libitum*; besides that, since calves had free access to hay and were housed on straw, an uptake of hay and straw was also possible, the quantity of which was not measured. The amount of starter ingested is one of the key factors when determining the weaning age. Early consumption of starter dry matter is more important for systems in which the goal is early weaning and the lowest cost rearing programme (Davis & Drackley, 1998; Drackley, 2005). In the present study, weaning was finished at the age of 75 days when the starter intake of all treatment groups was above 1 kg day^{-1} in accordance with the starter producers' recommendations.

CONCLUSIONS

The results of the present study show that the whey-derived acidifier can be used in both - divided and undivided - milk feeding systems for pre-weaned calves' milk acidification having no adverse effects on animal health and growth. Various products containing highly concentrated acids are available on the market for the acidification of animal feed and must be diluted before being added to the milk feed. The advantage of the newly-developed product is that it is derived from food by-products, relatively easier to use and safer than other - more concentrated - products. Comparing acidified milk feeding methods, we concluded that the largest amount of unconsumed milk and the biggest differences among individual animals of leftover milk amounts were observed with the divided method for acidified-milk feeding (EG-1); therefore, it is recommendable to extend the feeding time and raise the acidified milk temperature to reduce the amount of leftover milk by younger calves. As for the second - the undivided method - it saves the time and labour required for feeding, as well as reduces the loss of milk; yet consumption of the starter was lower. As results observed regarding growth performance and health indices of all dietary treatment groups of calves were similar, we could anticipate that the acidification benefits would be greater when providing unpasteurised milk, or during the hottest weather when the risks of milk spoilage are greater, as previously concluded by other researchers (Jones & Heinrichs, 2014; Coelho et al., 2020). Therefore an in-depth study of the tested acidifier efficiency in other production, housing, and more extreme conditions would be useful.

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REFERENCES

Adams, M.C., Luo, J., Rayward, D., King, S., Gibson, R. & Moghaddam, G.H. 2008. Selection of a novel direct-fed microbial to enhance weight gain in intensively reared calves. *Anim. Feed Sci. Technol.* **145**(1–4), 41–52. doi.org/10.1016/j.anifeedsci.2007.05.035

Aiello, S.E. & Moses, M.A. 2016. *Merck veterinary manual - Summary,* 11th ed., Aiello, S.E., Moses, M.A., Allen, D.G. (eds), The Wiley Merck & Co. Inc.: Kenilworth, NJ, USA, p. 50.

Akins, M.S. 2016. Dairy Heifer Development and Nutrition Management. *Vet. Clin. N. Am. Food animal practice* **32**(2), 303–317. doi.org/10.1016/j.cvfa.2016.01.004

Ali, R., Saravia, F., Hille-Reichel, A., Gescher, J. & Horn, H. 2021. Propionic acid production from food waste in batch reactors: Effect of pH, types of inoculum, and thermal pre-treatment, *Bioresour. Technol.* **319**, 1–7, 124166. doi.org/10.1016/j.biortech.2020.124166

Anderson, N.G. 2013 Group housing and feeding of milk-fed calves: Moving closer to nature's way. Ontario Ministry of Agriculture, Food and Rural Affairs (blog). *http://www.omafra.gov.on.ca/english/livestock/dairy/facts/grouphousing.html.* Accessed 20.12.2021.

Antone, U., Liepins, J., Zagorska, J. & Cinkmanis, I. 2021. Fermentation of milk whey permeate with different dairy propionibacteria strains. **In** Černiauskienė J. (ed): *Rural development: challenges for sustainable bioeconomy and climate change: proceedings of the 10th international scientific conference,* 21-23 September, 2021, Vytautas Magnus University Agriculture Academy, Kaunas, Lithuania, pp. 1–7, ISSN 1822-3230, 2345-0916.

Appleby, M.C., Weary, D.M. & Chua, B. 2001. Performance and feeding behaviour of calves on ad libitum milk from artificial teats. *Appl. Anim. Behav. Sci.* **74**, 191–201. doi.org/10.1016/S0168-1591(01)00171-X

Aschenbach, J.R., Zebeli, Q., Patra, A.K., Greco, G., Amasheh, S. & Penner, G.B. 2019. Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow*. *J. Dairy Sci.* **102**(2), 1866–1882, (*Presented as part of the Ruminant Nutrition Platform Session: Rumen Function and Health at the ADSA Annual Meeting, Knoxville, Tennessee, June 2018). doi.org/10.3168/jds.2018-15243

Atkinson, R.L., Kratzer, F.H. & Stewart, G.F. 1957. Lactose in Animal and Human Feeding: A Review. *J. Dairy Sci.* **40**(9), 1114–1132. doi.org/10.3168/jds.S0022-0302(57)94603-9

Audic, J., Chaufer, B. & Daufin, G. 2003. Non-food applications of milk components and dairy co-products: A review. *Lait* **83**, 417–438. doi.org/10.1051/lait:2003027

Azzaz, H.H., El-Sherbiny, M., Murad, H.A. & Ebeid, H.M. 2019. Propionibacteria in Ruminant's Diets: An Overview. *J. Appl. Sci.* **19**, 166–172. doi.org/10.3923/jas.2019.166.172

Bai, J.A. & Rai, V.R. 2015. Application of Dairy Propionic Acid Bacteria in Fermented Foods and Probiotics. **In** Bai, J.A., Rai, V.R. (eds), *Beneficial Microbes in Fermented and Functional Foods,* CRC Press Taylor & Francis Group: Boca Raton, Florida, USA, pp. 551–569.

Bain, P.J. Liver. 2011. **In** Latimer, K.S. (ed) *Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology,* 5th ed., John Wiley & Sons, Ltd.: Chichester, UK, p. 216.

Bargeman, G. 2003. Separation technologies to produce dairy ingredients. **In** Smit G. (ed) *Dairy processing. Improving quality.* CRC, Abington, USA, pp. 366–390.

Breen, J.E., Down, P.M., Kerby, M., Bradley, A.J. & Green, M.J. 2012. Restoring the dairy herd: rearing youngstock and replacing cows. **In** Green, M., Bradley, A., Breen, J. et al. (eds) *Dairy Herd Health,* CABI Publishing: London, UK, pp. 35–60.

Cardo, L. DVM, 2016. Improving calf health in Denmark, Dairy Global (blog). *https://www.dairyglobal.net/Calves/Articles/2016/10/Improving-calf-health-in-Denmark-2901491W/.* Accessed 11.02.2022.

Celi, P., Cowieson, A.J., Fru-Nji, F., Steinert, R.E., Kluenter, A.-M. & Verlhac, V. 2017. Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. *Anim. Feed Sci. Technol.* **234**, 88–100. doi.org/10.1016/j.anifeedsci.2017.09.012

Chen, Y., Gao, Y., Yin, S., Zhang, S., Wang, L. & Qu, Y. 2020. Effect of acidified milk feeding on the intake, average daily gain and fecal microbiological diversity of Holstein dairy calves. *Asian-australas. J. Anim. Sci.* **33**(8), 1265–1272. doi.org/10.5713/ajas.19.0412

Coelho, M.G., Silva, F.L.M., Silva, M.D., Silva, A.P., Cezar, A.M., Slanzon, G.S., Miqueo, E., De Toledo, A.F. & Bittar, C.M.M. 2020. Acidified milk for feeding dairy calves in tropical raising systems. *J. Anim. Feed Sci.* **29**(3), 215–223. doi:10.22358/jafs/127690/2020

Dai, D., Qiu, K., Zhang, H.J., Wu, S.G., Han, Y.M., Wu, Y.Y., Qi, G.H. & Wang, J. 2021. Organic Acids as Alternatives for Antibiotic Growth Promoters Alter the Intestinal Structure and Microbiota and Improve the Growth Performance in Broilers. *Front. Microbiol.* **11**, 618144. doi.org/10.3389/fmicb.2020.618144

Davis, C.L. & Drackley, J.K. 1998. *The development, Nutrition, and Management of the Young Calf.* Iowa State University Press, Ames, Iowa. https://www.cabdirect.org/cabdirect/abstract/19981414219. Accessed 20.12.2021.

Diao, Q., Zhang, R. & Fu, T. 2019. Review of Strategies to Promote Rumen Development in Calves. *Animals* **9**(8), 490. doi.org/10.3390/ani9080490

Diebold, G. & Eidelsburger, U. 2006. Acidification of diets as an alternative to antibiotic growth promoters. **In** Barug, D., Dejong, J., Kies, A.K., Verstegen, M.W.A. (eds), *Antimicrobial growth promoters. Where do we go from here?* Wageningen Academic Publishers, pp. 311–327.

Drackley, J.K. 2005. Does Early Growth Affect Subsequent Health and Performance of Heifers? **In** Garnsworthy, P.C. (ed): *Advances in dairy technology: proceedings of the Western Canadian Dairy Seminar,* March 8–11, 2005, Red Deer, Alberta, Canada*,* 17, 189–205.

Dunn, O.J. 1964. Multiple comparisons using rank sums. *Technometrics* **6**(3), 241–252. doi.org/10.1080/00401706.1964.10490181

Eckersall, P.D. 2008. Proteins, Proteomics and Dysproteinemias. **In** Kaneko, J.J., Harvey, J.W., Bruss, M.L. (eds), *Clinical Biochemistry of Domestic Animals,* 6th ed., Academic Press: p. 146.

El-Shewy, A.A. 2016. Whey as a Feed Ingredient for Lactating Cattle. *Science International* **4**, 80–85. doi.org/10.17311/sciintl.2016.80.85

El-Tanboly, E. 2017. Recovery of Cheese Whey, a by-Product from the Dairy Industry for use as an Animal Feed. *J. nutri. health food eng.* **6**(5), 148–154. doi.org/10.15406/jnhfe.2017.06.00215

EC (European Comission) 2017. EU One Health Action Plan against Antimicrobial Resistance. *https://ec.europa.eu/health/amr/action_eu_en.* Accessed 24.05.2020.

European Food Safety Authority (EFSA) Scientific Opinion on the safety and efficacy of Propionibacterium acidipropionici (CNCM MA 26/4U) as a silage additive for all species, *EFSA Journal* **10**(5), 2673. *http://www.efsa.europa.eu/en/efsajournal/pub/2673.* Accessed 25.02.2022. doi.org/10.2903/j.efsa.2012.2673

EWPA. 2020. Whey in animal nutrition. A valuable ingredient (This brochure is a joint publication of the members of EWPA, the European Whey Products Association, Brussels, Belgium), Available online: www.euromilk.org/ewpa (accessed on 22 May 2021).

Geiger, A.J., Parsons, C.L.M., James, R.E. & Akers, R.M. 2016. Growth, intake, and health of Holstein heifer calves fed an enhanced preweaning diet with or without post-weaning exogenous estrogen. *J. Dairy Sci.* **99**, 3995–4004. doi.org/10.3168/jds.2015-10405

Gupta, A. & Srivastava, A.K. Continuous propionic acid production from cheese whey using in situ spin filter. 2001. *Biotechnol Bioprocess Eng.* **6**, 1–5. doi.org/10.1007/bf02942242

Hill, T.M., Bateman, H.G., Aldrich, J.M., Quigley, J.D. & Schlotterbeck, R.L. 2013. Evaluation of ad libitum acidified milk replacer programs for dairy calves. *J. Dairy Sci.* **96**(5), 3153–3162. doi.org/10.3168/jds.2012-6132

House, J.K., Smith, G.W., McGuirk, S.M., Gunn, A.A. & Izzo, M. 2015. Manifestations and Management of Disease in Neonatal Ruminants. **In** Smith, B.P. (ed). *Large Animal Internal Medicine*, 5th ed., Elsevier Mosby, Ltd.: St Louis, Missouri, USA, p. 301.
Jasper, J. & Weary, D.M. 2002. Effects of ad libitum milk intake on dairy calves. *J. Dairy Sci.* **85**, 3054–3058. doi.org/10.3168/jds.S0022-0302(02)74391-9
Ježek, J. 2007. The dynamics of serum immunoglobulin concentrations and hematological and biochemical parameters in the period to the age of 24 weeks in differently reared calves, *Univerza v Ljubljani, Veterinarska fakulteta*, Ljubljana, Slovenija, p. 172.
Jones, C.M. & Heinrichs, J. 2014. Feeding Acidified Milk to Calves. PennStateExtension (blog). *https://extension.psu.edu/feeding-acidified-milk-to-calves.* Accessed 20.02.2022.
Kertz, A.F., Prewitt, L.R. & Everett, J.P. Jr. 1979. An Early Weaning Calf Program: Summarization and Review, *J. Dairy Sci.* **62**(11), 1835–1843. doi.org/10.3168/jds.S0022-0302(79)83508-0
Kessell, A. 2015. Bovine Haemetology and Biochemistry. **In** Cockcroft, P.D. (ed), *Bovine Medicine (3d. ed.)* John Wiley & Sons, Ltd.: Chichester, UK. https://doi.org/10.1002/9781118948538.ch16
Knowles, T.G., Edwards, J.E., Bazeley, K.J., Brown, S.N., Butterworth, A. & Warriss, P.D. 2000. Changes in the blood biochemical and haematological profile of neonatal calves with age. *Vet. Rec.* **147**(21), 593–598. doi.org/10.1136/vr.147.21.593
Kraft, W. 1999. Hämatologie. **In** Kraft, W., Dürr, U.M. (eds), *Klinische Labordiagnostik in der Tiermedizin,* Schattauer, Stuttgart, Germany, pp. 43–77.
Kraft, W. & Dürr, U.M. 1999. Harnapparat. **In** Kraft, W., Dürr, U.M. (eds), *Klinische Labordiagnostik in der Tiermedizin*, Schattauer, Stuttgart, Germany, pp. 169–200.
Kwak, H.S., Lee, W. & Lee, M. 2012. Revisiting lactose as an enhancer of calcium absorption. *Int. Dairy J.* **22**, 147–151. doi.org/10.1016/J.IDAIRYJ.2011.09.002
Latvietis, J. 2013. Lopbarība (Forage), Latvijas Lauksaimniecības universitāte (Latvia University of Agriculture), Jelgava, Latvia, pp. 213–217 (in Latvian).
Leal, L.N., Doelman, J., Keppler, B.R., Steele, M.A. & Martín-Tereso, J. 2021. Preweaning nutrient supply alters serum metabolomics profiles related to protein and energy metabolism and hepatic function in Holstein heifer calves. *J. Dairy Sci.* **104**(7), 7711–7724. doi: 10.3168/jds.2020-19867. Epub 2021 Apr 23. PMID: 33896629.
Li, L., Qu, J., Xin, X., Yin, S. & Qu, Y. 2019. Comparison of Reconstituted, Acidified Reconstituted Milk or Acidified Fresh Milk on Growth Performance, Diarrhea Rate, and Hematological Parameters in Preweaning Dairy Calves. *Animals* **9**, 778. doi.org/10.3390/ani9100778
Long, S.F., Xu, Y.T., Pan, L., Wang, Q.Q., Wang, C.L., Wu, J.Y., Wu, Y.Y., Han, Y.M., Yun, C.H. & Piao, X.S. 2018. Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. *Anim. Feed Sci. Technol.* **235**, 23–32. doi.org/10.1016/j.anifeedsci.2017.08.018
Lorenz, I., Mee, J.F., Earley, B. & More, S.J. 2011. Calf health from birth to weaning. I. General aspects of disease prevention. *Ir. Vet. J.* **64**(1), 10. doi.org/10.1186/2046-0481-64-10
Manurung, S. 2012. *Fermented Whey Permeate for Piglets: As a Strategy to Reduce Post Weaning Diarrhea.* Ph.D. Thesis, Technical University of Denmark, Copenhagen, Denmark, 141 pp.
Miller-Cushon, E. 2015. Intensified pre-weaning calf feeding programs: Impacts on growth and behavior, University of Florida, In *Proceedings of the 2015 Florida Ruminant Nutrition Symposium,* Gainesville, FL, USA, 2–4 February, pp. 79–89.
Mohri, M., Sharifi, K. & Eidi, S. 2007. Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults. *Res Vet Sci.* **83**(1), pp. 30–39. doi.org/ 10.1016/j.rvsc.2006.10.017
Partanen, K.H. & Mroz, Z. 1999. Organic acids for performance enhancement in pigs. *Nutr. Res. Rev.* **12**, 117–145. doi.org/10.1079/095442299108728884

Poonam, Pophaly, S.D., Tomar, S.K., De, S. & Singh, R. 2012. Multifaceted attributes of dairy propionibacteria: a review. *World J. Microbiol. Biotechnol.* **28**(11), 3081–3095. doi.org/10.1007/s11274-012-1117-z

Rabah, H., Rosa do Carmo, F.L. & Jan, G. 2017. Dairy Propionibacteria: Versatile Probiotics. *Microorganisms* **5**(2), 24. doi.org/10.3390/microorganisms5020024

Ranaei, V., Pilevar, Z., Khanegha, A.M. & Hosseini, H. 2020. Propionic Acid: Method of Production, Current State and Perspectives. *Food Technol Biotechnol.* **58**(2), 115–127. doi: 10.17113/ftb.58.02.20.6356

Sardoabi Shiasi, R., Alikhani, M., Hashemzadeh, F., Khorvash, M., Mirzaei, M. & Drackley, J.K. 2021. Effects of different planes of milk feeding and milk total solids concentration on growth, ruminal fermentation, health, and behavior of late weaned dairy calves during summer. *J. Animal Sci. Biotechnol.* **12**, 96. doi.org/10.1186/s40104-021-00615-w

Schwarzkopf, S., Kinoshita, A., Kluess, J., Kersten, S., Meyer, U., Huber, K., Dänicke, S. & Frahm, J. 2019. Weaning Holstein Calves at 17 Weeks of Age Enables Smooth Transition from Liquid to Solid Feed. *Animals* **9**(12), 1132. doi.org/10.3390/ani9121132.

Stämpfli, H. & Oliver-Espinosa, O. 2015. Clinical Chemistry Tests. **In** Smith, B.P. (ed), *Large Animal Internal Medicine*, 5th ed., Elsevier Mosby Publishers, Ltd.: St Louis, Missouri, USA, p. 350–371.

Stratton-Phelps, M. & Maas, J. 2015. Alterations in Body Weight and Size. **In** Smith, B.P. (ed), *Large Animal Internal Animal Medicine,* 5th ed., Elsevier Mosby, Ltd.: St Louis, Missouri, USA, pp. 131–133.

Teixeira, A.G.V., Stephens, L., Divers, T.J., Stokol, T. & Bicalho, R.C. 2015. Effect of crofelemer extract on severity and consistency of experimentally induced enterotoxigenic Escherichia coli diarrhea in newborn Holstein calves, *J. Dairy Sci.* **98**, 8035–8043. doi.org/10.3168/jds.2015-9513

Van Amburgh, M. 2017. Optimizing Nutrition and Management of Calves and Heifers for Lifetime Productivity, In *2017 Western Dairy Management Conference Proceedings,* Reno, NV, USA, 28 February – 2 March 2017, pp. 198–210. *http://wdmc.org/2017/Full2017.pdf.* Accessed 11.02.2022.

Vargha, A. & Delaney, H.D. 1998. Kruskal-Wallis test and stochastic homogeneity. *J. Educ. Behav. Stat.* **23**(2), 170–192. doi.org/10.3102/10769986023002170

Widyastuti, Y.R. & Febrisiantosa, A. 2014. The Role of Lactic Acid Bacteria in Milk Fermentation, *Food Sci. Nutr.* **5**(4), 435–442. doi.org/10.4236/fns.2014.54051

Yu, K., Canalias, F., Solà-Oriol, D., Arroyo, L., Pato, R., Saco, Y., Terré, M. & Bassols, A. 2019. Age-Related Serum Biochemical Reference Intervals Established for Unweaned Calves and Piglets in the Post-weaning Period. *Front Vet. Sci.* **6**, 123. doi: 10.3389/fvets.2019.00123

Zarate, G., Babot, J.D., Argañaraz-Martínez, E., Lorenzo-Pisarello, M.J. & Perez Chaia, A. 2011. Dairy propionibacteria: Technological importance and probiotic potential for application on human and animal nutrition. In Filip, R. (ed), *Multidisciplinary Approaches on Food Science and Nutrition for the XXI Century*, Kerala, India, 175–213.

Zhang, H., Wang, Y., Chang, Y., Luo, H., Brito, L. F., Dong, Y., Shi, R., Wang, Y., Dong, G. & Liu, L. 2019. Mortality-Culling Rates of Dairy Calves and Replacement Heifers and Its Risk Factors in Holstein Cattle. *Animals* **9**(10), 730. doi.org/10.3390/ani9100730

Zhang, R., Diao, Q.Y., Zhou, Y., Yun, Q., Deng, K.D., Qi, D. & Tu, Y. 2017. Decreasing the pH of milk replacer containing soy flour affects nutrient digestibility, digesta pH, and gastrointestinal development of preweaned calves. *J. Dairy Sci.* **100**(1), 236–243. doi.org/10.3168/jds.2016-11374

Zou, Y., Wang, Y., Deng, Y., Cao, Z., Li, S. & Wang, J. 2017. Effects of feeding untreated, pasteurized and acidified waste milk and bunk tank milk on the performance, serum metabolic profiles, immunity, and intestinal development in Holstein calves. *J. Anim. Sci. Biotechnol.* **8**(1), Article nr 53. doi.org/10.1186/s40104-017-0182-4

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Composition and cheese suitability of milk from local Ukrainian cows and their crossbreedings with Montbeliarde breed

O.O. Borshch[1,2,*], S. Ruban[2], O.V. Borshch[1], L. Kosior[1], M. Fedorchenko[1], L. Bondarenko[1] and V. Bilkevich[1]

[1]Bila Tserkva National Agrarian University, Chair of Technology Milk and Meat Production, UA09117 Bila Tserkva, Ukraine
[2]National University of Life and Environmental Science of Ukraine, Chair of Genetics, Breeding and Reproductive Biotechnology, UA03041 Kyiv, Ukraine
[*]Correspondence: borshcha@outlook.com

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Abstract. The aim of this work was to compare the qualitative composition of milk and its suitability for cheese processing at cows of local Ukrainian Red-Spotted breed (URS) and their crossbreeds with Montbeliarde (MO) breed. The research was conducted at commercial farm in the Vinnytsia region, Ukraine (48° 57′01″ n.l., 28° 47′09″ e.l). At farm, two groups of purebred and crossbred first lactation cows-analogues with a population of 20 heads in each were formed. The use of crossbreeding cows URS × Montbéliarde breed had a positive effect on the milk composition and cheese suitability. It was established that local purebred cows exceeded purebred counterparts in daily milk yield by 2.47 kg. When the content of fat, protein and lactose in milk was higher in crossbreed group by 0.19, 0.19 and 0.12%, respectively. In addition, crossbreed cows surpassed purebred counterparts for the energy value of 1 kg of milk and theoretically possible output of rennet cheese by 0.142 MJ and 0.61 kg. The duration of the coagulation phase of milk obtained from crossbred cows was shorter than that of purebred analogues by 1.54 minutes.

Key words: crossbreeding, local Ukrainian dairy breed, Montbeliarde, milk, suitability for cheese processing.

INTRODUCTION

Breeding of cattle is directed at improving the genetic value of animals and providing opportunities for future generations to produce milk in a more efficient way. Crossbreeding or interbreeding has a positive effect on the profitability of milk production, the reproduction, the health of dairy cows, and on the composition and properties of milk (Dezetter et al., 2015; Hazel et al., 2017). For this reason, crossbreeding programs in dairy cattle breeding have been widespread in recent decades in many countries. Thus, in the period from 2003 to 2014, in the United States, the number of cows obtained from crossbreeding increased from 0.5 to 4.5% (Hazel et al., 2017). In recent years, crossbreeding has been actively implemented in a number of other countries, such as India (Singh, 2016), New Zealand (Sneddon et al., 2016), Ireland

(Hazel et al., 2017) and Ukraine (Borshch et al., 2018). Holstein (HO) cattle are the most common milk breed in the world because of the ability to produce a large amount of milk.

El-Tarabany (2015) has detected that the crossbreeds of Brown Swiss and HO were less susceptible to metabolic disorders and to changes in their daily diet compared with pure breed HO. The results of the research conducted at the HO breed in different environmental conditions indicated a reduction in the productivity of the firstborn (Malchiodi et al., 2014; Hazel et al., 2017).

Hazel et al. (2017) reported that at the crossbreeds of the Montbeliarde and HO and Brown Swiss and HO breeds, milk contained more fat and protein compared with purebred HO. Blöttner et al. (2011) found no differences in the cast of fat and protein for the first three lactations at the crossbreeds of Brown Swiss and HO breeds compared to purebreeding HO.

One of the most important indicators that characterizes dairy raw materials, along with the content of fat, protein, and lactose, is the ability for processing into butter and cheese depending on the amino acid and fatty acid composition (Mapekula et al., 2011; Sun et al., 2014; Borshch et al., 2019).

The production of cheese is an important sector of milk processing, and the technological indicator of the cheese cast percentage (the amount of cheese obtained from a certain amount of processed milk expressed in percents) is the most important economic indicator for the dairy industry and, indirectly, for determining the price of milk (Cipolat-Gotet et al., 2013; Stocco et al., 2018; Amalfitano et al., 2020; El Jabri et al., 2020). The cheese industry is one of the most dynamic consumer segments with steady growth in production and consumption (Bankole et al., 2021; Mota et al., 2022; Sanchez et al., 2022). Cheese production is the most important technological parameter in the dairy industry in many countries, for example, in Italy, where almost 75% of milk is used for cheese production (Bittante et al., 2014). Thus, cheese output is an important feature of the Italian dairy industry (Bittante et al., 2013; Cecchinato et al., 2015).

The most popular dairy breeds in Ukraine are Ukrainian Black-Spotted (UBS) and Ukrainian Red-Spotted (URS). These breeds are common in all regions and climatic zones of the country. URS breed was created by crossing red-spotted local cattle with Simmental and Holstein breed. The main problems for the breed are the composition of milk and the duration of productive longevity (Borshch et al., 2021).

The aim of this work was to compare the qualitative composition of milk and its suitability for cheese processing at cows of local Ukrainian Red-Spotted breed and their crossbreeds with Montbeliarde breed.

MATERIAL AND METHODS

The research was conducted at commercial farm in the Vinnytsia region, Ukraine (48° 57′01″ n.l., 28° 47′09″ e.l). In farm holds the cows of the Ukrainian Red-Spotted (URS) breed and the first-generation of crossbred cows, which has been got thanks to the breeding with MO breed. At farm, two groups of purebred and crossbred first lactation cows-analogues with a population of 20 heads in each were formed. Indicator of average productivity is 22.70 ± 1.07 kg day^{-1}, days in milking - 92 ± 4 days. Dairy cows were kept loosely in brick barn for 100 heads. Parameters of placements (Length × Width × Height): 76×12×6 m. On the dairy farm, the cows were milked twice a day, from 06:00 to 08:00 and from 16:00 to 18:00. Accounting for the milk productivity of experimental cows was carried out according to daily and monthly milk yields. Cows being milked in the milking parlour with a capacity of 2 × 6 on the installation 'Tandem' (Bratslav, Ukraine).

Cows fed total mixed ration (Table 1). Distribution of feed takes place twice a day (at 09.00 and 19.00 hours).

Table 1. Ingredients of diet fed to dairy cows

Item	Amount (kg)
Alfalfa hay	1
Barley straw	2
Corn silage	17.5
Bean haylage	10.5
Molasses	1.2
Compound feed[1]	6

Where: [1]Compound feed: 23% corn, 20% wheat, 18% barley, 15% peas, 5% oats, 5% wheat bran, 5% sunflower cake, 5% soybean meal, 2% feed phosphates, 1% NaCl, 1% premix.

Samples of milk (500 mL) were selected during evening milking from each cow and stored without adding preservative in a refrigerator at a temperature of 4 °C for a period of 14 hours. Indicators of fat, protein, lactose content in milk, as well as density and freezing temperatures were determined in a milk analyzer (Milkotester Lactomat Rapid S, Bulgaria).

Milk suitability for cheese was evaluated according to the rate of formation of a clot under the action of the rennet, and its quality - after the rennet-fermenting sample. The rate of formation of the clot was determined as follows: 20 mL of milk heated to 35 °C and 4 mL of a 0.03% solution of the rennet (Podpuszczka, Poland) were added to the sterile test tube. Prior to this, the rennet agent was dissolved in water. The contents were thoroughly mixed and placed in a water bath (MEMMERT WPE 45, Germany) at 35 °C. Time was fixed from the moment when milk was added to the rennet until the milk clotting. The end of the coagulation was considered the moment of formation of a dense clot that does not fall out of the tube with its careful overturning.

The rennet-fermenting test was carried out in such a sequence: 30 mL of milk was poured into the test tubes and then 1 mL of 0.5% solution of the rennet was added to each test tube, mixed well and placed in a water bath at a temperature of 38 °C for 12 hours, after which it was removed from the bath and examined. The assessment of the milk quality was carried out according to the characteristics of the formed clot. To class I the clots with the following features were assigned: a clot with a smooth surface, elastic to the touch, without eyes on a longitudinal section, floating in transparent whey; to the II class: a soft to touch clot, with single eyes (1–10), torn apart, but not scattered; to the III class: a clot with numerous eyes, spongy, soft to the touch, has come to the surface or instead of a clot a flatter mass has formed.

The net energy content (NEL) of milk was estimated by means of the following equation, derived from that proposed by the NRC (2001):

$$NEL = 0.0929 \times \text{fat, \%} + 0.0547 \times \text{protein, \%} + 0.0395 \times \text{lactose, \%} \qquad (1)$$

where NEL is the gross energy of one kg of milk.

The NEL values obtained were converted to MJ kg^{-1}.

Predict the theoretical yield of rennet cheese was calculated according to Brito et al. (2002):

$$Y = 1.037 + 1.433 \times P_M + 1.71 \times F_M \qquad (2)$$

where Y – theoretical yield of rennet cheese, kg 100 kg of milk; P_M – protein content of milk, g 100 g of milk; F_M – fat content of milk, g 100 g of milk.

All data are presented as the means ± standard error of the mean. Student's *t*-test was used to estimate statistical significance of the obtained values. Data were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. These computations were performed using the STATISTICA software (Version 11.0, 2012).

RESULTS AND DISCUSSION

The productivity of crossbred cows was slightly inferior to the productivity of purebred analogues (Table 2). Thus, the average daily milk yield of URS × MO cows was 2.24 kg less than that of thoroughbred analogues. Instead, local cows significantly surpassed purebred analogues by the indicators of fat and protein in milk. The fat and protein content of the milk of crossbreed URS × MO cows was 0.19 and 0.19% higher than that of purebred cows. The content of milk in lactose of local cows was also higher than that of purebred analogues: by 0.12%. The amount of milk fat and protein affects the energy value of 1 kg of milk and theoretical yield of rennet cheese. According to the indices, the crossbreed's cows excelled the local analogues by 0.142 MJ and 0.61 kg, respectively.

Table 2. Composition and properties of milk at cows of different genotypes

Indicators	URS	URS × MO
Average daily milk yield, kg	23.44 ± 0.53	$20.97 \pm 0.46^{**}$
Mass fraction of fat, %	4.09 ± 0.05	$4.28 \pm 0.05^{*}$
Mass fraction of protein, %	3.15 ± 0.04	$3.34 \pm 0.05^{**}$
Mass fraction of lactose, %	4.34 ± 0.03	$4.46 \pm 0.04^{*}$
Energy value of 1 kg of milk, MJ	3.027 ± 0.015	$3.169 \pm 0.019^{***}$
Theoretical yield of rennet cheese, kg 100 kg of milk	12.28 ± 0.08	$12.89 \pm 0.13^{***}$

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ as compared with URS group. URS: Ukrainian Red-Spotted dairy breed; MO: Montbeliarde breed.

Crossbred cows often have lower daily milk yields than purebred cows (Dechow et al., 2007; Heins & Hansen, 2012; Hazel et al., 2014; Hazel et al., 2021). The results of our studies not coincide with the research results of Puppel et al. (2017), it is indicated that the crossbreeds of HO × MO breeds had a higher average daily productivity than purebred HO. However, Saha et al. (2017) report that the crossbreed first generation of HO × MO breed dominated the purebred HO cows by the indicators of the milk yield. In researches of Sneddon et al. (2016), they indicate that the heterosis effect after crossing the HO × Jersey breeds at New Zealand's farms had a positive effect on the cast of cheese and butter. At the same time, the productivity of such crossbreeds was lower than at purebred HO cows.

Numerous studies confirm the influence of breed on nutrient content in milk (Rafiq et al., 2016; Liang et al, 2018; Borshch et al., 2019). The breeds of cows are also notable for technological properties of milk, such as the duration of rennet coagulation, phases of gel creation, the size and number of fat balls, different constants of milk fat and the composition of its fractions (Fox et al., 2017). Such important indicators as protein content, rennet coagulation and quality of the rennet clot are determined primarily by breed and race of cows.

The duration of the coagulation phase of milk obtained from crossbred cows was shorter than that of purebred analogues by 1.54 minutes (Table 3). Indicators of the freezing point and milk density are of great importance in the technology of preparation of dairy products. In the studied samples of milk, these indicators corresponded to the requirements for raw materials for cheese-making. Established breed differences of the considered indicators have a character of tendency and are not statistically significant.

When creating a rennet-fermentation test, clots were classified as classes I or II. From the 20 investigated samples of milk of cows of URS breed 16 (80%) are classified as the I and 4 (20%) of the II class; 18 (90%) samples of milk of crossbred cows of URS × MO corresponded to the I and 2 (10%) to the II class.

Table 3. Characteristic of cheese suitability and physical-chemical properties of milk at cows of different genotypes

Indicators	URS	URS × MO
Coagulation time, min	12.88 ± 0.306	11.34 ± 0.326***
Freezing point, °C	-0.555 ± 0.0028	-0.551 ± 0.0033
Density, g cm^{-3}	1.0257 ± 0.002	1.0291 ± 0.004
Characteristic of a rennet clot according to the class, %:		
I	80	90
II	20	10

***$P < 0.001$ as compared with URS group. URS: Ukrainian Red-Spotted dairy breed; MO: Montbeliarde breed.

Our studies coincide with the data of Puppel et al. (2017), which indicate that the phase of milk coagulation at crossbreeds of the Polish Holstein with Brown Swiss and MO breeds was shorter than that of purebred Polish Holstein. Similar data were also obtained in studies by Saha et al. (2017) in which it was indicated that the duration of the coagulation phase at pure breed HO is longer than that of the HO × MO breeds.

CONCLUSIONS

The use of crossbreeding local Ukrainian cows (Ukrainian Red-and-Spotted) with Montbéliarde breed had a positive effect on the milk composition and cheese suitability. It has been established that in the milk of crossbred cows the mass fraction of fat, protein and lactose in the average essentially prevailed the pure-breed analogues (by 0.19; 0.19 and 0.12%). Besides crossbred cows dominated pure-breed analogues by the energy value of 1 kg of milk and theoretically possible output of rennet cheese by 0.142 MJ and 0.61 kg, respectively. The duration of the coagulation phase of milk obtained from crossbred cows was shorter than that of purebred analogues by 1.54 minutes. The daily milk yield in the group of purebred cows was 2.47 kg higher than in crossbreed cows.

REFERENCES

Amalfitano, N., Stocco, G., Maurmayr, A., Pegolo, S., Cecchinato, A. & Bittante, G. 2020. Quantitative and qualitative detailed milk protein profiles of 6 cattle breeds: Sources of variation and contribution of protein genetic variants. *Journal of Dairy Science* **103**(12), 11190–11208. doi: 10.3168/jds.2020-18497

Bankole, I.R., Mahamat, S.A., Mazou M., Lokossou, S.C. & Tchobo, F.P. 2021. Cheese-making Ability of Plumeria alba and Plumeria rubra Latex: Milk Clotting and Proteolytic Activities. *American Journal of Chemical Engineering* **9**(3), 47–52. doi: 10.11648/j.ajche.20210903.11

Bittante, G., Cipolat-Gotet, C. & Cecchinato, A. 2013. Genetic parameters of different measures of cheese yield and milk nutrient recovery from an individual model cheese-manufacturing process. *Journal of Dairy Science* **96**(12), 7966–7979. doi: 10.3168/jds.2012-6517

Bittante, G., Ferragina, A., Cipolat-Gotet, C. & Cecchinato, A. 2014. Comparison between genetic parameters of cheese yield and nutrient recovery or whey loss traits measured from individual model cheese-making methods or predicted from unprocessed bovine milk samples using Fourier-transform infrared spectroscopy. *Journal of Dairy Science* **97**(10), 6560–6572. doi: 10.3168/jds.2014-8309

Blöttner, S., Heins, B.J., Wensch-Dorendorf, M., Hansen, L.B. & Swalve, H.H. 2011. Short communication: A comparison between purebred Holstein and Brown Swiss Holstein cows for milk production, somatic cell score, milking speed, and udder measurements in the first 3 lactations. *Journal of Dairy Science* **94**(10), 5212–5216. doi: 10.3168/jds.2011-4255

Borshch, A.A., Borshch, A.V., Lutsenko, M.M., Merzlov, S.V., Kosior, L.T., Lastovska, I.A. & Pirova, L.V. 2018. Amino acid and mineral composition of milk from local Ukrainian cows and their crossbreedings with Brown Swiss and Montbeliarde breeds. *Journal of the Indonesian Tropical Animal Agriculture* **43**(3), 238–246. doi: 10.14710/jitaa.43.3.238-246

Borshch, O.O., Borshch, O.V., Kosior, L.T., Lastovska, I.A. & Pirova, L.V. 2019. The influence of crossbreeding on the protein composition, nutritional and energy value of cow milk. *Bulgarian Journal of Agricultural Science* **25**(1), 117–123.

Borshch, O.O., Ruban, S., Borshch, O.V., Malina, V., Fedorchenko, M., Kosior, L. & Korol-Bezpala, L. 2021. Productivity, milk composition and reasons for leaving the herds of Ukrainian local cows and their crossbreeds with Brown Swiss and Montbeliarde breeds during five lactations. *Roczniki Naukowe Zootechniki* **48**(2), 205–216.

Brito, C., Niklitschek, L., Molina, L.H. & Molin, I. 2002. Evaluation of mathematical equations to predict the theoretical yield of Chilean Gouda cheese. *International Journal of Dairy Technology* **55**(1), 32–39. doi: 10.1046/j.1364-727X.2001.00035.x

Cecchinato, A., Albera, A., Cipolat-Gotet, C., Ferragina, A. & Bittante, G. 2015. Genetic parameters of cheese yield and curd nutrient recovery or whey loss traits predicted using Fourier-transform infrared spectroscopy of samples collected during milk recording on Holstein, Brown Swiss, and Simmental dairy cows. *Journal of Dairy Science* **98**(7), 4914–4927. doi: 10.3168/jds.2014-8599

Cipolat-Gotet, C., Cecchinato, A., De Marchi, M. & Bittante, G. 2013. Factors affecting variation of different measures of cheese yield and milk nutrient recovery from an individual model cheesemanufacturing process. *Journal of Dairy Science* **96**(12), 7952–7965. doi: 10.3168/jds.2012-6516

Dechow, C.D., Rogers, G.W., Cooper, J.B., Phelps, M.I. & Mosholder, A.L. 2007. Milk, fat, protein, somatic cell score, and days open among Holstein, Brown Swiss, and their crosses. *Journal of Dairy Science* **90**(7), 3542–3549. doi: 10.3168/jds.2006-889

Dezetter, C., Leclerc, H., Mattalia, S., Barbat, A., Boichard, D. & Ducrocq, V. 2015. Inbreding and Crossbreeding parameters for production and fertility traits in Holstein, Montbeliearde and Normande cows. *Journal of Dairy Science* **98**(7), 4904–4913. doi: 10.3168/jds.2014-8386

El Jabri, M., Trossat, P., Wolf, V., Beuvier, E., Rolet-Répécaud, O., Gavoye, S., Gaüzère, Y., Belysheva, O., Gaudillière, N., Notz, E., Grosperrin, P., Laithier, C. & Delacroix-Buchet, A. 2020. Mid-infrared spectrometry prediction of the cheese-making properties of raw Montbéliarde milks from herds and cheese dairy vats used for the production of Protected Designation of Origin and Protected Geographical Indication cheeses in Franche-Comté. *Journal of Dairy Science* **103**(7), 5992–6002. doi: 10.3168/jds.2019-17491

El-Tarabany, M.S. 2015. Effects of non-lactating period length on the subsequent calving ease and reproductive performance of Holstein, Brown Swiss and the crosses. *Animal Reproduction Science* **158**, 60–67. doi: 10.1016/j.anireprosci.2015.04.008

Fox, P.F., Guinee, T.P., Cogan, T.M. & Mcsweeney, P.L.H. 2017. Fundamentals of Cheese Science. *Second Edition*. Springer, New York, USA. pp. 34–60.

Hazel, A.R., Heins, B.J. & Hansen, L.B. 2017. Production and calving traits of Montbeliarde Holstein and Viking Red Holstein cows compared with pure Holstein cows during first lactation in 8 commercial dairy herds. *Journal of Dairy Science* **100**(5), 4139–4149. doi: 10.3168/jds.2016-11860

Hazel, A.R., Heins, B.J., Seykora, A.J. & Hansen, L.B. 2014. Production, fertility, survival, and body measurements of Montbéliarde-sired crossbreds compared with pure Holsteins during their first 5 lactations. *Journal of Dairy Science* **97**(4), 2512–2525. doi: 10.3168/jds.2013-7063

Hazel, A.R., Heins, B.J. & Hansen, L.B. 2021. Herd life, lifetime production, and profitability of Viking Red-sired and Montbéliarde-sired crossbred cows compared with their Holstein herdmates. *Journal of Dairy Science* **104**(3), 3261–3277. doi: 10.3168/jds.2020-19137

Heins, B.J. & Hansen, L.B. 2012. Short communication: Fertility, somatic cell score, and production of Normande × Holstein, Montbeliarde × Holstein, and Scandinavian Red × Holstein crossbreds versus pure Holsteins during their first 5 lactations. *Journal of Dairy Science* **95**(2), 918–924. doi: 10.3168/jds.2011-4523

Liang, K., Zhao, Y., Han, J., Liu, P., Qiu, J., Zhu, D., Qin, Y., Lu, L. & Wang, X. 2018. Fatty acid composition, vitamin A content and oxidative stability of milk in China. *Journal of Applied Animal Research* **46**(1), 566–571. doi: 10.1080/09712119.2017.1360186

Mapekula, M., Chimonyo, M. & Mapiye, C. 2011. Changes in Metabolites Concentration in Nguni and Crossbred Calves on Natural Pasture. *Asian Australasian Journal of Animal Sciences* **24**(11), 1569–1576. doi: 10.5713/ajas.2011.11050

Malchiodi, F., Cecchinato, A., Penasa, M., Cipolat-Gotet, C. & Bittante, G. 2014. Milk quality, coagulation properties, and curd firmness modeling of purebred Holsteins and first-and second-generation crossbred cows from Swedish Red, Montbeliarde, and Brown Swiss bulls. *Journal of Dairy Science* **97**(7), 4530–4541. doi: 10.3168/jds.2013-7868

Mota, L.F.M., Giannuzzi, D., Bisutti, V., Pegolo, S., Trevisi, E., Schiavon, S., Gallo, L., Fineboym, D., Katz, G. & Cecchinato, A. 2022. Real-time milk analysis integrated with stacking ensemble learning as a tool for the daily prediction of cheese-making traits in Holstein cattle. *Journal of Dairy Science* **105**(5), 4237–4255. doi: 10.3168/jds.2021-21426

NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National. Academy Press, 2101 Constitution Avenue, N.W., Lockbox 285, Washington. pp. 13–28.

Puppel, K., Bogusz, E., Gołebiewski, M., Nałecz-Tarwacka, T., Kuczynska, B., Slosarz, J., Budzinski, A., Solarczyk, P., Kunowska-Slosarz, M. & Przysucha, T. 2017. Effect of Dairy Cow Crossbreeding on Selected Performance Traits and Quality of Milk in First Generation Crossbreds. *Journal of Food Science* **83**(1), 229–236. doi: 10.1111/1750-3841.13988

Rafiq, S., Huma, N., Pasha, S., Sameen, A., Mukhtar, O. & Khan, M.I. 2016. Chemical Composition, Nitrogen Fractions and Amino Acids Profile of Milk from Different Animal Species. *Asian Australasian Journal of Animal Sciences* **29**(7), 1022–1028. doi: 10.5713/ajas.15.0452

Sanchez, M.P., Tribout, T., Fritz, S., Wolf, V., Laithier, C., Brochard, M. & Boichard, D. 2022. Opportunities for genomic selection of cheese-making traits in Montbéliarde cows. *Journal of Dairy Science* **105**(6), 5206–5220. doi: 10.3168/jds.2021-21558

Saha, S., Malchiodi, F., Cipolat-Gotet, C., Bittante, G. & Gallo, L. 2017. Effects of Cossbreeding of Holsteins Cows with Montbeliarde and Swedish Red in First and Second Generation on Cheese Yield Traits. *Agriculturae Conspectus Scientificus* **82**(3), 241–244.

Singh, C.V. 2016. Cross-breeding in Cattle for Milk Production: Achievements, Challenges and Opportunities in India-A Review. *Advances in Dairy Research* **4**(3), 158. doi: 10.4172/2329-888X.1000158

Sneddon, N.W., Lopez-Villalobos, N., Davis, S.R., Hickson, R.E., Shalloo, L. & Garrick, D.J. 2016. Estimates of genetic and crossbreeding parameters for milk components and potential yield of dairy products from New Zealand dairy cattle. *New Zealand Journal of Agricultural Research* **59**(1), 79–89. doi: 10.1080/00288233.2015.1131723

Stocco, G., Cipolat-Gotet, C., Gasparotto, V., Cecchinato, A. & Bittante, G. 2018. Breed of cow and herd productivity affect milk nutrient recovery in curd, and cheese yield, efficiency and daily production. *Animal* **12**(2), 434–444. doi: 10.1017/S1751731117001471

Sun, Q., Lv, J.P., Liu, L., Zhang, S.W., Liang, X. & Lu, J. 2014. Comparison of milk samples collected from some buffalo breeds and crossbreeds in China. *Dairy Science & Technology* **94**(4), 387–395. doi: 10.1007/s13594-013-0159-9

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Study of the controlled motion process of an agricultural wide span vehicle fitted with an automatic driving device

V. Bulgakov[1], J. Olt[2,*], S. Pascuzzi[3], S. Ivanovs[4], V. Kuvachov[5], F. Santoro[3], Ia. Gadzalo[6], V. Adamchuk[7] and M. Arak[2]

[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony Str., UA03041 Kyiv, Ukraine
[2]Estonian University of Life Sciences, Institute of Forestry and Engineering, 56 Kreutzwaldi Str., EE51006 Tartu, Estonia
[3]University of Bari Aldo Moro, Department of Agricultural and Environmental Science, Via Amendola, 165/A, IT70125 Bari, Italy
[4]Latvia University of Life Sciences and Technologies, Cakstes Blvd. 5, LV3001 Jelgava, Latvia
[5]Dmytro Motornyi Tavria State Agrotechnological University, 18[B] Khmelnytsky Ave, UA72310 Melitopol, Zaporozhye Region, Ukraine
[6]National Academy of Agrarian Sciences of Ukraine, 9 Mykhailo Omelyanovych-Pavlenko Str., UA01010 Kyiv, Ukraine
[7]Institute of Mechanics and Automation of Agricultural Production of the National Academy of Agrarian Sciences of Ukraine, 11 Vokzalna Str., Glevakha stl, Vasylkivsky Dist., UA08631 Kyiv Region, Ukraine
[*]Correspondence: jyri.olt@emu.ee

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Abstract. The aim of research is to analyse the process of the wide span vehicle motion on the treads of the permanent process track with the use of the traction method of turn. The completed studies have proved that the plane-parallel motion of a wide span vehicle solely with the use of the traction method of turn on the treads of the permanent process track requires a significantly smaller difference between the moments applied to the wheels on the right side and on the left side of the vehicle, as compared to that of a crawler tractor. This difference is in proportion to the width of its wheel base that has a relatively smaller length. It has been established that the use of solely traction-based turn does not provide for the adjustment of the motion trajectory through the lateral (plane-parallel) displacement of the fore-and-aft axis of the agricultural wide span vehicle. The oscillations in the lateral displacement of the agricultural wide span vehicle in the process of its motion are low-frequency ones. The main variance spectrum of these oscillations is concentrated within the range of frequencies of 0–2 s^{-1}. At the same time, the maximum transverse displacement of the agricultural wide span vehicle equal to 1.3 10^{-5} m N^{-1}, occurs also at low frequencies. In view of the fact that the main variance spectrum of the oscillations of the tangential forces applied to the wheels on the left and right sides of the agricultural wide span vehicle is concentrated exactly within a low frequency range, only the high accuracy of the system using a laser beam can ensure its satisfactory steerability.

Key words: accuracy, automatic driving, controlled traffic farming, wide span vehicle.

INTRODUCTION

The future development of agricultural wide span vehicles suggests the automation of their plane-parallel motion on the treads of the permanent process track (Kingwell & Fuchsbichler, 2011). This aspect becomes especially topical, when wide span vehicles are turned using the traction method (drive wheels on the right and left sides have different rates of revolution).

Farming with the use of a permanent process track implies the allocation of special zones in the field area intended for the motion of agricultural machinery and segregated from the zones containing cultivated plants (Nevens & Reheul, 2003; Hamza & Anderson, 2005; Chan et al., 2006; Gasso et al., 2013; Pedersen et al., 2013; Antille et al., 2015; Chamen, 2015; Keller et al., 2019). With such a layout, high grip-and-traction characteristics of its running gear are achieved due to the latter's contact with the dry and hard surface of the soil. That is, in terms of its functional purpose, the field area is divided into the fertile (agronomic) and process zones (Raper, 2005; Kingswell & Fuchsbichler, 2011; Onal, 2012; Antille et al., 2019).

In terms of engineering, it is most feasible to implement the above-mentioned farming strategy by way of developing the new design layout of the tractor, so called wide span (gantry) vehicle (Onal, 2012; Bulgakov et al., 2019). Its principal difference from other types of power units is that it travels on the treads of the permanent process track. The distance between the treads is equal to the span of the tractor and all the agricultural implements are situated within this span (Bulgakov et al., 2018). The worldwide experience of the use of wide span vehicles has proved that they have the following advantages in comparison with conventional tractors: the power savings in soil tillage processes reach 55%; the cost of crop drilling operations is reduced by 40%; the tillage quality and the soil structure are improved; the loss of the field area under the travelling agricultural machinery is reduced to a minimum; high accuracy is achieved in the positioning of the tools; the field operations become highly automated; the yield of the cultivated plants increases by 7–10% (Bulgakov et al., 2017).

On the other hand, the tractor steering control issues come into importance in case of wide span vehicles. In view of the very wide transverse wheel base, it becomes difficult for the driver-operator to control the motion trajectories of the wheels on the left and right sides. At the same time, it is common knowledge that the steering systems of today's wheeled vehicles are usually tailored for manual control. Also, these systems can be divided into two classes: they use either kinematic or forced principle of turning. The kinematic type of turning implies the angular displacement of either the steer wheels (front wheels, rear wheels or both of them simultaneously) relative to the frame of the machine or one part of the mobile unit's frame relative to its other part in the horizontal plane (articulated frame). The turning by (traction) force is implemented by way of rotating the wheels on different sides of the machine at different rates. The steering systems based on the use of steer wheels are the most widely used ones. That said, when all the wheels of the machine are steerable, they can simultaneously turn on the front axle and the rear axle to the opposite sides or to the same side. Sometimes combined kinematic-and-traction layouts are implemented or proposed in order to improve the steerability of mobile machines (Chebrolu et al., 2017; Pascuzzi et al., 2020; Kuvachov et al., 2021). However, the design and functioning conditions of the agricultural wide span vehicles intended for controlled traffic farming systems differ to some extent from those of other state-of-the-art wheeled machines. The assumptions established in the classical theory of motion for mobile power units are not applicable to the discussed agricultural wide span vehicles.

At the same time, it ought to be noted that considerable developments have recently been observed in the implementation of self-driving systems for mobile power and transport units (Pascuzzi et al., 2020). Depending on the result of the measurement, an automatic action is taken, which is aimed at reducing the said difference down to an acceptably small value. The above-mentioned self-control systems can work on the basis of different principles of operation. In particular, they can use GPS systems, short- and long-range radars, short-, middle- and long-range distance finders based on visible-light and infrared lasers, video cameras that monitor the forward view panorama and analyse the obtained images, etc. (Ji & Zhou, 2014; Cerruto et al., 2018).

The problem of automating the process of driving mobile machines amounts to the problem of developing the set of devices that will control the motion of the agricultural wide span vehicle on the treads of the permanent process track without an operator (Yang et al., 2015). Solving this problem has proved to be a rather difficult task. First of all, due to the relatively limited possibility of manoeuvring in motion and the action of a great number of the random perturbing factors that create all kinds of possible situations, to which the system has to respond by putting in action the respective controls (steering gear, brakes, engine control etc.). However, the authors believe that the principle of turning by force (traction) can be efficient in the automated control of the gantry tractor travelling within the limiting dimensions of the treads of the permanent process track. As is known, this principle is implemented by rotating the wheels on different sides of the machine at different rates of revolution.

The use of GPS equipment for the automated steering of mobile agricultural integrated machines has gained great popularity. The technical excellence of the state-of-the-art instrumentation in terms of its accuracy in reproducing the pre-set motion trajectories of the mobile machines is very impressive (Griepentrog, 2009; Bakker et al., 2011; Longo & Muscato, 2013; Zhu et al., 2016; Guerrieri et al., 2019).

As it is known from the classical tractor theory, for any mobile power tool, the ratio of its longitudinal base to the transverse base - $(L \cdot K^{-1})$, called the relative support base, is an important parameter characterizing its stable turns during the technological process in agricultural production. The significant by magnitude and direction external impacts from the soil and the cultivated plants tend to constantly change the trajectory of the movement of the agricultural equipment. For most serial agricultural tractors and other power tools, used in agricultural production, industry and transport, the value of the relative support base, as a rule, is greater than 1. However, the layout scheme of any wide span agricultural vehicle, due to its wide track and small wheelbase, a priori assumes that this value is less than 1. Because of this, the value of the stabilizing moment in the onboard method of turning any skeleton agricultural vehicle, caused by the moment of the lateral forces of interaction of its wheels with the supporting surface, is much less than the value of the moment of the forces caused by the difference in the sum of the driving moments, supplied to the wheels of its left and right sides in a horizontal plane. The process of the onboard method of turning such power tools with a small relative support base is insufficiently studied. For a more complete understanding of the process of the onboard method of turning a wide span agricultural vehicle, theoretical and experimental investigations are required.

The aim of this work was to theoretically analyze the dependencies of the dynamic and kinematic parameters that rule the optimal swerving of the developed agricultural wide span vehicle for changing its trajectory.

MATERIALS AND METHODS

An agricultural wide span vehicle (Fig. 1) has a number of features that significantly distinguish it from other types of power vehicles used in agriculture, industry and transport. This is, first of all, a significant by its dimensions transverse base (track) - K, formed by a frame, at the ends of which there are the left and the right sides. Since the sides of the vehicle are located at a considerable distance from each other, this makes it necessary to provide an individual drive for their running wheels installed in pairs at each side in the form of the front and the rear driving wheels, moving along the same track (Bulgakov et al., 2020a). The stability of the movement of this wide span agricultural vehicle must be ensured by appropriate dimensions of the longitudinal base - L, which is especially important since the working bodies of the agricultural machines are located on the frame between the two sides or strictly on the transverse axis of symmetry of the agricultural vehicle, or at some distance from this axis.

Figure 1. The agricultural wide span vehicle during the test (Bulgakov et al., 2020b).

A wide span agricultural vehicle makes a plane-parallel movement in a horizontal plane, parallel to the field surface, strictly following the traces of the technological track. However, in reality, in the process of the movement, under the influence of various external disturbances there are characteristic deviations of the wide span agricultural vehicle from the rectilinear movement along the traces of a constant technological track in a horizontal plane. They cause deviations from the given direction not different magnitude and direction. In this case the following takes place: Δx – its transverse deviation, $\Delta \varphi$ – its angular heading deviation. The nature of the disturbances fully depends on the design scheme of the wide span agricultural vehicle and its technological purpose.

To describe the plane-parallel movement of the wide span agricultural vehicle with an onboard method of its rotation, we will draw up an equivalent diagram in a horizontal plane that will simulate this type of its movement. In a simplified schematic representation of the wide span agricultural vehicle, we denote by the corresponding letters the characteristic points of the unit considered. These will be: point S_t – the center of mass of the agricultural vehicle; L_1 and L_2 – the centers of the front and rear wheels of the left side of the agricultural vehicle, respectively; R_1 and R_2 – the centers of the front and rear wheels on the starboard side, respectively; C – the point of attachment to the agricultural vehicle of the agricultural machine. The considered wide span agricultural vehicle has transverse and longitudinal axes of symmetry (Bulgakov et al., 2018), which, intersecting at S_t, form its center of mass (Fig. 2). Let us indicate on the equivalent diagram the design parameters of this wide span agricultural vehicle: K – the transverse base (track) of the agricultural vehicle (the distance between the longitudinal axes of the left and right sides); L – longitudinal base of the agricultural vehicle (distance between the centers of the front and rear wheels of the left and right sides; b – distance from the point of attachment C of the agricultural machine to the longitudinal axis of the left side; l_t – distance from the center of the rear wheel of the left and right sides to the transverse axis of symmetry of the agricultural vehicle passing through its center masses (point S_t).

Further, for an analytical description of the plane-parallel movement of the agricultural vehicle, we introduce the necessary coordinate systems xOy. First we will show the fixed Cartesian coordinate system xOy, rigidly connected with the surface of the field. Here axis Oy of the indicated coordinate system is directed towards the forward movement of the agricultural vehicle, axis Ox – to the right, in the direction of the movement of this wide span unit. We assume that the discussed agricultural vehicle, equipped with some kind of an agricultural machine, performs uniform forward movement at speed V_0 on the rut relative to the fixed horizontal plane xOy (which is connected with the plane of the field surface) (Fig. 2).

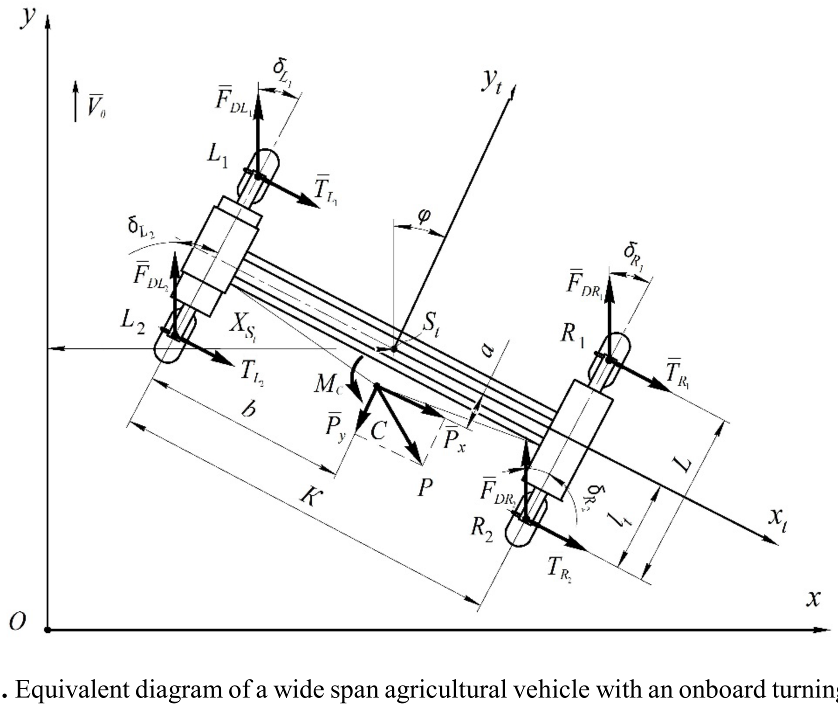

Figure 2. Equivalent diagram of a wide span agricultural vehicle with an onboard turning method during its plane-parallel movement in a horizontal plane.

We will also introduce an additional rectangular Cartesian coordinate system $x_tS_ty_t$, rigidly connected with the agricultural tool, the starting point of which is located at its center of mass (point S_t). In addition, we will direct axis S_ty_t of this coordinate system along the longitudinal axis of symmetry of the agricultural tool, axis S_tx_t will be directed to the right in the course of its movement along the transverse axis of symmetry of the agricultural device.

When performing an agricultural technological process, the skeleton of a wide span agricultural vehicle deviates from its original position under the impact of external random disturbances, receiving additional speeds. As a result, the forward transverse movement of the agricultural vehicle along axis Ox and its rotation around the center of mass (point S_t) at a certain angle φ (heading angle) relative to the coordinate system xOy in the horizontal plane begins. Therefore, the previously indicated coordinate system $x_t S_t y_t$ serves to describe the rotation of the agricultural tool around point S_t by angle φ in plane xOy, where φ is its heading angle, namely, the angle between axes Oy and $S_t y_t$ (Fig. 2). In addition, the origin of the coordinate system $x_t S_t y_t$ (point S_t), associated with the skeleton of the wide span agricultural vehicle, moves in a transverse direction (along axis Ox). The position of point S_t at an arbitrary point of time t relative to axis Oy is indicated by abscissa X_{S_t} (Fig. 2). This means that in the process of relative movement of the wide span agricultural vehicle, its center of mass S_t moves in the direction of axis xO, which is characterized by a change in the abscissa X_{S_t}. Thus the considered agricultural tool in relation to plane xOy has two degrees of freedom, which correspond to two generalized coordinates, namely: angle φ of its rotation around the center of mass, and a change in abscissa X_{S_t} of its center of mass S_t.

The external forces acting upon the agricultural vehicle during its plane-parallel movement in a horizontal plane (Fig. 2) include:

1) the driving tangential forces F_{DL_1}, F_{DL_2}, F_{DR_1} and F_{DR_2} of the wheels of the left and right sides of the agricultural vehicle, applied, respectively, the points L_1, L_2 and R_1, R_2 that form slip angles δ_{L_1}, δ_{L_2}, and δ_{R_1}, δ_{R_2}, with its longitudinal axis of symmetry;

2) the lateral forces T_{L_1}, T_{L_2}, T_{R_1} and T_{R_2}, applied, respectively, at points L_1, L_2, and R_1, R_2;

3) the longitudinal P_y and transverse P_x components of traction resistance, applied at point C, that constitute the main moment M_C of forces, from the side of the agricultural implements.

For a mathematical description of the lateral interaction of any mobile power tool with an agricultural background, the 'lateral slip' hypothesis in a linear interpretation is most often used. In this case, to determine the lateral horizontal forces at the points of contact of the wheels with the soil, the tire slip resistance coefficients are used (Bulgakov et al., 2020a and 2020b). Using this provision, for this bridge agricultural vehicle, we can write:

$$\begin{aligned} T_{L_1} &= k_{L_1} \cdot \delta_{L_1}, \\ T_{L_2} &= k_{L_2} \cdot \delta_{L_2}, \\ T_{R_1} &= k_{R_1} \cdot \delta_{R_1}, \\ T_{R_2} &= k_{R_2} \cdot \delta_{R_2}, \end{aligned} \tag{1}$$

where k_{L_1}, k_{L_2} and, k_{R_1}, k_{R_2} are the slip resistance coefficients of the left and the right sides of the agricultural vehicle, respectively.

The method for deriving expressions for calculating slip angles δ_{L_1}, δ_{L_2}, and δ_{R_1}, first of all involves determining the absolute values of the velocities of points L_1, L_2 and R_1, R_2. These speeds can be determined from the expressions (Fig. 3):

$$\begin{aligned} \overline{V}_{L_1} &= \overline{V}_0 + \overline{V}_{S_t} + \overline{V}_{L_1 S_t}, \\ \overline{V}_{L_2} &= \overline{V}_0 + \overline{V}_{S_t} + \overline{V}_{L_2 S_t}, \\ \overline{V}_{R_1} &= \overline{V}_0 + \overline{V}_{S_t} + \overline{V}_{R_1 S_t}, \\ \overline{V}_{R_2} &= \overline{V}_0 + \overline{V}_{S_t} + \overline{V}_{R_2 S_t}, \end{aligned} \tag{2}$$

where $\bar{V}_0$ is the vector of the linear forward speed of the movement of the wide span agricultural vehicle relative to plane xOy; $\bar{V}_{S_t}$ – the linear velocity vector of the center of mass of the agricultural vehicle relative to plane xOy; $\bar{V}_{L_1S_t}$, $\bar{V}_{L_2S_t}$ and $\bar{V}_{R_1S_t}$, $\bar{V}_{R_2S_t}$ are the linear velocity vectors of centers L_1, L_2 and R_1, R_2 of each wheel of the agricultural vehicle relative to its center of mass (point S_t) in a horizontal plane (Fig. 2).

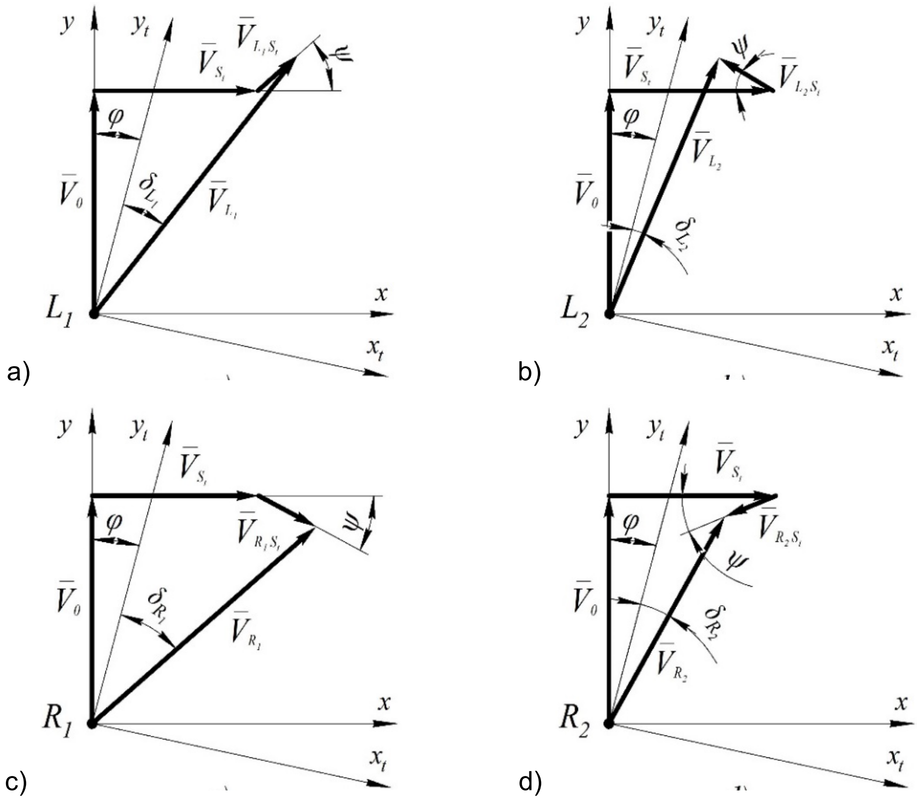

Figure 3. The vector sums of the speeds of the centers of the wheels of the wide span agricultural vehicle: a) – the front wheel of the left side with center L_1; b) – the rear wheel of the left side with center L_2; c) – the front wheel of the starboard side with center R_1; d) – the rear wheel of the starboard side with the center R_2.

To determine the tangents of the slip angles of the wheels (points L_1, L_2, and R_1), it is necessary to project the sums of vectors (Fig. 3) on and axes S_tx_t and S_ty_t, and take the ratio of the obtained sums of projections. As it is evident from Fig. 3, the sums of the projections of the velocity vectors, shown in Fig. 3, a; 3, b; 3, c and 3, d will be respectively equal:

$$\begin{aligned} V_{L_1x_t} &= -V_o \cdot \sin\varphi + V_{S_t} \cdot \cos\varphi + V_{L_1S_t} \cdot \cos(\varphi+\psi), \\ V_{L_2x_t} &= -V_o \cdot \sin\varphi + V_{S_t} \cdot \cos\varphi - V_{L_2S_t} \cdot \cos(\varphi-\psi), \\ V_{R_1x_t} &= -V_o \cdot \sin\varphi + V_{S_t} \cdot \cos\varphi + V_{R_1S_t} \cdot \cos(\varphi-\psi), \\ V_{R_2x_t} &= -V_o \cdot \sin\varphi + V_{S_t} \cdot \cos\varphi - V_{R_2S_t} \cdot \cos(\varphi+\psi), \end{aligned} \quad (3)$$

where ψ is the angle between the velocity vectors $\bar{V}_{L_1S_t}$, $\bar{V}_{L_2S_t}$, $\bar{V}_{R_1S_t}$, and $\bar{V}_{R_2S_t}$, and the horizontal axis.

In addition, taking into account the smallness of angles φ and ψ, we assume that $\sin\varphi \approx \varphi$, $\cos\varphi \approx 1$, and $\cos(\varphi + \psi) \approx 1$. Therefore, expressions (3) can be written in a more simplified form. Namely:

$$\begin{aligned} V_{L_1 x_t} &= -V_{\text{o}} \cdot \varphi + V_{S_t} + V_{L_1 S_t}, \\ V_{L_2 x_t} &= -V_{\text{o}} \cdot \varphi + V_{S_t} - V_{L_2 S_t}, \\ V_{R_1 x_t} &= -V_{\text{o}} \cdot \varphi + V_{S_t} + V_{R_1 S_t}, \\ V_{R_2 x_t} &= -V_{\text{o}} \cdot \varphi + V_{S_t} - V_{R_2 S_t}. \end{aligned} \tag{4}$$

Further, it is obvious that the projection of the velocity vector $\bar{V}_{L_1 y_t}$ of center L_1 of the front wheel of the left side of the agricultural vehicle on axle $S_t y_t$ will be equal to:

$$V_{L_1 y_t} = V_{\text{o}} \cdot \cos\varphi + \dot{X}_{S_t} \cdot \sin\varphi \tag{5}$$

Considering that in the second term on the right side of expression (5) the product of two small quantities is a small quantity of a higher order, there is every reason to write that $\bar{V}_{L_1 y_1} \approx V_0$.

In a similar way we have:

$$V_{L_1 y_t} = V_{L_2 y_t} = V_{R_1 y_t} = V_{R_2 y_t} = V_{\text{o}} \tag{6}$$

Next, we determine the values (modules) of the vectors included in expression (2). It's obvious that:

$$\left|\bar{V}_{\text{o}}\right| = V_{\text{o}}, \; \left|\bar{V}_{S_t}\right| = \dot{X}_{S_t}. \tag{7}$$

To determine the values of the linear speeds of rotation of centers L_1, L_2, R_1, and R_2 of the wheels of the agricultural vehicle relative to its center of mass (point S_t), we find the radii of the tuns of points L_1, L_2, R_1, and R_2 relative to point S_t. As it evident from Fig. 3, the specified radii will be equal to:

$$\begin{aligned} R_{L_1} &= R_{R_1} = \frac{1}{2}\sqrt{4 \cdot \left(L - l_t\right)^2 + K^2}, \\ R_{L_2} &= R_{R_2} = \frac{1}{2}\sqrt{4 \cdot l_t^2 + K^2}. \end{aligned} \tag{8}$$

Then, taking into account expression (8), we obtain:

$$\begin{aligned} \left|V_{L_1 S_t}\right| &= \left|V_{R_1 S_t}\right| = \frac{1}{2}\sqrt{4 \cdot \left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}, \\ \left|V_{L_2 S_t}\right| &= \left|V_{R_2 S_t}\right| = \frac{1}{2}\sqrt{4 \cdot l_t^2 + K^2} \cdot \dot{\varphi}. \end{aligned} \tag{9}$$

After substitution of expressions (7) and (9) into expression (4), we will have:

$$\begin{aligned} \left|V_{L_1 x_t}\right| &= \left|V_{R_1 x_t}\right| = -V_{\text{o}} \cdot \varphi + \dot{X}_{S_t} + \frac{1}{2}\sqrt{4 \cdot \left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}, \\ \left|V_{L_2 x_t}\right| &= \left|V_{R_2 x_t}\right| = -V_{\text{o}} \cdot \varphi + \dot{X}_{S_t} - \frac{1}{2}\sqrt{4 \cdot l_t^2 + K^2} \cdot \dot{\varphi}. \end{aligned} \tag{10}$$

Thus, on the basis of the obtained expressions (6) and (10), it is possible to determine the values of the tangents of the slip angles δ_{L_1}, δ_{L_2}, and δ_{R_1}, δ_{R_2}, and, considering the smallness of these angles, and the slip angles themselves of all the four wheels of the considered agricultural vehicle. Namely:

$$\tan\delta_{L_1} = \tan\delta_{R_1} \approx \delta_{L_1} \approx \delta_{R_1} = \frac{V_{L_1 x_t}}{V_{L_1 y_t}} = \frac{V_{R_1 x_t}}{V_{R_1 y_t}} =$$
$$= \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\cdot\left(L-l_t\right)^2 + K^2}\cdot\dot{\varphi} - V_o\cdot\varphi}{V_o},$$
$$\tan\delta_{L_2} = \tan\delta_{R_2} \approx \delta_{L_2} \approx \delta_{R_2} = \frac{V_{L_2 x_t}}{V_{L_2 y_t}} = \frac{V_{R_2 x_t}}{V_{R_2 y_t}} =$$
$$= \frac{\dot{X}_{S_1} - \frac{1}{2}\sqrt{4\cdot l_t^2 + K^2}\cdot\dot{\varphi} - V_o\cdot\varphi}{V_o}. \tag{11}$$

Since the slip angles δ_{L_1}, δ_{L_2}, and δ_{R_1}, δ_{R_2}, are directed in the other direction with respect to the positive direction of change of the heading angle φ (Fig. 2), their values must be negative. With this in mind, we finally obtain:

$$\delta_{L_1} = \delta_{R_1} = \varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\cdot\left(L-l_t\right)^2 + K^2}\cdot\dot{\varphi}}{V_o},$$
$$\delta_{L_2} = \delta_{R_2} = \varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4\cdot l_t^2 + K^2}\cdot\dot{\varphi}}{V_o}. \tag{12}$$

Having determined the slip angles δ_{L_1}, δ_{L_2}, and δ_{R_1}, δ_{R_2}, we find the lateral forces:

$$T_{L_1} = k_{L_1}\cdot\left[\varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\cdot\left(L-l_t\right)^2 + K^2}\cdot\dot{\varphi}}{V_o}\right],$$
$$T_{L_2} = k_{L_2}\cdot\left[\varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4\cdot l_t^2 + K^2}\cdot\dot{\varphi}}{V_o}\right],$$
$$T_{R_1} = k_{R_1}\cdot\left[\varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\cdot\left(L-l_t\right)^2 + K^2}\cdot\dot{\varphi}}{V_o}\right],$$
$$T_{R_2} = k_{R_2}\cdot\left[\varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4\cdot l_t^2 + K^2}\cdot\dot{\varphi}}{V_o}\right]. \tag{13}$$

From the study of automatic driving and driving wheels (Bulgakov et al., 2021), it is known that different turning torques M_{ki} must be applied, which will provide different values of the tangential forces F_{Di}:

$$F_{Di} = \frac{\left(M_{ki} - M_{fi}\right)}{r_i}, \tag{14}$$

where M_{ki} is the turning torque applied to the i-th wheel; r_i is the dynamic radius of the i-th wheel; M_{fi} is the rolling resistance moment of the i-th wheel.

Due to the difference in the sum of the driving moments M_k applied to the left and right wheels of the agricultural wide span vehicle, a torque M_p appears in a horizontal plane, given by Bulgakov et al. (2021):

$$M_p = \frac{1}{2}K\left(F_{DL_1} + F_{DL_2} - F_{Dr_1} - F_{Dr_2}\right) \neq 0, \tag{15}$$

where F_{DL_1}, F_{DL_2} and F_{DR_1}, F_{DR_2} are the tangential forces applied, respectively, to the front and rear wheels of the left and right sides of the agricultural wide span vehicle.

The appearance of the torque M_p causes a change in the direction of the motion of the agricultural wide span vehicle. The resistance to turning of the machine causes a moment of lateral interaction forces of the wheels with the supporting surface M_T, which can be considered a stabilizing moment, the main moment M_C of forces acting from the side of the agricultural implements, the moment of the transverse component P_x of the traction resistance of agricultural implements and the moment of inertia forces M_J:

$$M_p = M_T + M_C + M_J = \frac{1}{2}L\cdot\left(T_{L_1} + T_{R_1} - T_{L_2} - T_{R_2}\right) + M_C + P_x\cdot a + J_m\cdot\ddot{\varphi}\,, \tag{16}$$

where M_T is the torque caused by the lateral interaction forces of the wheels with the supporting surface; M_J is the moment of inertia; T_{L_1}, T_{L_2}, and T_{R_1}, T_{R_2} are the lateral forces applied, respectively, to the front and the rear wheels of the left and right sides of the agricultural wide span vehicle; M_C – the main moment of forces acting upon from the side of the agricultural machine; J_m – is the moment of inertia of the agricultural wide span vehicle in a horizontal plane; $\ddot{\varphi}$ is the angular acceleration of the agricultural wide span vehicle in a horizontal plane.

When the turn is stable, the following equality is satisfied:

$$\frac{1}{2}K\cdot\left(F_{DL_1} + F_{DL_2} - F_{DR_1} - F_{DR_2}\right) = \frac{1}{2}L\cdot\left(T_{L1} + T_{R1} - T_{L2} - T_{R_2}\right) + M_C + P_x\cdot a + J_m\cdot\ddot{\varphi}\,. \tag{17}$$

By substituting expressions (13) into expression (17), after some transformations, the following differential equation is obtained:

$$\begin{aligned} J_m\cdot\ddot{\varphi} = {} & \frac{1}{2}K\cdot\left(F_{DL_1} + F_{DL_2} - F_{DR_1} - F_{DR_2}\right) - \frac{1}{2}L\cdot\left\{\left(k_{L_1} + k_{R_1}\right)\cdot\left[\varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{\left(L - l_t\right)^2 + K^2}\cdot\dot{\varphi}}{V_o}\right] - \right. \\ & \left. -\left(k_{L_2} + k_{R_2}\right)\cdot\left[\varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{l_t^2 + K^2}\cdot\dot{\varphi}}{V_o}\right]\right\} - M_C - P_x\cdot a. \end{aligned} \tag{18}$$

Eq. (18) is a differential equation for the turn of the wide span agricultural vehicle around its center of mass (point S_t) in its plane-parallel motion.

Further we will compose a differential equation for the transverse displacement X_{S_t} of the center of mass S_t of the considered agricultural vehicle, using the basic law of dynamics, which, in this case, will be written in the following form:

$$M\cdot\ddot{X}_{S_t} = \sum_{k=1}^{n} F_{kx}\,, \tag{19}$$

where M is the mass of the agricultural vehicle; $\ddot{X}_{S_t}$ – acceleration of the center of mass S_t

of the agricultural vehicle in the direction of axis Ox; $\sum_{k=1}^{n} F_{kx}$ – the sum of the projections of all the external forces acting upon the unit under consideration, on axis Ox.

Using the equivalent circuit, shown in Fig. 3, we can find the sum of the projections of all the external forces on axis Ox. We have:

$$\begin{aligned}\sum_{k=1}^{n} F_{kx} &= -F_{DL_1} \cdot \sin\left(\delta_{L_1} - \varphi\right) - F_{DL_2} \cdot \sin\left(\delta_{L_2} - \varphi\right) - \\ &-F_{DR_1} \cdot \sin\left(\delta_{R_1} - \varphi\right) - F_{DR_2} \cdot \sin\left(\delta_{R_2} - \varphi\right) + \\ &+\left(T_{L_1} + T_{L_2} + T_{R_1} + T_{R_2}\right)\cos\varphi + P_x \cdot \cos\varphi - P_y \cdot \sin\varphi.\end{aligned} \tag{20}$$

Taking into account the smallness of angles φ, δ_{L_1}, δ_{L_2}, δ_{R_1} and δ_{R_2}, expression (20) can be written in a more simplified form:

$$\begin{aligned}\sum_{k=1}^{n} F_{kx} &= -F_{DL_1} \cdot \left(\delta_{L_1} - \varphi\right) - F_{DL_2} \cdot \left(\delta_{L_2} - \varphi\right) - F_{DR_1} \cdot \left(\delta_{R_1} - \varphi\right) - \\ &-F_{DR_2} \cdot \left(\delta_{R_2} - \varphi\right) + T_{L_1} + T_{L_2} + T_{R_1} + T_{R_2} + P_x - P_y \cdot \varphi.\end{aligned} \tag{21}$$

By substituting expressions (12) and (13) into expression (21) and performing a series of transformations, we will obtain the final expression for the sum of the projections of all external forces, acting upon the discussed agricultural vehicle, to axis Ox:

$$\begin{aligned}\sum_{k=1}^{n} F_{kx} &= \left(F_{DL_1} + F_{DR_1}\right) \cdot \left[\frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}}{V_o}\right] + \\ &+\left(F_{DL_2} + F_{DR_2}\right) \cdot \left[\frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4 \cdot l_t^2 + K^2} \cdot \dot{\varphi}}{V_o}\right] + \\ &+\left(k_{L_1} + k_{R_1}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}}{V_o}\right] + \\ &+\left(k_{L_2} + k_{R_2}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4 l_t^2 + K^2} \cdot \dot{\varphi}}{V_o}\right] + P_x - P_y \cdot \varphi.\end{aligned} \tag{22}$$

Substituting expression (22) into Eq. (19), we obtain the desired differential equation for the transverse movement X_{S_t} of the considered agricultural tool in the direction of axis Ox:

$$M \cdot \ddot{X}_{S_t} = \left(F_{DL_1} + F_{DR_1}\right) \cdot \left[\frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] +$$
$$+\left(F_{DL_2} + F_{DR_2}\right) \cdot \left[\frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4 \cdot l_t^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] +$$
$$+\left(k_{L_1} + k_{R_1}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] + \quad (23)$$
$$+\left(k_{L_2} + k_{R_2}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4 l_t^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] + P_x - P_y \cdot \varphi .$$

Combining differential Eqs (23) and (18) into one system of differential equations, we obtain a mathematical model of a plane-parallel movement of the wide span agricultural vehicle with an onboard method of its turning. We have:

$$\left.\begin{array}{l} M \cdot \ddot{X}_{S_t} = \left(F_{DL_1} + F_{DR_1}\right) \cdot \left[\frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] + \left(F_{DL_2} + F_{DR_2}\right) \cdot \left[\frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4 \cdot l_t^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] + \\ +\left(k_{L_1} + k_{R_1}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{4\left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] + \left(k_{L_2} + k_{R_2}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{4 l_t^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] + P_x - P_y \cdot \varphi , \\ J_m \cdot \ddot{\varphi} = \frac{1}{2} K \cdot \left(F_{DL_1} + F_{DL_2} - F_{DR_1} - F_{DR_2}\right) - \frac{1}{2} L \cdot \left\{ \left(k_{L_1} + k_{R_1}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} + \frac{1}{2}\sqrt{\left(L - l_t\right)^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] - \right. \\ \left. -\left(k_{L_2} + k_{R_2}\right) \cdot \left[\varphi - \frac{\dot{X}_{S_t} - \frac{1}{2}\sqrt{l_t^2 + K^2} \cdot \dot{\varphi}}{V_o} \right] \right\} - M_C - P_x \cdot a. \end{array}\right\} \quad (24)$$

If we consider that the slip resistance coefficients of the front and the rear wheels of the left and the right sides of the agricultural vehicle are equal, then we can assume as a first approximation that:

$$k_{L_1} = k_{L_2} = k_L \text{ and } k_{R_1} = k_{R_2} = k_R , \quad (25)$$

then, substituting values (25) into the system of differential equations (24), after a series of transformations, we obtain a system of differential equations of the following form:

$$
\left.
\begin{aligned}
&M\cdot\ddot{X}_{S_t}=\left(F_{DL_1}+F_{DR_1}\right)\cdot\left[\frac{\dot{X}_{S_t}+\frac{1}{2}\sqrt{4\left(L-l_t\right)^2+K^2}\cdot\dot{\varphi}}{V_o}\right]+\left(F_{DL_2}+F_{DR_2}\right)\cdot\left[\frac{\dot{X}_{S_t}-\frac{1}{2}\sqrt{4\cdot l_t^2+K^2}\cdot\dot{\varphi}}{V_o}\right]+\\
&+2\left(k_L+k_R\right)\cdot\left(\varphi-\frac{\dot{X}_{S_t}}{V_o}\right)+\frac{1}{2}\cdot\left(k_L+k_R\right)\cdot\left[\frac{\sqrt{4\left(L-l_t\right)^2+K^2}-\sqrt{4l_t^2+K^2}}{V_o}\right]\cdot\dot{\varphi}+P_x-P_y\cdot\varphi,\\
&J_m\cdot\ddot{\varphi}=\frac{1}{2}K\cdot\left(F_{DL_1}+F_{DL_2}-F_{DR_1}-F_{DR_2}\right)+\frac{1}{2}\cdot\frac{\left(k_L+k_R\right)\cdot L^2}{V_o}\cdot\dot{\varphi}-M_C-P_x\cdot a.
\end{aligned}
\right\}
\tag{26}
$$

For the case when the front and the rear wheels of the right and the left sides of the agricultural vehicle are at the same distance from its transverse axis of symmetry, which finds its reflection in the design of the wide span agricultural vehicle, developed by us (see Fig. 1), then we can assume that $l_t = L^{-2}$. Then the system of differential equations (26) takes the final form:

$$
\left.
\begin{aligned}
&M\cdot\ddot{X}_{S_t}=\left(F_{DL_1}+F_{DR_1}\right)\cdot\left[\frac{\dot{X}_{S_t}+\frac{1}{2}\sqrt{L^2+K^2}\cdot\dot{\varphi}}{V_o}\right]+\left(F_{DL_2}+F_{DR_2}\right)\cdot\left[\frac{\dot{X}_{S_t}-\frac{1}{2}\sqrt{L^2+K^2}\cdot\dot{\varphi}}{V_o}\right]+\\
&+2\left(k_L+k_R\right)\cdot\left(\varphi-\frac{\dot{X}_{S_t}}{V_o}\right)+P_x-P_y\cdot\varphi,\\
&J_m\cdot\ddot{\varphi}=\frac{1}{2}K\cdot\left(F_{DL_1}+F_{DL_2}-F_{DR_1}-F_{DR_2}\right)+\frac{1}{2}\cdot\frac{\left(k_L+k_R\right)\cdot L^2}{V_o}\cdot\dot{\varphi}-M_C-P_x\cdot a.
\end{aligned}
\right\}
\tag{27}
$$

Numerical calculation of the system of equations (27) shows that already during the first 0.6 s from the moment of the created difference of the moments applied to the wheels of the left and the right sides of the wide scan agricultural vehicle in a horizontal plane, its heading angle φ changes to 6 degrees. And also further this value increases exponentially. But at the same time it should be taken into account that after some time of the side turn it is necessary to return the axis of the wide span agricultural vehicle into an opposite direction (exit the turn). That is, in order to return the parallel-displaced agricultural vehicle to the preset trajectory, it is necessary to introduce an additional orientation error in the form of a turn of its skeleton when entering the turn, which should be eliminated at the exit from the turn.

Therefore, manual correction of the trajectory of the movement of a bridge agricultural vehicle with a side turn will only be accompanied by its worse orientation relative to the preset trajectory. Hence it follows that by its onboard turn it is practically impossible to carry out controlled movement with its transverse displacement without an additional turn. It means that the use of only a side turn does not allow to make correction of the trajectory of the movement by a lateral (plane-parallel) displacement of the longitudinal axis of the wide span agricultural vehicle. In case a displacement of the longitudinal axis of the wide span agricultural vehicle from the preset trajectory arises without turning it (for example, as result of its movement on a transverse slope), restoration of the preset trajectory of the movement will be accompanied by turning of its longitudinal axis, and this will require next correction by turning in the opposite direction.

RESULTS AND DISCUSSION

The experimental amplitude-frequency characteristic of oscillations of the heading angle φ (Fig. 2) of the wide span agricultural vehicle showed (Fig. 4) that in the operating range frequencyof 0–2 s^{-1}, the highest value that the amplitude reaches is 2.6 10^{-5} rad N^{-1}, applied, respectively, to the wheels of its left and right sides, which is observed at low frequencies ω. At frequencies of oscillations of the control action of about 2 s^{-1}, the amplitude of oscillations of the heading angle φ of the agricultural tool does not exceed 1.0 10^{-5} rad N^{-1}.

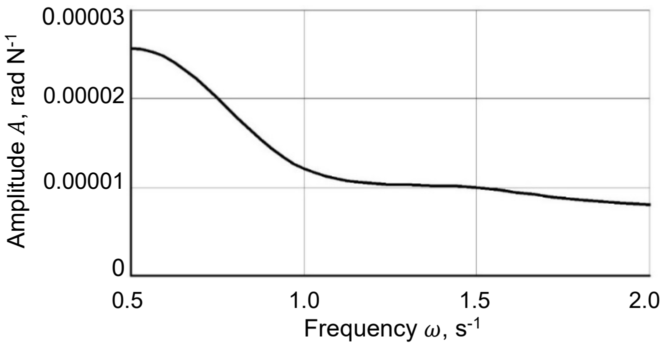

Figure 4. Experimental amplitude-frequency characteristic of fluctuations of the heading angle φ when the agricultural vehicle is working out the difference in tangential forces, applied, respectively, to the wheels of its left and right. Sides.

Oscillations of the transverse displacement of the wide span agricultural vehicle during its movement are also of low frequency (Fig. 5). The main dispersion spectrum of these oscillations is also concentrated in the frequency range 0–2 s^{-1}. The standard of oscillations of this parameter is ± 0.05 m.

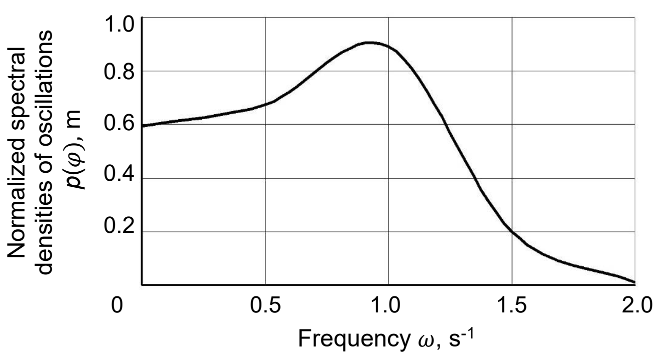

Figure 5. Normalized spectral densities of oscillations of the linear transverse displacement *Xs* of agricultural tool.

The experimental amplitude-frequency characteristic of oscillations of the linear transverse displacement *Xs* of the wide span agricultural vehicle during working out of the control action (Fig. 6) showed that in the operating frequency range of 0–2 s^{-1} oscillations of the input signal its maximum displacement, equal to $1.3 \cdot 10^{-5}$ m N^{-1}, is also observed at low frequencies.

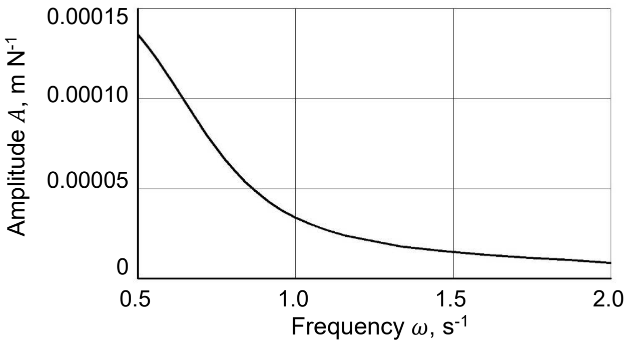

Figure 6. Experimental amplitude-frequency characteristic of oscillations of the linear transverse displacement *Xs* when working out the control action by the wide span agricultural vehicle.

Since the main range of dispersions of oscillations of the tangential forces, applied, respectively, to the wheels of the left and the right sides of the wide span agricultural vehicle, is concentrated exactly at low frequencies, therefore, only the high accuracy of the automated system for driving it along the tracks of a constant tramline, using a laser beam, will allow it to have satisfactory controllability.

CONCLUSIONS

The completed research has proved that the plane-parallel steering of the agricultural wide span vehicle with the use of solely the traction (forced) method of its turning on the treads of the permanent process track requires a significantly smaller difference in the moments applied to the wheels on the right and left sides, as compared to the conventional crawler tractor. The said difference is in proportion to the width of its wheel base at its relatively smaller length.

The smallest power consumption in the process of turning an agricultural wide span vehicle with the use of the traction (forced) method is achieved, when the lateral deformations of the tyres on the wheels of its left and right sides are equal in absolute magnitude, but have opposite directions. To ensure such type of deformations, the traction (forced) turning of the agricultural machine has to be done in such a way that its instantaneous centre of turn is on the axis of transverse symmetry of its chassis.

It has been proved theoretically that already in the first 0.6 s after a difference in the moments applied to the wheels on the right and left sides of the agricultural wide span vehicle has arisen, the heading angle of the vehicle in the horizontal plane changes by 6 deg. Thereafter, this value increases exponentially. In practical terms, that means that it would hardly be possible to perform the controlled motion with a lateral displacement without an additional angular displacement. Hence, the use of solely the traction turning does not provide for the adjustment of the motion trajectory with a lateral (plane-parallel) displacement of the agricultural wide span vehicle's fore-and-aft axis. The oscillations of its transverse displacement in the course of its motion are low-frequency ones. The main variance spectrum of these oscillations is concentrated within the frequency range of 0–2 s^{-1}. The standard oscillations of this parameter are within ± 0.05 m.

Within the operating frequency range of 0–2 s^{-1} of the control action, the maximum lateral displacement of the agricultural wide span vehicle equal to 1.3 10^{-5} m N^{-1} occurs also at lower frequencies.

In view of the fact that the main variance spectrum of the oscillations of the tangential forces applied to the wheels on the left and right sides of the agricultural wide span vehicle is concentrated exactly within a low frequency range, only the high accuracy of the system using a laser beam can ensure its satisfactory steerability.

REFERENCES

Antille, D.L., Chamen, W.C.T., Tullberg, J.N. & Lal, R. 2015. The potential of controlled traffic farming to mitigate greenhouse gas emissions and enhance carbon sequestration in arable land: a critical review. - In *Transactions of the ASABE* **58**(3), 707–731. doi: 10.13031/trans.58.11049

Antille, D.L., Peets, S., Galambošová, J., Botta, G.F., Rataj, V., Macak, M., Tullberg, J.N., Chamen, W.C.T., White, D.R., Misiewicz, P.A., Hargreaves, P.R., Bienvenido, J.F. & Godwin, R.J. 2019. Review: Soil compaction and controlled traffic farming in arable and grass cropping systems. *Agronomy Research* **17**(3), 653–683. doi: 10.15159/AR.19.133

Bakker, T., Van, A.K. & Bontsema, J. 2011. Autonomous navigation using a robot platform in a sugar beet field. *Biosystems Engineering* **109**(4), 357–368.

Bulgakov, V., Adamchuk, V., Kuvachov, V., Arak, M. & Olt, J. 2017. Study into movement of wide span tractors (vehicles) used in controlled traffic farming. *Proceedings of the 28th DAAAM International Symposium "28th DAAAM International Symposium on Intelligent Manufacturing and Automation"* (Ed. B. Katalinic), 08–11th November 2017, Zadar, Croatia, EU. Vienna, Austria: DAAAM International Vienna, pp. 0199−0208, doi: 10.2507/28th.daaam.proceedings.027.

Bulgakov, V., Kuvachov, V. & Olt, J. 2019. Theoretical Study on Power Performance of Agricultural Gantry Systems. *Proceedings of the 30th International DAAAM Symposium 'Intelligent Manufacturing & Automation'*, Zadar, 23.–26.10.2019. Ed. B. Katalinic. Vienna, Austria: DAAAM International, **28**(1), 0167−0175, doi: 10.2507/30th.daaam.proceedings.022.

Bulgakov, V., Melnik, V., Kuvachov, V. & Olt, J. 2018. Theoretical Study on Linkage Unit of Wide Span Tractor. *Proceedings of the 29th International DAAAM Symposium "Intelligent Manufacturing & Automation"* (Ed. B. Katalinic). 2018 October 24–27, Zadar, Croatia. Vienna, Austria: DAAAM International, 29(1):0180−0189. doi: 10.2507/29th. daaam.proceedings.026.

Bulgakov, V., Olt, J., Kuvachov, V., Chernovol, M., Sviren, M. & Kolomiiets, S. 2020a. A study of the interaction between soil and the pneumatic wheels of agricultural gantry systems. *Agraarteadus / Journal of Agricultural Science* **31**(1), 3–9. doi: 10.15159/jas.20.03

Bulgakov, V., Olt, J., Kuvachov, V. & Smolinskyi, S. 2020b. A theoretical and experimental study of the traction properties of agricultural gantry systems. *Agraarteadus / Journal of Agricultural Science* **31**(1), 10–16. doi: 10.15159/jas.20.08

Bulgakov, V., Pascuzzi, S., Ivanovs, S., Kuvachov, V., Postol, Y., Santoro, F. & Melnyk, V. 2021. Study of the steering of a wide span vehicle controlled by a local positioning system. *Journal of Agricultural Engineering* **52**(3), LII:1144. doi: 10.4081/jae.2021.1144

Cerruto, E., Manetto, G., Santoro, F. & Pascuzzi, S. 2018. Operator Dermal Exposure to Pesticides in Tomato and Strawberry Greenhouses from Hand-Held Sprayers. *Sustainability* **10**, 2273, doi:10.3390/su10072273

Chamen, T. 2015. Controlled traffic farming – from world wide research to adoption in Europe and its future prospects. *Acta Technologica Agriculturae Nitra* **3**, 64–73. doi: 10.1515/ata-2015-0014

Chan, K.Y., Oates, A., Swan, A.D., Hayes, R.C., Dear, B.S. & Peoples, M.B. 2006. Agronomic consequences of tractor wheel compaction on a clay soil. *Soil & Tillage Research* **89**, 13–21.

Chebrolu, N., Lottes, P., Schaefer, A., Winterhalter, W., Burgard, W. & Stachniss, C. 2017. Agricultural robot dataset for plant classification, localization and mapping on sugar beet fields. *The International Journal of Robotics Research* **36**, 1045–1052. doi: 10.1177/0278364917720510

Gasso, V., Sørensen, C.A.G. & Oudshoorn, F.W. 2013. Controlled traffic farming: A review of the environmental impacts. *European Journal of Agronomy* **48**, 66–73.

Griepentrog, H.W. 2009. Safe and reliable: Further development of a field robot. *Precision Agriculture* 9, 857–866.

Guerrieri, A.S., Anifantis, A.S., Santoro, F. & Pascuzzi, S. 2019. Study of a Large Square Baler with Innovative Technological Systems that Optimize the Baling Effectiveness. *Agriculture (Switzerland)* **9**(5), 86. doi: 10.3390/agriculture9050086

Hamza, M.A. & Anderson, W.K. 2005. Soil compaction in cropping systems - a review of the nature, causes and possible solutions. *Soil & Tillage Research* **82**, 121–145.

Ji, C. & Zhou, J. 2014. Current situation of navigation technologies for agricultural machinery. *Transactions of the Chinese Society for Agricultural Machinery* **45**(9), 44–54.

Keller, T., Sandin, M.; Colombi, T., Horn, R. & Or, D. 2019. Historical increase in agricultural machinery weights enhanced soil stress levels and adversely affected soil functioning. *Soil & Tillage Research* **194**, 104293. doi: 10.1016/j.still.2019.104293

Kingwell, R. & Fuchsbichler, 2011. A.The whole-farm benefits of controlled traffic farming: An Australian appraisal. *Agricultural Systems* **104**(7), 513–521.

Kuvachov, V., Bulgakov, V., Adamchuk, V., Kaminskiy, V., Melnik, V. & Olt, J. 2021. Experimental research into new harrowing unit based on gantry agricultural implement carrier. *Agronomy Research* **19**(1), 126–135. doi: 10.15159/AR.20.239

Longo, D. & Muscato, G. 2013. Design and simulation of two robotic systems for automatic artichoke harvesting. *Robotics* **2**(4), 217–230. doi: 10.3390/robotics2040217

Nevens, F. & Reheul, D. 2003. The consequences of wheel-induced soil compaction and subsoiling for silage maize on a sandy loam soil in Belgium. *Soil & Tillage Research* **70**, 175–184.

Onal, I. 2012. Controlled traffic farming and wide span tractors. *Journal of Agricultural Machinery Science* **8**(4), 353–364.

Pascuzzi, S., Anifantis, A.S. & Santoro, F. 2020. The concept of a compact profile agricultural tractor suitable for use on specialised tree crops. *Agriculture (Switzerland)* **10**, 123. doi:10.3390/agriculture10040123

Pedersen, H.H., Sorensen, C.G., Oudshoorn, F.W. & McPhee, J.E. 2013. User requirements for a wide span tractor for controlled traffic farming. – International Commission of Agricultural and Biological Engineers, Section V. CIOSTA XXXV Conference 'From Effective to Intelligent Agriculture and Forestry', Billund, Denmark, 3–5 July 2013

Raper, R.L. 2005. Agricultural traffic impacts on soil. *Journal of Terramechanics* **42**, 259–280.

Yang, L., Luo. T., Cheng, X., Li, J. & Song. Y. 2015. Universal autopilot system of tractor based on Raspberry Pi. *Transactions of the Chinese Society of Agricultural Engineering* **31**(21), 109–115.

Zhu, Q., Gao, G. & Niu, W. 2016. The automatic navigation driving system of agricultural machinery. *Modern Agriculture* **5**, 65–67.

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https://doi.org/10.15159/AR.22.052

Tracing of the rapeseed movement by using the contrast point tracking method for DEM model verification

B. Černilová[1,*], J. Kuře[1], M. Linda[1] and R. Chotěborský[2]

[1]Czech University of Life Sciences Prague, Faculty of Engineering, Department of Electrical Engineering and Automation, Kamýcká 129, CZ165 21 Prague – Suchdol, Czech Republic
[2]Czech University of Life Science Prague, Faculty of Engineering, Department of Material Science and Manufacturing Technology, Kamýcká 129, CZ165 21 Prague – Suchdol, Czech Republic.
[*]Correspondence: cernilova@tf.czu.cz

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Abstract. For designing the efficient storage and transport of rapeseed, it is necessary to follow the rules of bulk material, know its properties, and use appropriate equipment. The mentioned properties of bulk material are essential for simulating the numerical model and obtaining the parameters of the geometry particle tracing during the rapeseed manipulation. In order to determine the angle of repose and mechanical properties of rapeseed, the results of previous experiments were used. The aim of this paper was to propose the methodology for calculating the traces of individual particles using the contrast point method during a real test to calibrate and verify a numerical model of the same system as in a real test. RockyDEM software was used to create the numerical model of rapeseed. The numerical model was used to test the flow analysis of rapeseed particles. The experiment measuring rapeseed particle traces was performed using an assembled experimental device. The rapeseed particles and the contrast point particles were poured out from the device, and a camera recorded the process. In parallel with the real experiment, the angle of repose test was performed on the same device to verify the numerical model. The results showed that the methodology is suitable for the DEM model verification with an accuracy of 3.77 mm in the X-axis, 0.55 mm in the Y-axis and 1.7 mm in the Z-axis. This confirmed that the proposed method is suitable for determining the surface behaviour of bulk material to verify the DEM model.

Key words: rapeseed, discrete element methods, particle tracing, angle of repose test, contrast point.

INTRODUCTION

Modelling and simulation are methods often used in science, economics, engineering praxis, and many other fields of human activity. Computer simulation is one of the most commonly used methods in agriculture because of its low design and testing costs. To effectively manipulate and store rapeseed, following a specific set of rules and using special equipment is necessary. This equipment has to be designed in such a way as to allow seamless transportation and storage of rapeseed. The numeric simulation using discrete element methods (DEM) allows for creating a preview of particle tracking geometry (of rapeseed). However, in order to achieve the correct calibration of the numerical model, it is necessary to obtain the mechanical properties of the rapeseed. By using known parameters and properties, it is possible to create a computer model and determine the correlation between the experiments and the model (Zhou et al., 2002; Asaf et al., 2007; Chen et al., 2013). For the bulk matter's basic properties, it is necessary to determine the angle of repose as well as the external and internal friction angle of said bulk material (Amšiejus et al., 2014). The internal and external friction angle can be calculated using shear tests (Schwedes & Schulze, 1990; Ucgul et al., 2015; Kotrocz & Kerenyi, 2017). The tests are performed using a special shear device. The test was also used by Kuře et al. (2019), who created and verified a numerical DEM model of rapeseed.

Lei et al. (2021) verified the rapeseed's DEM model by comparing the motion characteristics and seeding performance during the simulation and the bench test. The results confirmed the possibility of using DEM to determine the final position of rapeseed during seeding. By analysing the movement of particulate matter, it is possible to verify whether the movement in the DEM model corresponds. One of the many methods of motion analysis is image processing.

Image processing can be a useful tool for tracing surface objects. It is possible to record the movement of particles for later analysis by using a camera (Hoffmann et al., 2014; Kříž et al., 2016). Zhang et al. (2006) proposed two types of analysis, soil mass motion analysis for tracking soil displacement and soil particle tracking analysis for unique soil particle translation and rotation tracking. They used a basic algorithm based on cross–correlations of the frames to determine the corresponding locations of the traced point in successive frames and thus measure the displacement values. Image processing for particle tracing using the contrast point method was applied by Hardy et al. (2017), who simulated the effect of raindrops on soil erosion by using fluorescent coating on the upper particles in a soilbox.

The aim of this paper is to propose and use the methodology for calculating the traces of individual particles of rapeseed using the contrast point method during a real test. To calibrate and verify a numerical model of the same system as in a real test. For additional verification will be used the angle of repose test.

MATERIALS AND METHODS

An experimental device for measuring the angle of repose was used to trace the rapeseed grains during the pouring process. The process was filmed and then processed by the optical contrast point method. Then a simulation model was created, corresponding with rapeseed grains in their parameters. For verification and added calibration of the simulation model, a test measuring the angle of repose was performed for the model and a real experiment.

The material model and the physical input parameters were based on the numerical model from previous experiments (Kuře et al., 2019). The experiment aimed to create a numerical rapeseed model and verify it by comparing the static and dynamic friction in the real experiment and the model. Model calibration was made on a shear test using a shear device.

The rapeseed seeds (*Brassica napus*) of the Atora variety were used for the measurements. The used rapeseed properties were based on results from previous experiments (Kuře et al., 2019). The size of the rapeseeds was measured, which was 1.93 mm ± 0.214. The bulk density was 707 kg m^{-3} ± 5.469, and the moisture content was 3.5% ± 0.145. The moisture content of the rapeseed was verified by using the Memmert oven 100–800. The amount of the rapeseed was placed into the oven and dried to the moisture of 0%. From the weight difference before and after drying, the moisture was counted.

The experimental device for measuring the angle of repose

The device (Fig. 1) consisted of a camera (1), a tray (2), a cylinder (3), and construction (4), to which a cylinder was attached to a linear system. The cylinder was made of a transparent tube with an inner diameter of 100 mm, and an outer diameter of 110 mm. The cylinder was filled with particulate matter, which consisted of the rapeseed particles. After filling the cylinder with particles, the cylinder was lifted by a DC motor (5). The cylinder's lifted speed has been set at 0.00325 m s^{-1}. The speed depends on the gear ratio of the DC motor and the pitch of the thread. The speed mustn't be too high so that only the static behaviour of the substance is manifested. As the cylinder was lifted at a constant speed, its content (particulate matter) was spilt on the tray.

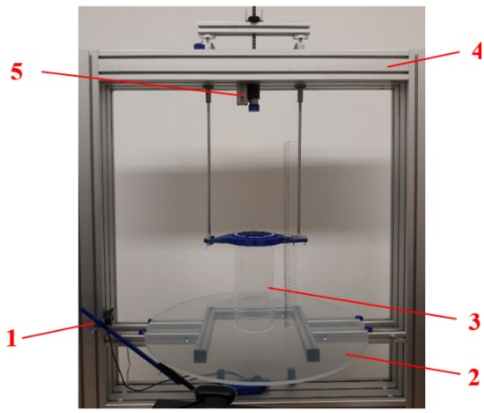

Figure 1. The equipment for measuring the angle of repose.

Tracing the particles by using the method of the contrast point

Polypropylene particles were chosen as contrast points for particle tracking. The polypropylene impact copolymers with the density of 900 kg m^{-3}, tensile stress at yield of 26 MPa, tensile strain at yield of 10% and the average size of 4.3×3.6×2.7 m was used. The cylinder of the experimental equipment was filled with particulate matter, which consisted of the rapeseed particles and nine polypropylene particles. Three polypropylene particles were placed at the approximate height of 80 mm at the centre of the camera's axis. Due to the spacing, the particles were approximately 20 mm apart. The other three particles were seated at the height of 120 mm. The last three particles were placed at the height of 160 mm. The cylinder was filled with rapeseed approximately at the high of 210 mm (Fig. 2).

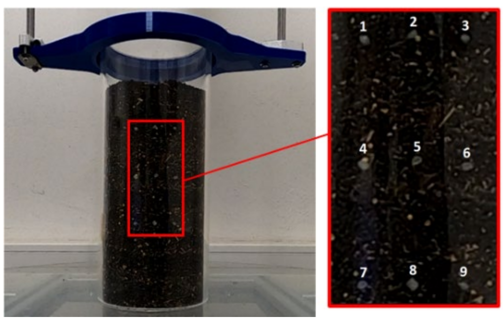

Figure 2. Single points of PP particles in rapeseed.

The used camera recorded the movement of polypropylene particles during the whole experiment. The record was evaluated using image analysis to determine the particle trajectory, visible throughout the entire recording duration. The camera was placed at the height of 80 mm above the level of the plate and recorded the entire course of the experiment from a distance of 360 mm. After recording, the video was saved in a MOV format.

DaVinci Resolve (16.1.2) software environment was used to obtain paths of polypropylene particles from video records. It was done using the DaVinci Resolve function called Tracker. The contrasting point was bounded as an image (Fig. 3). Then the Tracker monitored image movement during the video playback. This movement eventually created the path of motion of the contrast point image.

Figure 3. Bounded contrast point.

The coordinate system was shifted, and the [0;0] value on X and Y-axis was moved to the cylinder's centre on it's wall (Z-axis was not concerned, because it was calculated later), i.e., to the height where the cylinder contacts the plate. It was done so that the gathered data could be later compared with data gained from Rocky DEM. The path coordinate data (X-axis and Y-axis) were exported to MS Excel and processed. The resolution of the recorded images was 1,080×1,920 pixels. The resolution was later recalculated to millimetres, so the value of the particle movement was known. The polypropylene particles' position on the Z-axis was obtained by calculations (1) and (2).

$$z = r \cdot cos\alpha \tag{1}$$

$$\sin\alpha = \frac{x}{r} \tag{2}$$

where x (m) is the position of the particle on the X-axis; r (m) is the diameter of the cone's cross–section; α (°) is the angle between the r and z curves, and z is the particle's position on the Z-axis.

The diameter r was variable due to the conical shape of the spilt rapeseed, so it was necessary to determine its value for each position on the Z-axis. It was obtained using graphical measurements in the mathematical application GeoGebra software environment (GeoGebra, 2019). Subsequently, all coordinate values were known. The coordinate values of the contrast particle's initial positions were selected and used to select particles in the numerical model.

The numerical model

The numerical model was created in Rocky DEM Particle Simulator (4.2.0) environment (Fonte et al., 2015). The angle of repose experiment (described in The angle of repose experiment) calibrated the numerical model of spilling rapeseed. The property of particle interaction was set up according to previous experiments (Kuře et al., 2019). Input parameters of the materials, their interactions, and physics are listed in (Table 1.). The particles were chosen to be spherical, and their size was set up to 2 mm, which corresponds to the average rapeseed diameter. The hysteretic linear spring model is the mathematical model chosen for the normal force component. The resulting contact force can be expressed by Eq. (2) for the convergence of two particles and separately by the equation for the retraction of two particles (Kruggel-Emden et al., 2007). The model is described in detail in (Katinas et al., 2019).

$$F_n = \begin{cases} -k_z \cdot \delta, & \dot{\delta} \geq 0 \\ -k_o \cdot (\delta - \delta_0), & \dot{\delta} < 0 \end{cases} \quad (3)$$

where $F_n(N)$ – normal force; $k_z(N \cdot m^{-1})$ – spring load stiffness; $k_o(N \cdot m^{-1})$ – spring relief stiffness; $\delta\ (m)$ – overlap; $\delta_0\ (m)$ – overlap during the retraction of particles; $\dot{\delta}\ (m \cdot s^{-1})$ – instantaneous normal velocity.

Table 1. Input parameters of the numerical model

Simulation physics:		Source
Normal force	Hysteretic linear spring	(Rocky DEM technical manual, 2020)
Tangential force	Linear spring coulomb limit	(Rocky DEM technical manual, 2020)
Adhesive force	None	(Rocky DEM technical manual, 2020)
Rolling Resistance	0.28	Selected
Gravity	9.81 m s^{-2}	Selected
Material interaction properties between particles:		
Static friction	0.33	Selected
Dynamic friction	0.3	Selected
Tangential stiffness ratio	0.8	Selected
Restitution coefficient	0.3	Selected
Material properties:		
Bulk density	707 kg m^{-2}	(Kuře et al., 2019)
Young's modules	2.66 Mpa	(Kuře et al., 2019)
Poisson's ratio	0.3	Selected
Partical size	2 mm	(Kuře et al., 2019)
Lifting velocity of the cylinder in the Y-axis	0.00325 m s^{-1}	Selected

The cylinder and tray models were imported to Rocky DEM. The cylinder model was filled with the model of particles (Fig. 4), and the retraction velocity was set up to 0.00325 m s^{-1}. Once the model was set up, a calculation was followed by a simulation of spilling the rapeseed.

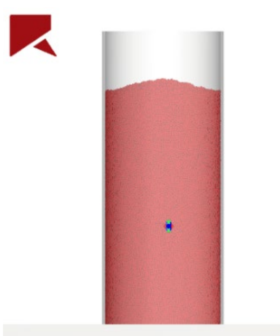

Figure 4. The cylinder model was filled with the model of particles.

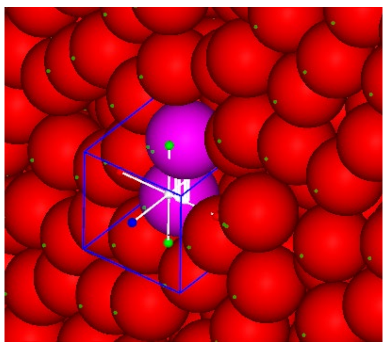

Figure 5. Two nearest particles correspond to the initial position of contrast points from the real test.

After the simulation, particle paths could be detected. According to the initial position of the particles from the real experiment, two particles from the simulation were selected. The model particles' positions were the closest to the position that corresponded to the position of the particles from the real experiment (Fig. 5). Each particle was marked, and its path was exported for the entire simulation course.

The angle of repose experiment

The measurement of the angle of repose experiment was used to verify the model of the particle tracing experiment. GeoGebra (2019) software environment was used to obtain said angles. Defining a horizontal line was the first step in measuring the angle of repose. A horizontal line was created by using two marginal points on the bottom of the cylinder. These points are visible in Fig. 6 as the point ‘S’ and ‘T’. The horizontal line is parallel to the bottom of the pile and is used as the adjacent side of the angle of repose. To count the angle of repose, it was also necessary to make the hypotenuse lines. Both sides of the pile's surface profile formed the hypotenuse lines. That formed the pile's profile into two right triangles from which the angles could be calculated. The angle between the surface lines of the pile and the horizontal line are the angles of repose, that is indicated in the Fig. 6.

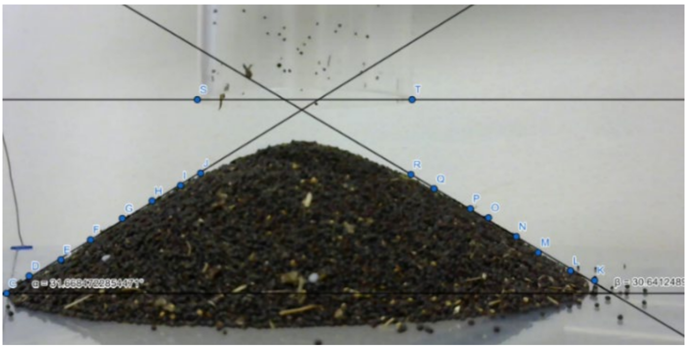

Figure 6. Measuring the angle of repose on the real system.

Subsequently, the script supported by the RockyDEM environment was used to determine the angle of repose. The pile was divided into 36 individual slices utilising the script (Fig. 7). Each slice was divided into separate sectors from the centre of the pile (Fig. 8). For each sector, a particle with the highest Y-axis value was identified. A line was created for each slice using linear regression, and its direction determined the given angle.

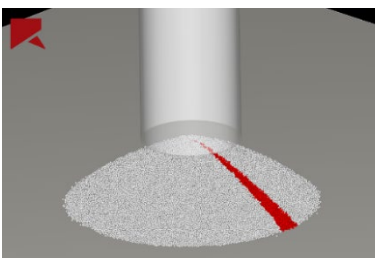

Figure 7. A circular section set aside for determining the angle of repose.

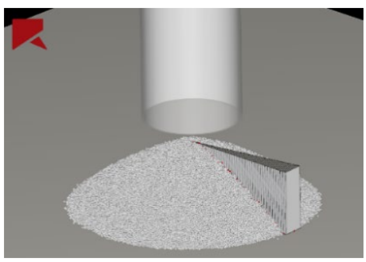

Figure 8. Division of a circular segment into sectors.

The statistical evaluation

Statistical methods F-Test: Two-Sample for Variances and subsequently t-Test: Two-Sample Assuming Equal Variances were used to compare the measurement results and the results obtained by simulations in RockyDEM. The variances and the expected values for the individual X, Y, and Z-axis between the measurement and the simulation were determined using these methods. Hypotheses about the conformity of the variances and the conformity of the Equal Variances were established. The level of significance was set up to a value of $\alpha = 0.05$. This value was the border value of the p-value from the F-Test and t-Test that confirmed their hypotheses. (Svatošová & Kába, 2013).

RESULTS AND DISCUSSION

The angle of repose test

After the experimental phase, captured images of the pile were evaluated. The resulting average angle of repose equals 30.51° ± 1.50°. See (Fig. 9) for the measured data. The graph contains the individual average data for each slice (Fig. 8) with relative deviation. The horizontal line marks the average angle of repose. The chart shows that a systematic error was made during the measuring. Image deflection has occurred during the rotation of the camera. Systematic error is a sine function. A slight tilt of the camera may have caused the error. Another possibility is that the centre of the cylinder was not attached exactly to the axis. From a statistical point of view, this error has no significant effect on the average value of the entire measurement. In comparison to Izli et al. (2009) the angle is greater. However, a grain with a different humidity was chosen in our case.

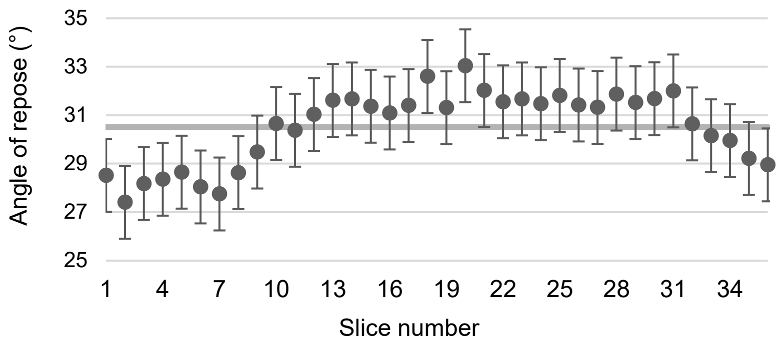

Figure 9. Graph of measured Angle of repose of pile of rapeseed after the real experiment.

Once the data evaluation was complete using RockyDEM rapeseed model script, an image containing the results was exported. The resulting angle of repose from the model equals 30.06° ± 0.5°. See Fig. 10 for the results. The part on the left shows the evaluation of angle of repose measurement. The result is an approximation of the maximum Y-axis values for the individual sectors. The part on the right shows the evaluation of the individual angles of repose, similar to Fig. 9. By considering the relative error, it can be said that the measured data and the data from the model correlate, and the error is in tolerance value.

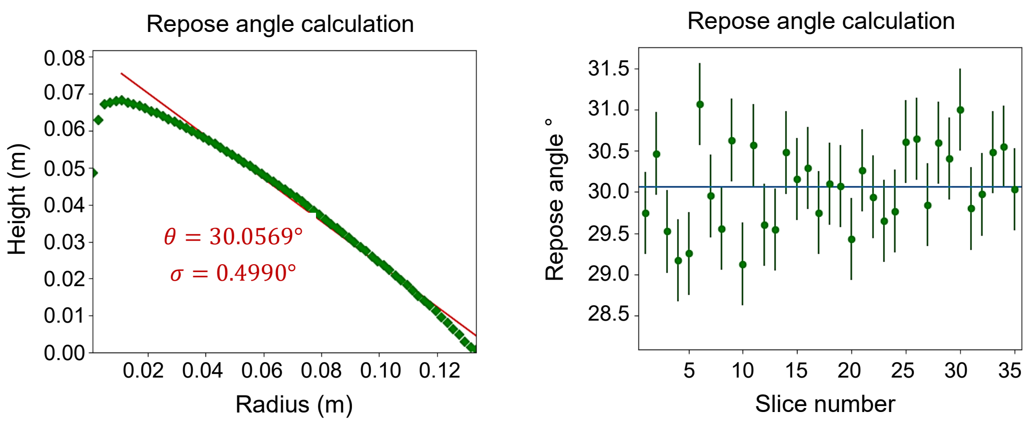

Figure 10. Graph of measured Angle of repose of pile of rapeseed model from RockyDEM.

The cone base (poured pile) in the model is 269 mm long. The base of the pile at the time of pouring was 293 mm long. Fig. 11 shows the proportional comparison between the poured pile and the rapeseed pile of the model. Because an angle of repose test verified the model's accuracy, it was possible to use the model to trace individual particles.

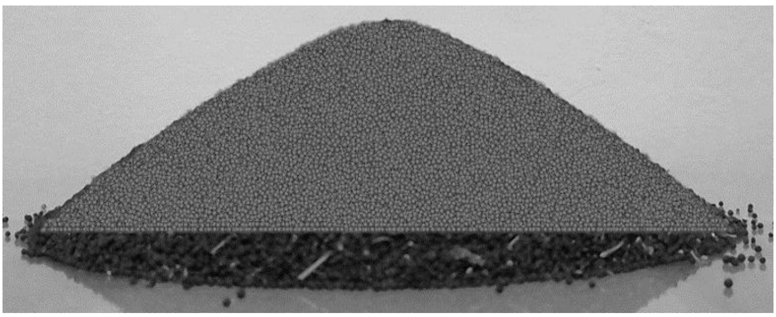

Figure 11. Comparison of rapeseed pile model and real test.

Results of the contrast point tracing

The data obtained and recalculated from DaVinci Resolve were compared with the data obtained from the model in Rocky DEM. To each particle traced in the real experiment were found two particles in the model, and their X, Y, and Z coordinates were exported to MS Excel. Fig. 12 compares the contrast point traces from the model and real test on the Y-axis and X-axis, and Fig. 13 shows the chart comparing the contrast point traces from the model and real test on the Y-axis and Z-axis. Fig. 12 and Fig. 13 show the trace the particle number 1. Seven out of nine particles could be traced from the measurements. For others, the trace failed due to poor visibility from the video due to particle overlap.

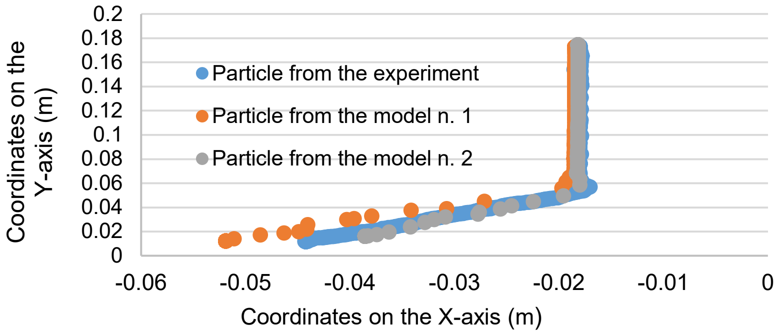

Figure 12. Comparison of contrast point traces from model and real test on X and Y-axis.

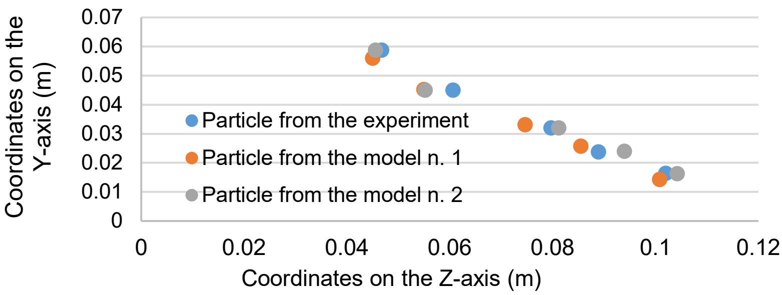

Figure 13. Comparison of contrast point traces from model and real test on Z and Y-axis.

The statistical evaluation

The results of statistical verification are seen in Table 2. These results show that seven traced particles out of nine were compared in each axis separately. The particle number refers to the order of the traced particle, according to Fig. 2. The *F*-Test column shows the resulting *p*-values that had values higher than the significance level of 0.05. This confirmed the hypothesis of equality of variances. The *t*-Test (the Equal Variances confirmation) column shows the resulting *p*-values that had values higher than the significance level of 0.05, except for particle 2 in X-axis. All comparisons were statistically significant except for particle 2 in X-axis. The average deviation was 3.77 m in X-axis, 0.55 mm in Y-axis and 1.7 mm in Z-axis.

Table 2. The results of statistical verification

Number of particle	Axis	*F*-Test	*t*-Test	Result
1	X	0.436	0.692	significant
	Y	0.486	0.991	significant
	Z	0.444	0.920	significant
2	X	0.071	0.017	insignificant
	Y	0.492	0.999	significant
	Z	0.427	0.870	significant
4	X	0.414	0.807	significant
	Y	0.498	0.975	significant
	Z	0.230	0.987	significant
5	X	0.105	0.287	significant
	Y	0.454	0.998	significant
	Z	0.326	0.995	significant
6	X	0.221	0.529	significant
	Y	0.310	0.999	significant
	Z	0.451	0.457	significant
8	X	0.329	0.079	significant
	Y	0.499	0.079	significant
	Z	0.484	0.074	significant
9	X	0.232	0.295	significant
	Y	0.232	0.992	significant
	Z	0.177	0.854	significant

DISCUSSION

The loose (particulate matter) consists of particles of various shapes and sizes but of the same loose particulate bulk density. Essentially, the particles create a statistical set, and therefore it is complicated to predict the matter's behaviour. Studying and monitoring the bulk material makes it possible to get an idea that helps predict the material's behaviour. However, the movement of a single particle can cause a domino effect of the entire mass of the matter. It is why tracing the particulate matter is difficult to measure and gain quality parameters.

The proposed method proved to be an affordable method for individual particulate matter movement surface monitoring. The video of particle movement is possible post-process with video processing software DaVinci Resolve. The method has been used, for example, by Hardy et al. (2017). However, they used fluorescent coating, so the light contrast was obtained, and data were post-processed by using Spyder (Scientific PYthon Development EnviRonment) script.

In order to preserve the maximal accuracy of the model, it is important to know not only the internal states of the system that is being examined but also take into consideration the external conditions affecting the system's behaviour. Incorrect entry values, poor computer model, or inappropriate selection of methods can lead to incorrect and misleading results. The length of the calculation, which is conditioned by the number and shape of the individual particulate particles, poses another risk. The more complex and numerous the material, the more time (multiplicatively) is needed to calculate the model. It is necessary to optimise the model parameters as well as the number of modelled particles and, at the same time, achieve sufficient model accuracy based on correlation verification with the real experiment (Wojtkowski et al., 2010).

The discrete element method can be used in the description of particulate particles, such as rapeseed. The given parameters are for moisture content of 6 % and the actual grain size of rapeseed. The possible change of boundary conditions must be considered when using a model with different grain ratios (Bravo et al., 2014).

CONCLUSION

This paper aimed to create the methodology for performing particle tracing using an optical contrast point method. The results obtained from the real test were statistically compared with the simulation and showed their statistical significance for all particles, except for one in one axis. The main conclusions can be summarised as follows:

- The proposed methodology is suitable for the verification of the DEM model.
- The trace of real particles from the original place is related to the average trace of the two nearest particles from the numerical model. The final position of traced particles was in the nearby area.
- The numerical model with relevant parameters can be used to predict the particulate matter movement with an accuracy of 3.77 mm in X-axis, 0.55 mm in Y-axis and 1.7 mm in Z-axis.

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REFERENCES

Amšiejus, J., Dirgeliene, N., Norkus, A. & Skuodis, Š. 2014. Comparison of sandy soil shear strength parameters obtained by various construction direct shear apparatuses. *Archives of Civil and Mechanical Engineering* **14**(2), 327–334. https://doi.org/10.1016/j.acme.2013.11.004

Asaf, Z., Rubinstein, D. & Shmulevich, I. 2007. Determination of discrete element model parameters required for soil tillage. *Soil and Tillage Research* **92**(1–2), 227–242. https://doi.org/10.1016/J.STILL.2006.03.006

Bravo, E.L., Tijskens, E., Suárez, M.H., Gonzalez Cueto, O. & Ramon, H. 2014. Prediction model for non-inversion soil tillage implemented on discrete element method. *Computers and Electronics in Agriculture* **106**, 120–127. https://doi.org/10.1016/j.compag.2014.05.007

Chen, Y., Munkholm, L.J. & Nyord, T. 2013. A discrete element model for soil-sweep interaction in three different soils. *Soil and Tillage Research* **126**, 34–41. https://doi.org/10.1016/j.still.2012.08.008

Fonte, C.B., Oliveira, J.A.A. & Almeida, L.C.De. 2015. DEM-CFD coupling: Mathematical medelling and case studies using Rocky DEM® and ANSYS Fluent®. *Elev. Int. Conf. CFD Miner. Process Ind, CSIRO Organization, 1–7.*

Hardy, R.A., James, M.R., Pates, J.M. & Quinton, J.N. 2017. Using real time particle tracking to understand soil particle movements during rainfall events. *CATENA* **150**, 32–38. https://doi.org/10.1016/J.CATENA.2016.11.005

Hoffmann, D., Rybka, A., Linda, M. & Kříž, M. 2014. Detection of anchoring columns in low trellis. *Agronomy Research* **12**(1), 73–80.

Izli, N., Unal, H. & Sincik, M. 2009. Physical and mechanical properties of rapeseed at different moisture content. *International Agrophysics* **23**(2), 137–145.

Katinas, E., Chotěborský, R., Linda, M. & Jankauskas, V. 2019. Wear modelling of soil ripper tine in sand and sandy clay by discrete element method. *Biosystems Engineering* **188**, 305–319. https://doi.org/10.1016/J.BIOSYSTEMSENG.2019.10.022

Kotrocz, K. & Kerenyi, G. 2017. Numerical discrete element simulation of soil directshear test. *Proceedings - 31st European Conference on Modelling and Simulation, ECMS 2017*, 510–515. https://doi.org/10.7148/2017-0510

Kříž, M., Linda, M., Svatoš, J. & Hromasová, M. 2016. Application of 3D cameras in agriculture when evaluating the quality of soil tillage. *Research in Agricultural Engineering* **62**(2), 39–49. https://doi.org/10.17221/4/2014-RAE

Kruggel-Emden, H., Simsek, E., Rickelt, S., Wirtz, S. & Scherer, V. 2007. Review and extension of normal force models for the Discrete Element Method. *Powder Technology* **171**(3), 157–173. https://doi.org/10.1016/j.powtec.2006.10.004

Kuře, J., Hájková, L., Hromasová, M., Chotěborský, R. & Linda, M. 2019. Discrete element simulation of rapeseed shear test. *Agronomy Research* **17**(2), 551–558. https://doi.org/10.15159/AR.19.034

Lei, X., Hu, H., Wu, W., Liu, H., Liu, L., Yang, W., Zhou, Z. & Ren, W. 2021. Seed motion characteristics and seeding performance of a centralised seed metering system for rapeseed investigated by DEM simulation and bench testing. *Biosystems Engineering* **203**, 22–33. https://doi.org/10.1016/J.BIOSYSTEMSENG.2020.12.017

Schwedes, J. & Schulze, D. 1990. Measurement of flow properties of bulk solids. *Powder Technology* **61**(1), 59–68. https://doi.org/10.1016/0032-5910(90)80066-8

Svatošová, L. & Kába, B. 2013. *Statistika*.

Ucgul, M., Fielke, J.M. & Saunders, C. 2015. Three-dimensional discrete element modelling (DEM) of tillage: Accounting for soil cohesion and adhesion. *Biosystems Engineering* **129**, 298–306. https://doi.org/10.1016/J.BIOSYSTEMSENG.2014.11.006

Wojtkowski, M., Pecen, J., Horabik, J. & Molenda, M. 2010. Rapeseed impact against a flat surface: Physical testing and DEM simulation with two contact models. *Powder Technology* **198**(1), 61–68. https://doi.org/10.1016/j.powtec.2009.10.015

Zhang, G., Liang, D. & Zhang, J.M. 2006. Image analysis measurement of soil particle movement during a soil–structure interface test. *Computers and Geotechnics* **33**(4–5), 248–259. https://doi.org/10.1016/J.COMPGEO.2006.05.003

Zhou, Y.C., Xu, B.H., Yu, A.B. & Zulli, P. 2002. An experimental and numerical study of the angle of repose of coarse spheres. *Powder Technology* **125**(1), 45–54. https://doi.org/10.1016/S0032-5910(01)00520-4

Agronomy Research **20**(3), 531–548, 2022
https://doi.org/10.15159/AR.22.054

The use of SSR-markers in rice breeding for resistance to blast and submergence tolerance

E. Dubina[1,2,*], P. Kostylev[3], S. Garkusha[1,2], M. Ruban[1], S. Lesnyak[1], Y. Makukha[1], S. Korzh[1], D. Nartymov[1] and O. Gorun[1]

[1]Federal Scientific Rice Centre, Laboratory of Informational, Digital and Biotechnologies, Belozerny 3, RU350921 Krasnodar, Russia
[2]Kuban State Agrarian University, Faculty of Agronomy, Department of Biotechnology, Biochemistry and Biophysics, Kalinina 13, RU350004 Krasnodar, Russia
[3]Agricultural research center 'Donskoy', Laboratory of Rice Breeding and Seed Production, Nauchny Gorodok 3, RU347740 Zernograd, Russia
[*]Correspondence: lenakrug1@rambler.ru

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Abstract. The identification of effective specialized DNA markers providing the clear control of target locus inheritance by the trait of submergence tolerance has been conducted. Among the studied set of microsatellite markers, two the most informative SSR-markers - RM 7481, PrC3 showed high efficiency in detecting intraspecific polymorphism of rice varieties and lines used in the work. With the use of these markers the clear genotype marking the obtained hybrid rice plants by this trait has been conducted and it is has been verified by phenotype evaluation as a result of laboratory trials. The plant samples carrying the target gene in heterozygous and homozygous state has been selected. About 400 backcrossed self-pollinated rice lines with introgressed and pyramided resistance genes *Pi-1, Pi-2, Pi-33, Pi-ta, Pi-b* to *Pyricularia oryzae Cav*. were obtained within the frameworks of program to develop genetic rice sources resistant to blast. The conducted testing for resistance to blast and the assessment by economically valuable traits have allowed to select the prospective rice samples. The plant samples of F2 and BC1F1 generations with combination of resistance to blast genes (*Pi*) and submergence tolerance gene (*Sub1A*) in homozygous and heterozygous state that is confirmed be the results of analysis of their DNA have been obtained. The obtained hybrid plants are being tested in breeding nurseries for a complex of economically valuable traits. The best plants will be selected and send to State Variety Testing system. Their involving in rice industry will reduce the use of plant protection chemicals against diseases and weeds, thereby increasing the ecology status of the rice industry.

Key words: rice, blast resistance genes, submergence tolerance genes, PCR analysis, DNA markers, rice varieties.

INTRODUCTION

Rice is the world's third most cultivated cereal crop. The Krasnodar region is the largest rice-growing area in Russia, having more than 80% of the country's sown area (Dubina, 2019). The main limiting biotic stress factors that prevent high rice yields and reduce plant productivity are diseases, weeds, and pests. Unfavourable climatic conditions (high air temperatures, lack of water (drought), etc.) also restrain the growth of rice yields and threaten the food security of the region (Kumar & Bhagwat, 2012).

The introduction of genes (functions) of resistance to biotic and abiotic stressors into rice varieties with high productivity that are adapted to local environmental conditions, marker control, and the pyramiding of several target genes in one genotype, are considered relevant and promising directions in breeding (Singh et al., 2009; Negrao et al., 2011).

Genetic resistance is a strategy that allows for the obtaining of high yields of, to some extent, more environmentally friendly rice that meets the requirements of the modern consumer without loss and risk (Zelenskiy, 2016).

The use of SSRs makes it possible to accelerate the breeding process, and therefore, today, molecular breeding technologies are one of the prioritized and dynamically developing scientific directions for breeding programs (Mackill & Ni, 2001; Dubina et al., 2015; Dubina, 2019).

At the International Rice Research Institute (IRRI), using DNA marker selection and phytopathological testing, a number of monogenic lines, 'IRBLs', were developed on a genetic basis for local breeding varieties, carrying resistance genes *to Pyricularia oryzae Cav.*: *Pi-ta, Pi-b, Pi-z, Pi-k, Pi-5, Pi-i, Pi-1, Pi-9,* and *Pi-zt* (Dubina, 2019). Such lines are worthwhile both for breeding blast resistant rice varieties and for monitoring the racial composition of the blast pathogen. A number of ABL lines with the resistance genes *Pi-ta, Pi-b, Pi-z,* and *Pi-40* were developed at the National Institute of Crop Science (NICS) in South Korea using DNA marker selection (Mackill & Ni, 2001; Choi et al., 2006).

However, these varieties in domestic breeding programs for this trait can only be used as donors, because their growing seasons are over 155–170 days (Zelenskiy, 2016). For rice cultivation in the Russian Federation, the growing season for such varieties should be no more than 125 days (Dubina et al., 2015). Therefore, the development of a domestic pool of varieties with genes for resistance to these traits is extremely necessary.

An important stage in the history of rice breeding for resistance to water stress (long-term flooding) was the identification of the *Submergence 1A* or *Sub1A* locus, which controls this trait (Catling, 1992; Xu et al., 1996; Ito et al., 1999; Xu et al., 2006). It regulates the response to ethylene and gibberellin, which limits carbohydrate intake and the state of shoot rest in conditions of flooding, and promotes tolerance to prolonged and deep submergence (Xu et al., 2000). The Sub1 region is within the markers CR25K and SSR1A and covers more than 182 thousand base pairs. This interval encodes three genes for ethylene response factors, designated *Sub1A, Sub1B*, and *Sub1C,* but only *Sub1A* increases submergence tolerance in plants (Mackill & Ni, 2001). Researchers from the University of California at Riverside (USA) found that *Sub1A* allowed rice plants to survive by growing new shoots after a dry period (Kende et al., 1998; Steffens et al., 2006). Work on the development of varieties for long-term flooding has been carried out for several decades at IRRI (Hattori et al., 2009). Recently, a number of different genes for resistance to long-term flooding have been studied, including *Sub1A*. This work is significant for short rice varieties such as Swarna (Collard et al., 2013). One variety has been released in the Philippines as PSB Rc68 (Septiningsih & Kretzschmar, 2015).

Despite the fact that there are no flooding problems associated with the prolonged flooding of rice fields in Russia, this gene can be used as a factor for controlling the harmful weeds of rice fields - the *Echinochloa* species (Dubina et al., 2017; Dubina et al., 2018). This strategy is not currently used in Russia due to several problems which are linked, firstly, to the deficiency of varieties carrying this gene, and secondly, to the high mortality of rice seedlings that do not have this gene on account of a lack of oxygen caused by raising the water level to combat weeds (Kostylev et al., 2015; Azarin et al., 2016; Azarin et al., 2017). Consequently, a rational approach to allowing rice defense without herbicides is to develop and grow varieties that are capable of anaerobic germination and are resistant to prolonged flooding (Steffens et al., 2006; Negrao et al., 2011).

Varieties with *Sub1A* developed by foreign colleagues can only be taken as donors in domestic breeding schemes for this trait due to their long growing seasons (Xu et al., 1996; Xu et al., 2000; Steffens et al., 2006; Xu et al., 2006; Hattori et al., 2009; Collard et al., 2013). This study was conducted with the following objectives: to identify of informative DNA-markers for blast resistance genes (*Pi-1, Pi-2, pi-33, Pi-ta, Pi-b*) and flooding tolerance (*Sub1A*); to introgress these genes in rice lines for the southern region of the Russian Federation; to identify promising lines with improved tolerance to blast and submergence. This research is of great importance for the development of fundamental and theoretical foundations, along with practical approaches to the use of SSR markers in rice breeding (Fukao et al., 2011; Kostylev et al., 2015; Mickelbart et al., 2015; Dubina et al., 2017; Dubina et al., 2018).

MATERIALS AND METHODS

Plant materials

Varieties of Asian origin, Khan Dan, Swarna, TDK-1, IR-64, CR 1009, Inbara-3, and BR-11, became donors for the introduction of *Sub1A,* a gene that confers tolerance to long-term flooding. The domestic rice varieties Lenaris, KP- 25, KP-163, and KP-24-15 were used as maternal forms. In the breeding program to increase blast immunity, the above-mentioned domestic varieties and breeding samples became the maternal forms, and the varieties and lines of foreign breeding became donors of target genes: BL-1 (*Pi-b*), C104-Lac (*Pi-1*), C101-A-51 (*Pi-2*), C101-Lac (*Pi-1, Pi-33*).

Breeding scheme

In the used breeding schemes, plants of donor and recipient forms, along with hybrid plants of BC (backcrossing) generations, were planted in growing vessels in artificial climate chambers (or in growing plots, depending on the season of the year) in triplicate with an interval of 3–10 days to synchronize flowering. Hybridization of rice plants was carried out by pneumocastration and pollination using the 'TVELL' method (Los, 1987).

Fig. 1 shows our proposed accelerated breeding scheme for developing a modern set of new genotypes resistant to biotic and abiotic stressors.

At the first stage of the breeding program, we crossed the domestic rice varieties and the rice varieties with resistance genes to *Pyricularia oryzae* (*Pi-ta, Pi-33, Pi-1, Pi- b,* and *Pi-2*) and also with the donor variety Khan Dan (paternal form), which possesses a gene for tolerance to long-term flooding, *Sub1A*, in its genotype. For each combination F1 generation was obtained. Then the first backcrossing event was carried out. We have planned to carry out two backcrossing events, since it is known that the replacement of the genome of the recurrent parent (RP) with backcrossing in BC2 is 87.5% (Jena et al., 2003; Dubina et al., 2018).

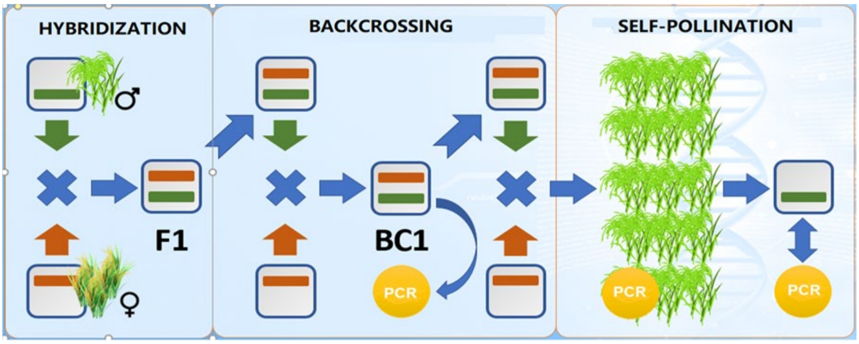

Figure 1. Scheme of introducing target genes into Russian rice varieties using molecular marking.

Note: F1 – the first generation hybrids; BC1 – the first backcrossing with the recurrent parent form; PCR – polymerase chain reaction.

Marker monitoring of the dominant allele of this transferred gene by PCR analysis was carried out at all stages of the breeding scheme, starting with BC1 (the first backcrossing event). Based on the results of DNA analysis, only plants that had dominant alleles of target resistance genes in their genotypes were selected for further study in the breeding nurseries for economically valuable traits.

Genome DNA extraction from the plant tissue

For molecular genetic studies, DNA samples of the analyzed rice plants were isolated from the freshly cut part of the leaf blade at the stage of 4–5 leaves by the CTAB method with some modifications (Murray, 1980).

PCR analysis for identification of *Pi*-genes

PCR was performed with 40–50 ng of DNA in a final volume of 25 µL. The following composition of the reaction mixture was used: 0.05 mM deoxyribonucleoside phosphates (dNTPs), 0.3 mM each primer, 25 mM KCL, 60 mM Tris-HCL (pH 8.5), 0.1% Triton X-100, 10 mM 2-mercaptoethanol, 1.5 mM $MgCl_2$, 1 unit of Taq-DNA polymerase with standard buffer (SibEnzyme).

DNA amplification was carried out under the following conditions: Initial DNA denaturation at 94 °C - 4 min. Thirty cycles: 1 min - denaturation at 94 °C; 1 min - primer annealing at 55 °C; 1 min elongation at 72 °C. Finally: 5 min at 72 °C. The primers used in the studies (Table 1) were synthesized by Syntol (Moscow).

DNA amplification was performed on Tertsik amplifiers (DNA technology, Moscow) and Bio Rad (made in Germany).

The amplification reaction products were separated by electrophoresis in 8% polyacrylamide gel (PAGE).

Visualization of the result of electrophoretic separation of PCR products was performed using ethidium bromide.

Table 1. Nucleotide sequences of primers for identifying blast resistance gene

Marker name	Gene	Nucleotide sequence of primers (5'→3')	
Rm 224	*Pi-1*	F	ATCGATCGATCTTCACGAGG
		R	TGCTATAAAAGGCATTCGGG
Rm 144		F	TGCCCTGGCGCAAATTTGATCC
		R	GCTAGAGGAGATCAGATGGTAGTGCATG
Rm 72	*Pi-33*	F	CCGGCGATAAAACAATGAG
		R	GCATCGGTCCTAACTAAGGG
Rm 310		F	CCAAAACATTTAAAATATCATG
		R	GCTTGTTGGTCATTACCATTC
Rm 527	*Pi-2*	F	GGCTCGATCTAGAAAATCCG
		R	TTGCACAGGTTGCGATAGAG
SSR140		F	AAGGTGTGAAACAAGCTAGCAA
		R	TTCTAGGGGAGGGGTGTGAA
Pi-ta	*Pi-ta*	F1	GCCGTGGCTTCTATCTTTACATG
		R1	ATCCAAGTGTTAGGGCCAACATTC
		F2	TTGACACTCTCAAAGGACTGGGAT
		R2	TCAAGTCAGGTTGAAGATGCATCGA

PCR analysis for identification of *Sub1A* gene

To identify *Sub1A* gene in hybrid rice plants we used microsatellite molecular markers with specific primers (Table 2) associated with the locus responsible for resistance to prolonged immersion of rice under water.

The amplification was carried out in the 'Tertsik' DNA amplifier under the following conditions: at the first stage - denaturation for 5 min at 94 °C, then at the second stage - 5 cycles according to the following protocol: denaturation - 35 s at 94 °C; annealing of primers - 45 s at 60 °C; elongation - 30 s at 72 °C. The final stage included elongation at 72 °C for 5 min.

The amplification reaction products were separated by electrophoresis in 2% agarose gel. The electrophoresis was carried out at a voltage of 120–130 V for an hour. To visualize the electrophoresis result, an agarose gel plate was placed in the GelDocXR (BioRad) and then was photographed in ultraviolet light.

Table 2. Nucleotide sequences of primers for identification of *Sub1A*

Marker name	Nucleotide sequence of primers (5'–3')	
Sub1A203	F	GATGTGTGGAGGAGAAGTGA
	R	GGTAGATGCCGAGAAGTGTA
Sub1A_6	F	GATGTGTGGAGGAGAAGTGA
	R	GGTAGATGCCGAGAAGTGTA
Sub1A_7	F	GATGTGTGGAGGAGAAGTGA
	R	GGTAGATGCCGAGAAGTGTA
RM7481	F	CGACCCAATATCTTTCTGCC
	R	CATTGGTCGTGCTCAACAAG
RM285	F	CTGTGGGCCCAATATGTCAC
	R	GGCGGTGACATGGAGAAAG
RM219	F	CGTCGGATGATGTAAAGCCT
	R	CATATCGGCATTCGCCTG
RM 464A	F	AACGGGCACATTCTGTCTTC
	R	TGGAAGACCTGATCGTTTCC
RM 285	F	CTGTGGGCCCAATATGTCAC
	R	GGCGGTGACATGGAGAAAG
Sub1A	F	CAGGAATAAGTAGGCACATCA
	R	GGACCAAGAACAAAGTCAAA
AEX	F	AGGCGGAGCTACGAGTACCA
	R	GCAGAGCGGCTGCGA
PrC1	F	TTGC GAGCTAGCTGTCTGAA
	R	TAGTCCACGCGCTAATGTGA
PrC3	F	CAATAAGACTCGGGCTGTGC
	R	TAGTCCACGCGCTAATGTGA
GnS2	F	CTTCTTGCTCAACGACAACG
	R	TCGATGGGGTCTTGATCTCT

The field trials

To assess for agricultural significant traits, the analyzed breeding rice plants with *Pi-* genes were sown on a rice irrigation system after predecessor of perennial grasses at the FSI ESOS 'Krasnaya' branch of 'Federal Scientific Rice Centre' (FSBSI), Krasnoarmeysky district. All agrotechnical works and phenological observations were conducted in pursuance of FSBSI's generally accepted methodology (Practical guidance…, 1980). The timing of the vegetation phases before flowering and full ripeness were taken into account. Lodging and shedding were estimated in the phase of full ripeness. Varietal weeding was conducted in order to remove atypical plants in the experimental area. For biometric analysis, 25 typical plants of promising samples were selected, which were distinguished by agricultural significant traits and their resistance to *Pyricularia oryzae* (Dospekhov, 1979; Podkin, 1981).

The phytopathology testing

Evaluation of rice donor lines BL-1 (*Pi-b*), C104-Lac (*Pi-1*), C101-A-51 (*Pi-2*), C101-Lac (*Pi-1, Pi-33*), and IR 83260-2-10-5-2-1-B (*Pi-40*), and breeding samples for resistance to the local population of *Pyricularia oryzae Cav.*, was carried out on a vegetation plot of FSBSI according to methodological guidelines Kovalenko, 1988). As susceptible controls, we used rice varieties Volgogradsky and Pobeda 65; as a resistant control, we used the rice variety Avangard.

Rice plants were infected with *P. oryzae* fungi culture in the phase of 4–5 leaves by spraying them with a suspension of its conidia by spraying from the sprinkler at a normal flow rate of a suspension of 0.5 mL per plant For better adhesion, 'Tween' was added to the suspension at the rate of 1 drop per liter of water (Fig. 2 (Kolomiets, 1990)).

Figure 2. Artificial infection of donor lines and breeding material with the phytopathogenic fungus *Pyricularia oryzae Cav.*

Note: a – the spraying of analyzed rice plants by suspension of *P. oryzae*; b – the plants, treated by the suspension of *P. oryzae*; c – the growing of the *P. oryzae* conidia on the slide in Petri dishes for the evaluation of the *P. oryzae* spores vitality by microscoping while rice plants infecting period.

The estimation of plants damaged by *Pyricularia oryzae* was conducted on the 14th day after inoculation, in accordance with the express method for assessing rice varietal resistance to blast. The assessment was carried out by taking into account two indicators: type of reaction (in points) and degree of damage (in percentages), using the ten-point scale of the International Rice Research Institute (Kovalenko, 1988; Kolomiets, 1990): (1) resistant, 0–1 points - the absence of damage, small brown spots, covering less than 25% of the total leaf surface; (2) medium resistant, 2–5 points - typical elliptical blast spots, 1–2 cm long, covering 26–50% of the total leaf surface; (3) non-resistant, 6–10 points - typical elliptical blast spots, 1–2 cm long, covering 51% and more of the total leaf surface.

The intensity of disease development (IDD,%) was calculated by the formula (1):

$$IDD = \sum \frac{a \cdot b}{9n} \tag{1}$$

where IDD – the intensity of disease development, %; $\sum(a \cdot b)$ – the sum of the products of the number of infected plants multiplied by the corresponding damage score; n – the number of recorded plants, pcs (pieces).

Depending on the damage score, all varieties were conventionally divided into 4 groups: (1) resistant; (2) intermediate; (3) susceptible; (4) highly susceptible.

The laboratory express method for submergence tolerance

Evaluation of donors and domestic rice varieties for tolerance to prolonged flooding was carried out in laboratory conditions. The seeds of hybrid plants were grown in test tubes (Skazhennik et al., 2009; Linh et al., 2013). Further, when the seeds germinated, the tubes were placed in an artificial climate chamber with a light regime of day for 12 h and night for 12 h at a temperature of 30 °C. After the sprouts reached a length of 2–3 cm (Fig. 3, a), the plants in the tubes were filled with water (Fig. 3, b) and on the 15th day the plants were assessed for tolerance to flooding (Fig. 3, c). The evaluation of the plants for submergence tolerance was conducted visually (Vergara et al., 1976; Catling, 1992; Manangkil et al., 2008).

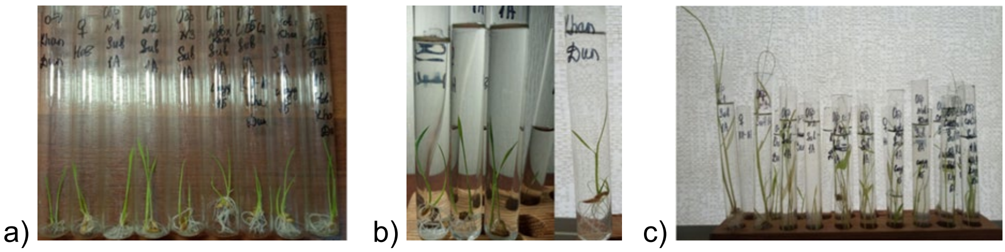

Figure 3. Laboratory-based express method testing the submergence tolerance of rice plants.
Note: a – growing hybrid rice plants seeds with Sub1A – gene in the tubes; b – the flooding sprouts with the water; c – the evaluation of hybrid plants for submergence tolerance.

Biometric rates (the plant height, the panicle length, the grain mass from one panicle, the mass of 1,000 grains) of experimental plants were counted by the software Microsoft Office Excel 2010 and STATISTICA 10.0 for Windows.

RESULTS AND DISCUSSION

Study of Sub1A genes/loci that control the defense reactions of rice plants to prolonged immersion under water - a factor in weed control

Recently, the usage of nature-saving technologies while growing agricultural crops and developing prospective germplasm resistant to stressors has been increased (Dubina et al., 2015, 2017, 2018).

Earlier, we mentioned that the *Sub1A* gene contributes resistance to prolonged flooding and that it could be used as a factor for combating weeds in rice cenosis.

Thirteen microsatellite markers were taken from the NCBI database (www.ncbi.nih.gov) for visualization of the donor and recessive alleles of the gene *Sub1A*. Their sequences are shown in the Materials and Methods section. For each specific pair of primers, protocols for the optimal composition of the reaction mixture and the amplification reaction program were developed, as a result of which, when performing SSR analysis under these conditions, the amplification products were clearly visualized (Figs 4–6).

Each molecular marker was tested on contrasting varieties: those both resistant (Khan Dan, TDK-1, CR 1009, Swarna, IR-64, BR-11, Inbara-3) and susceptible (KP-23, Contact, Boyarin, KP-25, KP-163, Flagman, Lenaris) to submergence tolerance.

It has been seen from the Fig. 4 that there is no polymorphism between analyzed plants by Sub1A203 locus. Therefore, this locus can't be used for the identification of the Sub1A-gene donor alleles.

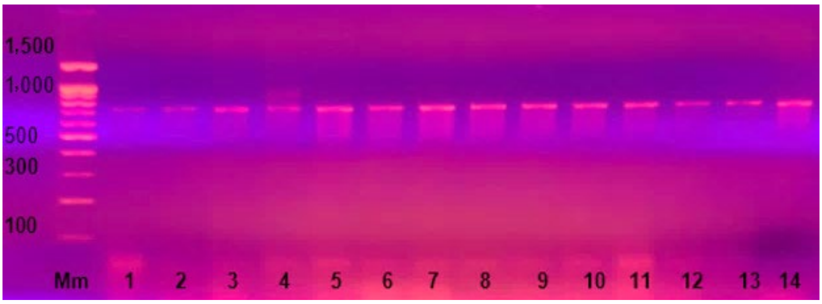

Figure 4. Visualization of amplification products at the Sub1A203 locus.

Note: Mm – molecular weight marker pBR322/BsuR I (supplier: Helicon, Russa), base pairs (bp); 1 – Khan Dan; 2 – TDK-1; 3 – CR 1009; 4 – Swarna; 5 – IR-64; 6 – BR-11; 7 – Inbara-3; 8 – KP-23; 9 – Contact; 10 – Boyarin; 11 – KP-25; 12 – KP-163; 13 – Flagman; 14 – Lenaris. The molecular mass is always measured in base pairs (bp).

Fig. 5 shows the allele variants of tolerant (1–7) and susceptible (8–14) rice varieties by the trait 'submergence tolerance'. The tolerant allele has PCR-product with size of 105 bp (base pairs), the susceptible one has 95 bp allele by this locus.

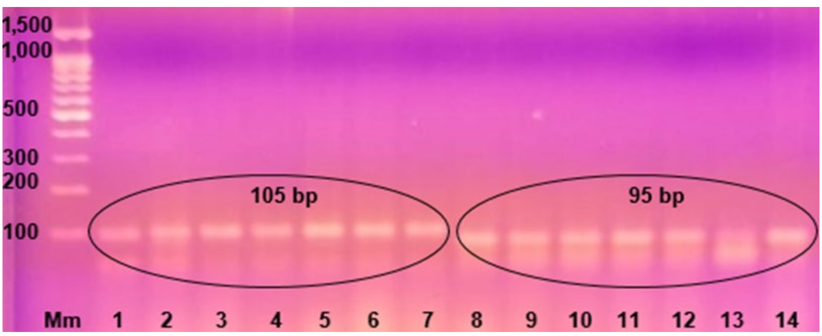

Figure 5. Visualization of amplification products at the RM 7481 locus.

Note: Mm – molecular weight marker pBR322/BsuR I (supplier: Helicon, Russia); 1 – Khan Dan; 2 – TDK-1; 3 – CR 1009; 4 – Swarna; 5 – IR-64; 6 – BR-11; 7 – Inbara-3; 8 – KP-23; 9 – Contact; 10 – Boyarin; 11 – KP-25; 12 – KP-163; 13 – Flagman; 14 – Lenaris.

Fig. 6 also demonstrates the allele variants of tolerant (1–7) and susceptible (8–14) rice varieties by the trait ‘submergence tolerance’. The tolerant allele has PCR-product with size of 703 bp, the susceptible one has 203 bp amplicon by this locus.

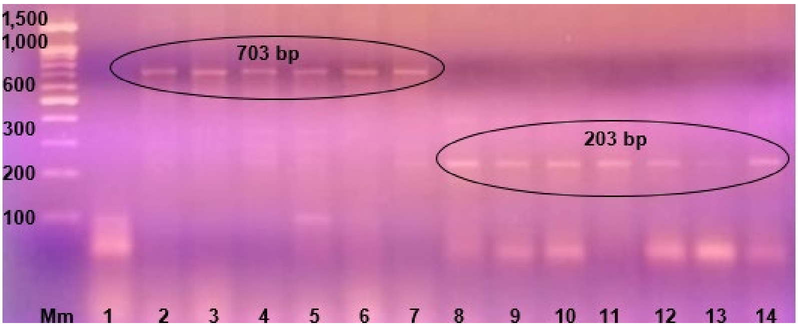

Figure 6. Visualization of amplification products at the PrC3 locus.

Note: Mm – molecular weight marker 100 bp + 1.5 Kb (supplier: comp. Syntol, Russia); 1 – Khan Dan; 2 – TDK-1; 3 – CR 1009; 4 – Swarna; 5 – IR-64; 6 – BR-11; 7 – Inbara-3; 8 – KP-23; 9 – Contact; 10 – Boyarin; 11 – KP-25; 12 – KP-163; 13 – Flagman; 14 – Lenaris.

The figures show that from the studied set of microsatellite markers, high efficiency in identifying the intraspecific polymorphism of the rice varieties and lines used in the work was shown by the two most informative SSR markers - RM 7481 and PrC3. The results were introduced into the breeding program for the development of rice varieties tolerant to prolonged flooding.

To compare molecular genetic studies, phenotypic analysis of parental forms was conducted, as described in the Materials and Methods section. As a result of the experiment, it was noted that the plants of the cultivated domestic rice varieties (maternal form) were elongated. This can be explained the fact that they belong to the *japonica* species, and it is known from literary sources (Vergara et al., 1976; Catling, 1992) that this species is characterized by the presence of genes (SK1/Sk2) which cause increased growth. This mechanism is based on the activation of ethylene accumulation, which reduces the amount of abscisic acid and increases the level of gibberellic acid, which cause increased growth of plants (Steffens et al., 2006; Hattori et al., 2009). For field conditions, this leads to the risk of the lodging of plants when water is discharged, and subsequent plant death.

In rice plants of the donor variety Khan Dan with the *Sub1A* gene, stunted growth was observed - i.e., they were in a state of rest. After 15 days in the full flooding regime, the water from the tubes was drained, and within 2–3 days the plants came out of the stressed state and began vegetation.

Recently, a BC1F2 population was obtained, which was verified by PCR analysis for the existence of the *Sub1A* gene in the genotype (Figs 7, 8).

From 184 plants of the hybrid combination Novator x Khan Dun, 143 plants carrying the target gene were selected, which were sown at the lysimetric site of the FSBSI for assessment by economically valuable traits. The selected plants with the *Sub1A* gene were involved in the backcross with recurrent parental forms. The work on saturating crosses was started with the aim of producing rice lines with a complex of valuable traits of the recurrent parental form, an effective *Sub1A* gene for submergence tolerance, and a vegetation period of up to 125 days.

Fig. 7 shows that hybrid plants No. 64/3, 64/5, 66/2, 66/3, 66/4, 66/5 have a specific allele of the Sub1A gene. Samples No. 64/3, 64/5 are homozygotes for this locus, and No. 66/2, 66/3, 66/4, 66/5 have maternal and paternal alleles. Plants that did not have the Sub1A gene in the genotype as a result of analysis of their DNA were rejected.

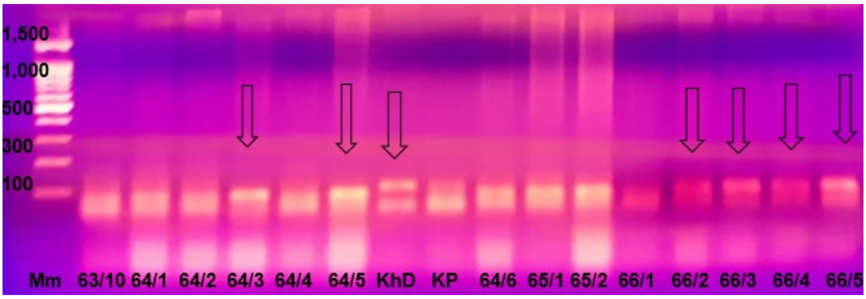

Figure 7. Results of PCR-analysis by RM7481locus.

Note: Mm – molecular weight marker 100 bp + 1.5 Kb (supplier - Syntol, Russia); 63/10–66/5 – hybrid plants; KhD – donor variety Khan Dan; KP – KP-163 line (maternal form;; the arrows show the dominant allele.

The electrophoresis gel in Fig. 7 shows that only the hybrid sample 37/8 had a dominant allele of the *Sub1A* gene in its genotype. The rest of the samples had a recessive allele and were thus rejected.

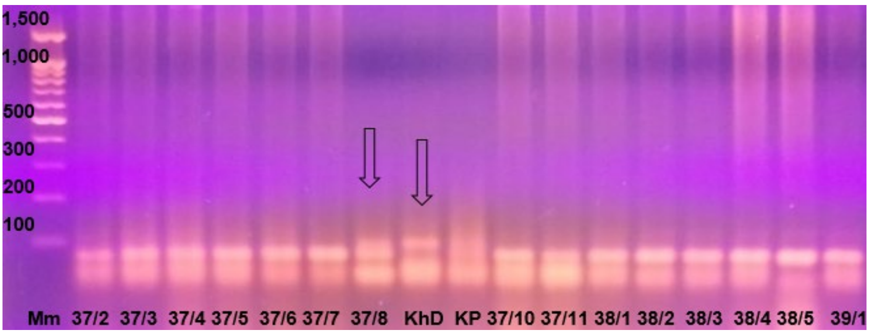

Figure 8. Results of PCR analysis by RM7481 locus.

Note: Mm – molecular weight marker 100 bp + 1.5 Kb (supplier: Syntol, Russia); 37/2–39/1 hybrid plants; KhD – donor variety Khan Dan; KP – KP-23 line (maternal form); the arrows show the dominant allele.

In the hybrids KP-163 × KhanDan and KP-23 × KhanDan, segregation took place in a ratio of 15:5 and 14: 6, respectively, or approximately 3: 1, i.e. close to Mendelian.

The deviations in segregation of the two combinations can be explained by the influence of selection and gene linkage. The best plants were selected to obtain F3-progenies.

The proposed accelerated scheme for the development of a modern set of rice lines carrying the target genes will make it possible to develop varieties that correspond to the environmental conditions of the South of Russia, with increased productivity, milled rice quality, resistance to *P. oryzae* and drought, and submergence tolerance, to combat weeds in the future. The proposed scheme will contribute to the production of environmentally friendly products, saving money for rice producing enterprises, since it will allow for a significant reduction in, if not a complete abandonment of the use of pesticides, which will increase the ecological status of the rice production and economy of the region. Ultimately, it will have a beneficial effect on the health and longevity of the nation.

Development of a modern set of rice lines with genes *Pi-ta, Pi-1, Pi-b, Pi-2, Pi-40*, and *Pi-33*, which controls rice protective reactions against the pathogen *Pyricularia oryzae Cav.*

Breeding rice varieties with increased resistance to blast disease (the most dangerous disease in the world), and introducing these into production, is becoming increasingly necessary.

In the program aimed at pyramiding blast resistance genes in one genotype based on the rice varieties Flagman and Snezhinka, and large grain lines with a short growing period, KP-163 and KP-24-15, in artificial climate chambers and on the growing plot of the FSBSI, about 400 backcross self-pollinated lines of the BC2F3 population with pyramided blast resistance genes ((*Pi-33, Pi-1, Pi-b, Pi-2*); (*Pi-1, Pi-33, Pi-2*); (*Pi-33, Pi-b*); (*Pi-1, Pi-2*)) were propagated.

Assessment of the donor rice lines BL-1 (*Pi-b*), C104-Lac (*Pi-1*), C101-A-51 (*Pi-2*), and C101-Lac (*Pi-1, Pi-33*), and breeding samples for the local population of *Pyricularia oryzae Cav*. was performed at a vegetation site of the FSBSI according to methodological guidelines (Los, 1987). As the susceptible control, we used the rice varieties Volgogradsky and Pobeda 65, and as the resistant control, we used the rice variety Avangard.

The results of assessing the breeding samples for blast resistance and some indicators for economically valuable traits are presented in Table 3.

Table 3. Some characteristics of rice breeding samples with genes *Pi-1, Pi-2, Pi-33, Pi-ta,* and *Pi-b,* grown on the growing plot of FSBSI in 2021

Line name/ origin	Vegetation period, days	Plant height, cm	Mass of 1,000 grains, g	IDD*, %
Flagman (St)	115–117	90–95	26.7–28.4	54.6–70.5
Avangard (St)	115–117	105–110	-	0
1/ Flagman × Bl-1/ Flagman	105–110	75–80	25.4–26.8	20.0
2/ Flagman × IR-36/ Flagman	110–115	80–85	27.8–29.3	12.6
3 /Flagman × C101-A-51	105–110	85–90	27.3–28.4	4.4
4 /Flagman x Bl-1/ Flagman	105–115	75–80	24.4–25.2	23.3
5 /Flagman × Bl-1/ Flagman	105–115	75–80	25.3–27.1	17.6
6 /Flagman × A-51/ Flagman	100–105	85–90	27.5–29.1	7.8
7 /Flagman x Bl-1/ Flagman)	105–110	80–85	25.3–27.3	18.7
8 [Fl × IR-36/ Fl] × [Fl× Bl-1/ F]	112–117	85–90	27.7–29.3	16.4
9/Flagman× A-51/ Flagman	100–105	85–90	28.4–30.1	6.8
10/(Flagman x Bl-1/ Flagman	105–110	80–85	25.6–26.6	17.6
11/Flagman x Bl-1/ Flagman	110–115	80–85	25.5–26.4	25.6
12 /Flagman × A-51/ Flagman	105-110	85–90	29.2–30.4	12.3
13 /Flagman × C101 Lac/ Flagman	110–115	80–85	27.3–28.1	17.4
14/Flagman × C101 Lac/ Flagman	110–115	80–85	28.4–29.2	13.3
15/Flagman× C101 Lac/ Flagman	110–115	80–85	26.9-28.1	10.2
16/Flagman × C101 Lac/ Flagman	110–115	85–90	27.6–28.9	18.5

17/Flagman × C101 Lac/ Flagman	110–115	85–90	27.7–29.3	10.8
19 [(Flagman x C101Lac\ Flagman)] × [(Flagman × A-51 \ Flagman)]	115–117	80-85	29.3–30.2	6.8
20 Flagman x Bl-1/ Flagman	110–115	80–85	25.4–27.3	25.6
21 [(Flagman x C101Lac\ Flagman)] × [(Fl. × A-51 \ Fl.]	115–117	80–85	27.8–29.2	5.6
22 [(Flagman x C101Lac\ Flagman)] × [(Flagman × A-51 \ Flagman)]	115–117	80–85	28.8–30.1	6.4
23 Flagman × IR-36/ Flagman	118–120	75–80	29.8–30.1	15.6
24 [(Flagman x IR-36\ Flagman)] × [(Flagman ×C101) \ Flagman]	115–117	80–85	28.6–30.1	12.3
25 [(Flagman x IR-36\ Flagman)] × [(Flagman ×C101) \ Flagman]	115–117	80–85	28.7–30.3	11.7
26 [(Flagman x IR-36\ Flagman)] × [(Flagman ×C101) \ Flagman]	115–117	85–90	23.2–25.4	11.5
27 [(Flagman x IR-36\ Flagman)] × [(Flagman×C101) \ Flagman]	115–117	85–90	23.5–25.5	10.8
28 Flagman x Bl-1\ Flagman	110–115	85–90	26.6–29.3	27.5
29 Flagman × A-51/ Flagman	115–120	85–90	28.3–30.3	12.1
30 Flagman x IR-36\ Flagman	115–117	77–80	30.1–30.4	15.6
31 Flagman x C101Lac/ Flagman	110–115	85–90	27.3–28.3	17.8
C101Lac-A-51 (*Pi-2*)	-	-	-	0.0
Bl-1 (*Pi-b*)	-	-	-	8.4
C101Lac	-	-	-	2.0
IR-36 (*Pi-ta*)	-	-	-	1.2
7-5-1 *(Pi-40)*	-	-	-	0.0

∗IDD – intensity of disease development; St – standard rice varieties; Avangard (St) – resistant rice variety.

Figs 9 and 10 show some results of the PCR analysis for the identification of the above-mentioned blast resistance genes in the hybrid material.

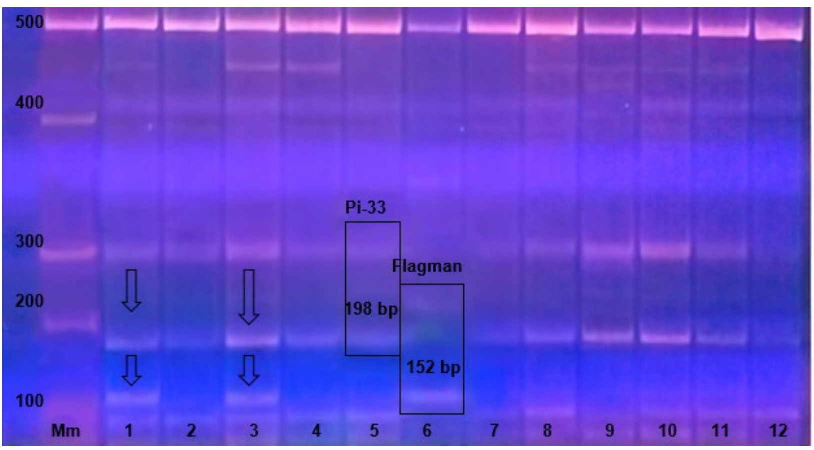

Figure 9. Electropherogram of genomic DNA amplification products of loci RM310 and RM72.
Note: 1–4, 7–12 – analyzed hybrid plants; 5 – donor line C101-Lac of the Pi-33 gene, which determines that the resistance gene is 198 bp; in varieties with a recessive allele, this is 152 bp; Flagman – recurrent parental form.

Fig. 9 shows that plants 2, 4, and 7–12 were homozygous for the dominant allele; plants 1 and 3 were heterozygotes.

Fig. 10 shows that plants 2, 7, and 9 were homozygous for the *Pi-b* gene.

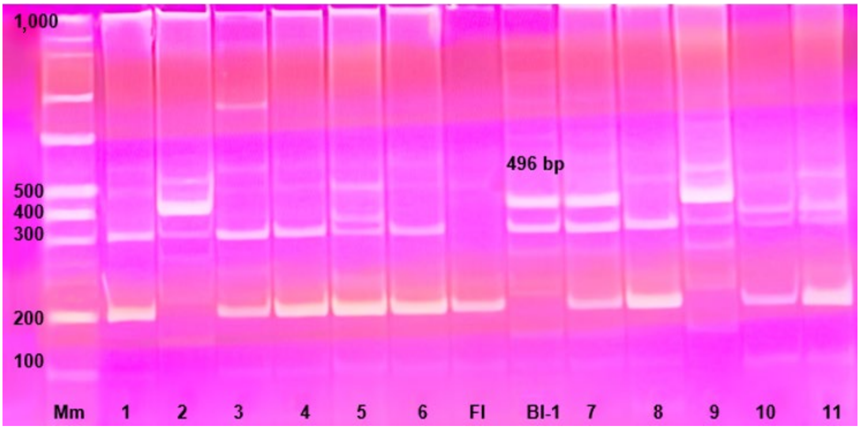

Figure 10. Electropherogram of genomic DNA amplification products of the *Pi-b* locus.

Note: 1–11 – analyzed hybrid plants; Fl – rice variety Flagman (maternal form); Bl-1 – line donor Bl-1 of the *Pi-b* gene, which determines that the resistance gene is 496 bp.

Thus, the results of PCR analysis (Fig. 10) show that samples 2 and 9 had four blast resistance genes in their genotypes (*Pi-1, Pi-2, Pi-33, Pi-b*); samples 4, 8 and 11 had three resistance genes (*Pi-1, Pi-2, Pi-33*); sample 7 had the genes *Pi-33* and *Pi-b*; sample 6 had genes *Pi-1* and *Pi-2*; samples 10 and 12 had the *Pi-33* gene.

The plant forms which had donor resistance alleles in their genotypes were selected and then sown in the field for evaluation by economically beneficial traits. Some of the results of this evaluation are presented in Table 3.

The data from the Table 3 demonstrate that the obtained plants have vegetation period of 110–120 days that corresponds growing conditions in the South of Russia. They have plant height of between 75 cm (undersized plants) and 90 cm (medium-sized), don't lie down and have the mass of 1,000 grains of between 25 g (medium-grained) and 30 g (coarse-grained). The resistance to blast rate is between 4.4 and 20% (for resistant plants) and between 25–27% (for susceptible plants).

Currently, samples with target genes in their genotypes are being studied in the field for agricultural valuable traits, and we also plan to conduct an assessment of blast resistance at test sites of a rice irrigation system after using a predecessor of perennial grasses. The best ones will be selected for further study. This will make it possible to develop varieties appropriate to the environmental conditions of the South of Russia. Increased productivity and blast resistance are two key traits they should possess. Rice resistance to diseases plays an important role in grain production, as it allows rice to be grown without the use of toxic fungicides, which would improve the ecological impact of the rice industry and the economy of the region.

Development of rice lines with complex resistance to blast and submergence tolerance

To diversify the gene pool of rice with complex blast resistance and submergence tolerance, we crossed rice lines with the genes *Pi-2* and *Pi-33* with the Khan Dan variety (*Sub1A*) to obtain breeding material with united genes for submergence tolerance and resistance to disease. The F_2 and BC_1F_2 generations were obtained using climatic chambers.

In order to increase the efficiency of MAS (marker-assisted selection), we developed multi-primer systems for the identification of two target genes at the same time. A protocol of DNA analysis was designed where the specific PCR products of target genes were visualized clearly.

Fig. 11 demonstrates the results of DNA marker systems for the simultaneous identification of two genes: *Pi-2* and *Sub1A*.

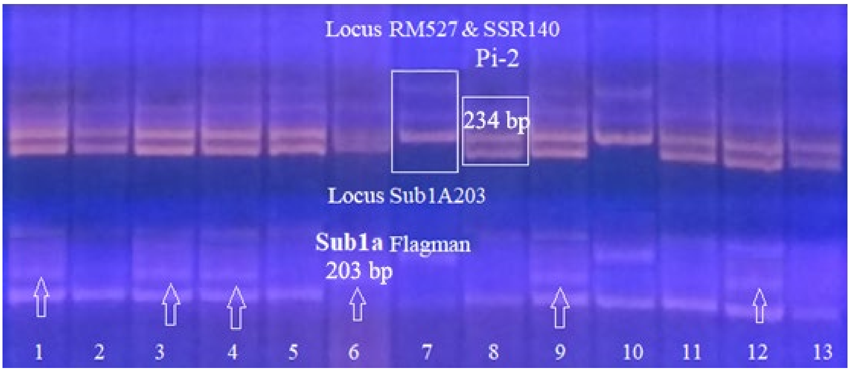

Figure 11. Results of visualization of PCR products of loci RM527 and SSR140 for Pi-2, showing that this resistance gene is 234 bp; and the products of the Sub1A203 locus for Sub1A, which show that this resistance gene is 203 bp in 8% PAAG.

Note: 1–4, 7–13 – analyzed hybrid plants of BC1F2 generation; 6 – rice variety Khan Dan, donor of the Sub1A gene; 7 – Flagman, recurrent parental form.

Fig. 11 shows that samples 3 and 12 had dominant alleles of the *Pi-2* and *Sub1A* genes in a homozygous state in their genotypes; samples 1, 4, 9, and 12 were homozygotes for the *Sub1A* gene and had *Pi-2* in their genotypes in a heterozygous state; sample 10 was recessive homozygous for the two target genes and was thus rejected. Clear identification on the electropherogram made it possible to reliably identify the presence of the dominant alleles of target genes.

The involvement of such varieties in rice production will help avoid the epiphytotic development of disease, preserving rice yields and obtaining environmentally friendly agricultural products.

Thus, through MAS (breeding with the use of molecular markers), an important national economic problem can be solved - namely, rice varieties with increased resistance to blast and high yields can be developed.

The identification of effective specialized DNA markers which provide clear control over the inheritance of the target locus was performed. Of the investigated set of microsatellite markers based on tolerance to long-term flooding, four of the most informative SSR markers, RM 7481, PrC1, Gns2, and PrC3, showed high efficiency in identifying the intraspecific polymorphism of the rice varieties and lines used in the work. The identities of the microsatellite markers RM 7481, PrC1, Gns2, and PrC3, linked to the trait 'tolerance to long-term flooding', were established. The results were introduced into the breeding program for the development of rice varieties tolerant to prolonged flooding (for weed control).

On the basis of the selected informative SSR markers, closely linked with the trait 'tolerance to prolonged flooding', clear genotype marking of the analyzed hybrid rice plants for this trait was carried out, which was confirmed by phenotype assessment during laboratory testing. Plants that had a target gene in a hetero- or homozygous state were selected.

Within the framework of the program for development of rice genetic resources resistant to biotic stressors (blast), about 400 backcross self-pollinated rice lines with introgressed and pyramided genes for resistance to *P. oryzae Cav.* (*Pi-1, Pi-2, Pi-33, Pi-ta, Pi-b,* and *Pi-40*) were obtained. A test for blast resistance and an assessment of the economically valuable traits of experimental plants with introgressive and effective *Pi* genes for the south of Russia were performed. Promising samples were selected.

To increase economic efficiency and to reduce labor costs, multi-primer systems were developed to identify two genes (*Pi and Sub1A*) in a hybrid material simultaneously. The optimal conditions for the amplification reaction were selected, by which the PCR products were clearly visualized.

Samples of F2 and BC1F1 generations with combined genes for blast resistance (*Pi*) and tolerance to long-term flooding (for weed control) (*Sub1A*) in homo- and heterozygous states were obtained, which was confirmed by DNA analysis. The conducted testing of the obtained rice breeding samples for resistance to prolonged flooding under experimental laboratory conditions allowed for the selection of tolerant rice forms in which the breeding processes can be studied according to the complex of economically valuable traits. Their use will reduce the application of plant protection chemicals against diseases and weeds, thereby increasing the ecological status of the rice industry.

CONCLUSIONS

1. As a result of the studies carried out using molecular marking based on PCR in combination with traditional breeding, early maturing rice lines with genes for resistance to flooding Sub 1A, suitable for cultivation in the south of Russia, were isolated.
2. Rice lines have been developed, the genotype of which contains effective blast resistance genes (*Pi-1*, *Pi-2*, *Pi-33*, *Pi-ta*, *Pi-b*). The intro-duction of such varieties into production will allow avoiding the epiphytotic development of the disease, preserving the biological productivity of rice and obtaining environmentally friendly agricultural products.
3. Samples of the F2 and BC1F2 generations were obtained with com-bined blast resistance (Pi) and prolonged flooding tolerance (*Sub1A*) genes as a factor in the control of weeds in homo- and heterozygous state, which is confirmed by the data of their DNA

analysis. The testing of the obtained rice breeding resources for resistance to prolonged flooding under laboratory conditions made it possible to select tolerant rice forms that will be studied in the breeding process for a complex of agronomically valuable traits.

The proposed accelerated scheme for the development of a modern set of rice lines carrying target genes will make it possible to breed varieties that correspond to the agro-climatic conditions of the South of Russia, with increased productivity, cereal quality, resistance to blast, drought, submergence tolerance as a factor in weed control in the future. This will contribute to the production of environmentally friendly products, to save money for rice-producing enterprises, as it will allow, if not completely but to significantly reduce the use of pesticides, that will increase the ecological status of the rice growing industry and improve the economy of the region. Ultimately, this will have a beneficial effect on the health and longevity of the nation.

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REFERENCES

Azarin, K.V., Usatov, A.V., Alabushev, A.V., Kostylev, P.I., Makarenko, M.S. & Kovalevich, A.A. 2016. Validation of SSR markers associated with Submergence Tolerance in Rice (Oryza sativa L.). *American Journal of Agricultural and Biological Sciences* **11**(4), 142–147. doi: 10.3844/ajabssp.2016.142.147

Azarin, K.V., Usatov, A.V. & Kostylev, P.I. 2017. Breeding of flood-tolerant rice (review). *Rice growing* **4**(37), 36–42 (in Russian).

Catling, H.D. 1992. *Rice in deep water*. International Rice Research Institute, Manila, Philippines. Macmillan, London, 542 pp.

Choi, H.C., Kim, Y.G., Hong, H.C., Hwang, H.G., Ahn, S.N., Han, S.S., Ryu, J.D. & Moon, H.P. 2006. Development of blast resistant rice multi-line cultivars and their stability to blast resistance and yield performance. *Korean J. Breed*. **38**, 83–89.

Collard, B.C.Y., Septiningsih, E. Das, S., Carandang, J., Pamplona, A., Sanchez, D., Kato, Y, Ye, G., Reddy, J., Singh, U., Iftekharuddaula, K., Venuprasad, R., Vera-Cruz, C.N., Mackill, D. & Ismail, A. 2013. Developing new flood-tolerant varieties at the International Rice Research Institute (IRRI). *SABRAO Journal of Breeding and Genetics* **45**(1), 42–56.

Dospekhov, B.A. 1979. *Methods of field experiment (with the basics of statistical processing of research results).* Ed. 4th, revised and enlarged Moscow, Kolos, 416 pp. (in Russian).

Dubina, E.V., Mukhina, Zh.M., Kharithonov, E.M., Shilovskiy, V.N., Kharchenko, E.S., Esaulova, L.V., Korkina, N.N., Maximenko, E.P. & Nikitina, I.B. 2015. Development of blast resistant rice germplasm using the technology of DNA-marking. *Genetics* **51**, 881–886.

Dubina, E.V., Shilovsky, V.N., Kostylev, P.I., Makukha, Yu.A, Ruban, M.G., Balyasnyi, I.V, Ham, Le.H., Tu, D.X. & Linh, Le. H. 2017. Sub1A gene in rice breeding for tolerance to flooding, as a fact of weeds control. *Rice growing* **35**, 20–26 (in Russian).

Dubina, E.V., Alabushev, A.V., Kostylev, P.I., Makukha, Yu.A, Ruban, M.G. Balyasnyi, I.V., Ham, Le.H., Tu, D.X. & Linh, Le.H. 2018. Introduction of the Sub1 gene into the Russian rice varieties using the polymerase chain reaction (PCR) methods. *African J. of Agr. Res.* **13**, 2757–2762.

Dubina, E.V. 2019. *DNA-technologies (molecular marking) in rice breeding and vegetable crops seed production*. PhD Thesis (Biology). All-Russian Rice Research Institute, Krasnodar, Russia, 275 pp. (in Russian).

Fukao, T., Yeung, E. & Bailey-Serres, J. 2011. The submergence tolerance regulator sub1a mediates crosstalk between submergence and drought tolerance in rice. *The Plant Cell*. **23**, 412–427.
Hattori, Y., Nagai, K., Furukawa, S.X., Song, X.J., Kawano, R. Sakakibara, H., Wu, J.Z., Matsumoto, T., Yoshimura, A, Kitano, H., Matsuoka, M., Mori, H. & Ashikari, M. 2009. The ethylene response factors Snorkel1 and Snorkel2 allow rice to adapt to deep water. *Nature* **460**, 1026–1031.
Ito, O., Ella, E. & Kawano, N. 1999. Physiological basis of submergence tolerance in rainfed lowland rice ecosystem. *Field Crops Research*, **64**, 75–90.
Jena, K.K., Moon, H.P. & Mackill, D.J. 2003. Marker assisted selection – a new paradigm in plant breeding. *Korean J. Breed*. **35**, 133–140.
Kende, H., Knaap, E. & Cho, H.T. 1998. Deepwater Rice: A Model Plant to Study Stem Elongation. *Plant Phys*. **4**, 1105–1110.
Kolomiets, T.M. 1990. *Selection of the rice starting material for immunity to blast*. Abstract of PhD thesis. Institute of Phytopathology, Moscow, 21 pp. (in Russian).
Kostylev, P.I, Redkin, A.A., Krasnova, E.V., Dubina, E.V. & Mukhina, Zh.M. 2015. Perspectives of using rice with Sub1 resistant to prolonged flooding in breeding Russian varieties. *Grain Economy of Russia* **4**(40), 37–42 (in Russian).
Kovalenko, E.D., Gorbunova, Yu.V. & Kovaleva, A.A. 1988. *Methodical guidelines for assessing the resistance of rice varieties to blast causative agent*, 30 pp. (in Russian).
Kumar, V. & Bhagwat, S.G. 2012. Microsatellite (SSR) Based Assessment of Genetic Diversity among the Semi-Dwarf Mutants of Elite Rice Variety WL112. *Int. J. of Plant Breed. and Gen.* **6**, 195–205.
Linh, T.H, Linh, L.H, Cuc, D.T.K., Ham, L.H. & Khanh, T.D. 2013. Improving submergence tolerance of Vietnamese rice cultivar by molecular breeding. *J. Plant Breed. Genet*. **1**(03), 157–168.
Los, G.D. 1987. A promising method for rice hybridization. *Agricultural biology* **12**, 15–17 (in Russian).
Mackill, D.J. & Ni, J. 2001. Molecular mapping and marker-assisted selection for major-gene traits in rice. *Rice genetic*. *Proceeding of the fourth international rice genetic symposium.* Los Banos, pp. 137–151.
Manangkil, O.E., Vu, H.T.Th., Yoshida, Sh., Mori, N. & Nakamura, Ch. 2008. A simple, rapid and reliable bioassay for evaluating seedling vigor under submergence in indica and japonica rice (Oryza sativa L.). *Euphytica* **163**, 267–274. doi: 10.1007/s10681-008-9645-1
Mickelbart, M.V., Hasegawa, P.M. & Bailey-Serres J. 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Nat Rev Genet*. **16**(4), 237– 251.
Murray, M.G. 1980. Rapid isolation of high molecular weight plant DNA. *Genomt* **40**, 379–378.
Negrao, S., Courtois, B., Ahmadi, N. & Abreu, I.A. 2011. Recent updates on salinity stress in rice: from Physiological to molecular responses. *Crit. Rev. in Plant Sci*. **30**, 329–377.
Podkin, O.V.1981. *Guidelines for the identification, accounting and methods of developing measures to combat rice diseases*. All-Russian Rice Research Institute, Krasnodar, Russia, 20 pp. (in Russian).
Practical guidance on intensive rice cultivation technology in Krasnodar region. 1986. All-Russian Rice Research Institute, Krasnodar, Russia, 38 pp. (in Russian).
Septiningsih, E.M. & Kretzschmar, T. 2015. Anaerobic germination-tolerant plants and related materials and methods. WO 2015087282 A1.
Singh, S., Mackill, D.J. & Ismail, A.M. 2009. Responses of Sub1 rice introgression lines to submergence in the field: yield and grain quality. *Nature Publ. Group*, 12–23.
Skazhennik, M.A., Vorobiev, N.V. & Doseeva, O.A. 2009. *Methods of physiological research in rice growing*. Methodical recommendations, All-Russian Rice Research Institute, Krasnodar, Russia, 23 pp. (in Russian).

Steffens, B., Wang, J. & Sauter, M. 2006. Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice. *Plants* **5**, 604–612.

Vergara, B.S., Jackson, M.B. & De Datta, S.K. 1976. *Deepwater rice and its response to deep-water stress*. In: Climate and Rice. International Rice Research Institute, Los Banos, Philippines, 301 pp.

Xu, K. & Mackill, D.J. 1996. A major locus for submergence tolerance mapped on rice chromosome 9. *Mol. Breed*. **2**, 219–224.

Xu, K., Xia, X., Fukao, T., Canlas, P., Maghirang-Rodriguez, R., Heuer, S., Ismail, A.I., Bailey-Serres, J., Ronald, P.C. & Mackill, D.J. 2006. Sub1A is an ethylene-response-factor like gene that confers submergence tolerance to rice. *Nature* **4**(42), 705–708.

Xu, K., Xu, X., Ronald, P.C. & Mackill, D.J. 2000. A high-resolution linkage map in the vicinity of the rice submergence tolerance locus Sub1. *Mol. Gen. Genet*. **2**(63), 681–689.

Zelenskiy, G.L. 2016. *Rice: the biology basis in the breeding and the agricultural technology*. Monography, KSAU, 238 spp.

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Content of biologically active substances in sweet cherry fruits at different stages of fruit development in the conditions of the living mulch

T. Gerasko[1,*], S. Pyda[2], Yu. Paschenko[1], I. Ivanova[1], L. Pokoptseva[1] and T. Tymoshchuk[3]

[1]Dmytro Motornyi Tavria State Agrotechnological University, Department of Plant Production and Horticulture, B. Khmelnitsky Av., 18, UA72310 Melitopol, Zaporizhia Region, Ukraine
[2]Volodymyr Hnatyuk Ternopil National Pedagogical University, Department of Botany and Zoology, Maksyma Krivonosa Str., 2, UA46000 Ternopil, Ternopil Region, Ukraine
[3]Polissia National University, Faculty of Agronomy, Department of Health of Phytocenoses and Trophology, 7, Staryi Blvd, UA10008 Zhytomyr, Ukraine
[*]Correspondence: tetiana.herasko@tsatu.edu.ua

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Abstract. Content of soluble solids, sugars, titrated acids, ascorbate, glutathione, phenolic substances, anthocyanins, total reducing activity of fruits tissues in sweet cherry fruits studied at different stages of fruit development during 2018 and 2019 in an organic sweet cherry orchard (*Prunus avium L. / Prunus mahaleb*) in the Southern Steppe of Ukraine. The aim of the research was to determine how the living much conditions (compared to bare fallow) affect the content of biologically active substances in sweet cherry fruits at different stages of ripening. It was determined that the fruits of sweet cherry accumulated significantly more ascorbate, phenolic substances and anthocyanins in the conditions of living mulch (compared to the fruits of the trees on bare fallow). So, at the stage of picking ripeness, the content of ascorbate in sweet cherry fruits in the conditions of living mulch was 29 and 22% more compared to bare fallow (respectively, in 2018 and 2019), phenolic substances - by 47 and 23%, anthocyanins - by 36 and 26%. The revealed regularities can be explained by stressful conditions of competition with natural herbs, which activate the synthesis of anti-stress, antioxidant biologically active substances in plant tissues (including fruits). Since it is the antioxidants of the fruits that have a physiological value for humans, it can be stated that the fruits grown in the conditions of living mulch have a higher therapeutic and preventive value than the fruits grown on bare fallow.

Key words: sweet cherry; living mulch; sugars, titrated acids, ascorbate, glutathione, phenols.

INTRODUCTION

All over the world, sweet cherries are valued not only for their taste, but also for their antioxidant properties, which help people with the treatment of many diseases, including diabetes, obesity, Alzheimer's disease, hypertension, cancer (Gonçalves et al., 2019; Fonseca et al., 2021). Organic sweet cherry fruits contain significantly more sugars, anthocyanins, flavonoids, compared to conventional fruits (Hallmann & Rozpara, 2017). Mulch is recognized as the most soil-saving practice in horticulture, especially for organic orchards, as it protects the soil from water and wind erosion (Gomez et al., 2008; Fidalski et al., 2010; Atucha et al., 2013) creates optimal conditions for soil microbiota (Yao et al., 2005; Zheng et al., 2018; Culumber et al., 2019), lowers soil temperature in summer and insulates soil in winter (Gu et al., 2016; Gerasko et al., 2021), retains soil moisture (due to the increase of organic matter and, accordingly, increases the water holding capacity of the soil) (Palese, 2014; Simões et al., 2014; Zheng et al., 2018). Thus, applying living mulch as an orchard floor management system meets the goal of leaving fertile soil for future generations and a strategy for the development of sustainable agriculture (Holden et al., 2017). But the emergence of sustainable agriculture requires the efforts of not only the farmers but also the scientists through the creation of a scientific base, namely, the need to determine the impact of living mulch on the physiological processes of fruit trees in detail, in order to have a clear idea of the 'pros' and 'cons' for producers. Regarding the effect of living mulch on the physiological state of fruit trees, and on the biochemical composition of fruits, the data is contradictory: according to E.E. Sánchez et al. (2007), living mulch improves the quality of the fruits of *Malus domestica Borkh.* in northern Patagonia and increases biologically active substances content; A. Atucha, I.A. Merwin & M.G. Brown (2011) indicate a decrease in fruit quality under the influence of living mulch; the results of studies by G.H. Neilsen et al. (2014) indicate no effect of living mulch on fruit quality. The physiological state of plants depends on their ability to withstand various stresses (Anjum et al., 2010). Using cover crops in orchards can create additional stress for fruit trees due to the competition with grasses (Atucha et al., 2011). Long-term research indicates that over time, trees overcome competition with grasses (Merwin, 2010; Atucha et al., 2011), but how this process works, what changes occur in the physiological parameters of fruit trees, is not yet definitively clarified. Information on the content of biologically active substances in sweet cherry fruits under living mulch conditions will help to understand the physiological changes that occur in the tissues of fruit trees and how they manage to overcome competition with herbs.

The aim of this research was to determine how the living mulch conditions affect the content of sugars, titrated acids, ascorbate, glutathione, phenolic substances, anthocyanins in sweet cherry fruits at different stages of ripening.

MATERIALS AND METHODS

The field experiment was set up in the research orchard of the Tavria State Agrotechnological University (Zelene village, Melitopol district, Zaporizhzhya region: 46 °46 'N, 35 °17 'E). The soil of the experimental site is chestnut, sandy, with light mechanical composition. Soil solution has a slightly alkaline reaction (pH ranges from 7.1 to 7.4). The humus content in the upper layer is 0.6%, the total water-soluble salt content is 0.015–0.024%. Mineral nitrogen was not detected, the content of P_2O_5 is 5.4; K_2O - 6.5 mg kg^{-1} of the soil. Sweet cherry is a traditional fruit crop for this region and has excellent fruit quality on such poor sandy soils.

The research site is located in the Southern Steppe of Ukraine, which climate is characterized as arid and very warm. Weather conditions in the years of the research (2018, 2019) had higher mean annual air temperature than long-term data by 1.3–1.6 °C. April of 2018 was warm and very dry. The drought also lasted in May and June of 2018. July of 2018 was satisfactory in terms of precipitation, but August this year was very dry as well (the amount of precipitation was 82% lower than the long-term average). The heavy rainfall in September 2018 was followed by draught in October (the amount of precipitation was 44% less than the long-term average). April and May of 2019 were satisfactory in terms of moisture supply (the amount of precipitation was 44 and 107% higher than the long-term average, respectively). But June of 2019 was abnormally hot and dry. In general, it can be stated that the conditions of fruit ripening (from the stage of petals falling to the picking ripeness) in 2018 were abnormally arid, and in 2019 the period of fruit ripening was satisfactory in terms of moisture.

The research was carried out sweet cherry trees of 'Dilemma' cultivar grafted on *Prunus mahaleb* seedlings, planted in 2011 (7×5 m). 'Dilemma' cultivar is medium-early, in the conditions of Melitopol ripens in early June, is used for fresh consumption. The fruits are convex-heart-shaped, the skin and flesh are dark red, have excellent sour-sweet taste.

The experiment was designed as a randomized complete block with two variants, in three replications (10 control trees per replication). Orchard floor management system in the experimental plot had two variants: bare fallow (tilling to a depth of 15 cm, manual weeding) and living mulch (natural grasses, mowing, mown mass remained in place). The rest of the technological operations in the orchard were identical in both variants. Synthetic fertilizers and chemical plant protection products were not used.

Fruit samples collection for analysis was performed during their ripening (May-early June) in the following stages: petal fall, stone hardening, partial reddening, picking maturity. In order to determine the biochemical composition, 30 intact fruits were selected in four replications from each variant of the experiment.

The collected data included: the content of soluble solids (SS, %), sugars (Sug, %), titrated acids (TA, %), phenolic substances (Phen, mg GA 100 g^{-1}), anthocyanins (Ant, mg 100 g^{-1}), ascorbic acid (AsA, mg 100 g^{-1}), glutathione (Glu, mg 100 g^{-1}) and the total reducing activity of fruits tissues (TRA, mL KIO_3 100 g^{-1}) in sweet cherry fruits.

The content of soluble solids and titrated acids was determined in accordance with the Methods of determining the quality of crop products (Methods…, 2021) by desktop refractometer IRF-451 62M.

Determination of the number of sugars (%) in fruits tissues was performed photometrically based on the ability of monosaccharides to reduce picric acid (2,4,6-trinitrophenol) to picramic acid, where the reaction product has an intense red colour. The calibration graph was prepared by glucose. The optical density was determined at a wavelength of 490 nm (Workshop …, 2001).

The total content of phenolic substances was determined photometrically using Folin-Chokalteu reagent (Yaman, 2022a; Yaman, 2022b). The optical density of the mixture was measured at a wavelength of 765 nm, which corresponds to the concentration of phenolic substances in terms of gallic acid (GA). Total amount of phenolic compounds was expressed in mg of gallic acid per 100 g of fresh mass (mg GA 100 g^{-1}).

Analysis of the anthocyanin content was performed as described by Francis (1982): 1 g of crushed tissue was suspended in 10 mL of 1.5 N HCl solution in 85% ethanol, transferred to a 50 mL volumetric flask and extracted for 13 hours in the refrigerator in the dark. The extracts were filtered (Whatman filter paper #1) and the absorbance was measured at λ = 535 nm. Determination of the content of ascorbic acid, glutathione and the total reducing activity of fruits tissues was performed on the reducing properties of ascorbate and glutathione, as described by Gorodniy (2006): a portion of fresh mass (2 g) was thoroughly grounded in a mortar with 8–10 mL of 5% HPO_3 solution, quantitatively transferred to a 50 mL volumetric flask, bringing the contents to the mark with distilled water. After shaking for 2–3 minutes, filtered through a dry folded filter into a dry flask. 5 mL of the filtrate was titrated with 0.001 n Tilman's paint solution (2,6-dichlorophenolindophenol) to a faint pink colour that does not disappear for 60 s. Simultaneously, the other 5 mL of filtrate (after adding 2–3 drops of 15% KI solution and 5 drops of 1% starch solution) was titrated with 0.001 n KIO_3 solution to a light blue colour, stable for 60 s.

For all analyses, the repeatability was trifold. The results were compared on the Tukey's mean separation test at a significance level of $P \leq 0.05$ and were processed by Pearson's correlation analysis using Minitab 19 software (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

The results of our research showed that the content of soluble solids in sweet cherry fruits ranged from 11.89 to 19.56% under bare fallow conditions and from 14.15 to 19.99% under living mulch conditions (Tables 1 and 2). Soluble solids content was significantly higher under living mulch conditions compared to bare fallow: by 19% in 2018 in the stage of petal fall, by 18% in the stage of partial reddening of fruits; in 2019 in the stages of petal fall, hardening of the stone and partial reddening of the fruits - respectively by 19, 20 and 18%. During fruit ripening, the content of soluble solids in the fruits gradually increased and became the highest in the stage of picking ripeness. Correlation analysis showed a strong direct positive correlation between soluble solids content and the content of sugars, phenolic substances and anthocyanins in sweet cherry fruits in both 2018 and 2019 (Table 3). In 2019, compared to 2018, the content of soluble solids decreased by 6% under the conditions of living mulch, by 12% - under bare fallow conditions.

Sugar content in sweet cherry fruits increased during ripening and ranged from 9.43 to 14.08% for bare fallow orchard floor management system and from 11.22 to 15.05% for living mulch. During the stages of petal fall and stone hardening during the two years of research and partial reddening of the fruits in 2019, a significant difference remained between the variants of the experiment - sugar content in the fruits was significantly higher for living mulch (by 18–37%). However, in the stage of picking ripeness, no significant difference in sugar content in the fruits between the variants of the experiment was observed (although in the conditions of living mulch this index tended to increase). The content of sugars in the fruits correlated the most with the content of phenolic substances and anthocyanins. In 2019, compared to 2018, the content of sugars in cherries in bare fallow conditions remained at the same level, under living mulch conditions - increased by 6%.

Table 1. Phytochemical composition of sweet cherry fruits under different orchard floor management systems in 2018, $\overline{M} \pm m$

Variant	SS, %	Sug, %	TA, %	Phen., mg (100 g)$^{-1}$	Ant., mg (100 g)$^{-1}$	AsA, g (100 g)$^{-1}$	Glu., mg (100 g)$^{-1}$	TRA, mL KIO_3 (100 g)$^{-1}$
Petal fall								
Bare fallow	13.20 ± 0.54	9.43 ± 0.24	1.01 ± 0.02	3.4 ± 0.11	0	10.1 ± 0.21	23.0 ± 0.15	7.5 ± 0.04
Living mulch	15.71 ± 0.33*	11.22 ± 0.27*	1.23 ± 0.02*	5.3 ± 0.12*	0	12.1 ± 0.28*	26.1 ± 0.22*	8.5 ± 0.03*
Hardening of the stone								
Bare fallow	14.05 ± 0.57	10.01 ± 0.39[a]	0.83 ± 0.03[a]	18.7 ± 0.35[a]	0	15.4 ± 0.34[a]	27.6 ± 0.34[a]	9.1 ± 0.18[a]
Living mulch	16.80 ± 0.69	12.22 ± 0.35*[a]	1.05 ± 0.02*[a]	26.4 ± 0.38*[a]	0	17.6 ± 0.35*[a]	29.2 ± 0.25*[a]	9.5 ± 0.20[a]
Partial reddening								
Bare fallow	15.90 ± 0.44[a]	11.36 ± 0.37[a]	0.78 ± 0.02[a]	59.1 ± 0.43[a]	4.93 ± 0.04	7.9 ± 0.48[a]	23.8 ± 0.53[a]	7.8 ± 0.08[a]
Living mulch	18.76 ± 0.49*[a]	13.40 ± 0.43*[a]	0.92 ± 0.03[a]	75.8 ± 0.44*[a]	5.95 ± 0.03*	9.5 ± 0.49*[a]	24.6 ± 0.41[a]	8.0 ± 0.09
Picking ripeness								
Bare fallow	19.56 ± 0.67[a]	13.93 ± 1.21[a]	0.64 ± 0.06[a]	67.1 ± 0.72[a]	7.36 ± 0.04[a]	7.3 ± 0.58	21.5 ± 0.42[a]	7.2 ± 0.09[a]
Living mulch	19.99 ± 0.22[a]	14.25 ± 1.27	0.72 ± 0.07[a]	98.9 ± 0.80*[a]	10.12 ± 0.23*[a]	9.4 ± 0.71*	22.3 ± 0.46[a]	7.3 ± 0.09[a]

*– significant difference between the variants at $P \leq 0.05$; [a] – significant difference at $P \leq 0.05$ in comparison with the previous stage of organogenesis. Phen: phenolic substances, GA: gallic acid.

Table 2. Phytochemical composition of sweet cherry fruits under different orchard floor management systems in 2019, $\overline{M} \pm m$

Variant	SS, %	Sug, %	TA, %	Phen., mg (100 g)$^{-1}$	Ant., mg (100 g)$^{-1}$	AsA, mg (100 g)$^{-1}$	Glu., mg (100 g)$^{-1}$	TRA, mL KIO_3 (100 g)$^{-1}$
Petal fall								
Bare fallow	11.89 ± 0.55	9.52 ± 0.46	1.09 ± 0.03	4.1 ± 0.23	0	13.6 ± 0.33	15.4 ± 0.23	5.2 ± 0.14
Living mulch	14.15 ± 0.54*	11.33 ± 0.48*	1.30 ± 0.03*	6.4 ± 0.29*	0	13.2 ± 0.35	17.9 ± 0.22*	6.1 ± 0.24*
Hardening of the stone								
Bare fallow	12.61 ± 0.68	10.11 ± 0.69	0.90 ± 0.02[a]	22.8 ± 0.45[a]	0	16.8 ± 0.34[a]	19.0 ± 0.16[a]	6.5 ± 0.45[a]
Living mulch	15.14 ± 0.74*	12.12 ± 0.65*	1.08 ± 0.04*[a]	32.1 ± 0.48*[a]	0	19.4 ± 0.56*[a]	22.1 ± 0.21*[a]	7.2 ± 0.39*[a]
Partial reddening								
Bare fallow	14.32 ± 0.85[a]	11.47 ± 0.64	0.84 ± 0.02[a]	72.7 ± 0.65[a]	7.65 ± 0.38	10.6 ± 0.32[a]	22.4 ± 0.53[a]	7.3 ± 0.21[a]
Living mulch	16.90 ± 0.91*[a]	13.53 ± 0.71*	1.02 ± 0.03*[a]	92.4 ± 0.59*[a]	9.21 ± 0.35*	10.3 ± 0.58[a]	24.5 ± 0.57[a]	7.5 ± 0.24
Picking ripeness								
Bare fallow	17.21 ± 0.67[a]	14.08 ± 1.14	0.73 ± 0.06[a]	89.3 ± 0.72[a]	12.05 ± 0.19a	8.4 ± 0.59[a]	20.4 ± 0.55[a]	6.7 ± 0.47[a]
Living mulch	18.75 ± 1.18[a]	15.05 ± 1.18	0.75 ± 0.04[a]	110.1 ± 0.75*[a]	15.23 ± 0.33*[a]	10.2 ± 0.87*	21.5 ± 0.61[a]	7.2 ± 0.55[a]

*– significant difference between the variants at $P \leq 0.05$; [a] – significant difference at $P \leq 0.05$ in comparison with the previous stage of organogenesis. Phen: phenolic substances, GA: gallic acid.

Organic acids affect the taste of fruits and perform several metabolic functions, including maintaining turgor pressure (Famiani et al., 2015; Walker et al., 2018), play a role in fruit ripening, absorbing Ca from pectins, which makes pectins soluble and thus help soften the fruits (Richter, 2001, p. 29). The content of titrated acids in sweet cherry fruits decreased during fruit ripening, reaching a minimum in the phase of picking ripeness and in bare fallow conditions ranged from 1.09 to 0.64%, in living mulch conditions - from 1.30 to 0.72%. During the stages of petal fall and stone hardening in 2018 and during the petal fall, stone hardening and partial fruit reddening phases in 2019, titrated acids content in fruits was significantly higher under the conditions of living mulch (by 20, 37, 18, 22 and 25%, respectively). In the phase of picking ripeness, the difference between the variants of the experiment was statistically insignificant both in 2018 and in 2019. The content of titrated acids in fruits mostly correlated with the content of phenolic substances and anthocyanins. In 2019, compared to 2018, the content of titrated acids in fruits increased by 17% under the conditions of bare fallow, by 14% - under living mulch conditions. There are reports in the scientific literature that the content of organic acids in sweet cherry fruits increases in the process of their ripening (Serrano et al., 2005; Tahir et al., 2013). In our study, however, the content of titrated acids in sweet cherry fruits decreased during ripening. It should be noted that the content of organic acids in the fruits was relatively low in our study. Probably, this is due to the peculiarities of the climatic conditions of our region and the peculiarities of the studied cultivar. After all, the content of

Table 3. Correlation coefficients (r^2) between the content of biologically active substances in sweet cherry fruits

Correlation coefficients between:	Year of the research	
	2018	2019
Soluble solids and sugars	0.99**	0.99**
Soluble solids and titrated acids	0.47*	0.27^{ns}
Soluble solids and ascorbate	0.28^{ns}	0.62*
Soluble solids and phenolic substances	0.51*	0.82**
Soluble solids and anthocyanins	0.74*	0.81**
Soluble solids and glutathione	0.11^{ns}	0.65*
Sugars and titrated acids	0.45*	0.31^{ns}
Sugars and ascorbate	0.28^{ns}	0.67*
Sugars and phenolic substances	0.78**	0.79**
Sugars and glutathione	0.57*	0.47*
Titrated acids and ascorbate	0.32^{ns}	0.51*
Titrated acids and phenolic substances	0.51*	0.61*
Titrated acids and glutathione	0.46*	0.21^{ns}
Ascorbate and phenolic substances	0.40*	0.72**
Ascorbate and glutathione	0.76**	0.36*
Phenolic substances and glutathione	0.27^{ns}	0.56*
Anthocyanins and sugars	0.72**	0.93**
Anthocyanins and titrated acids	0.58*	0.59*
Anthocyanins and ascorbate	0.54*	0.72*
Anthocyanins and phenolic substances	0.95**	0.95**
Anthocyanins and glutathione	0.36*	0.32*

Notes: **Correlation is significant at 0.01 levels; *Correlation is significant at 0.05 levels; ns correlation is not significant.

titrated acids in the fruits depends significantly on the characteristics of the sweet cherry cultivar (Corneanu et al., 2020). It has been reported that in arid and hot conditions during fruit ripening, the content of organic acids in sweet cherry fruits decreases (Pangelova, 1970; Skvareninova, 1997; Richter, 2001, pp. 34–36; Famiani et al., 2020): the acid content decreases during fruit ripening, as they are spent on energy and plasticity metabolism during intensive mesocarp growth (organic acid respiration). Regarding the decrease in the content of soluble solids, sugars, titrated acids in the fruits under bare fallow conditions, compared with living mulch, this is partly due to the tendency to increased fruit weight under bare fallow (Gerasko, 2020), as it was reported that increasing fruit weight may reduce the content of soluble solids, sugars, titrated acids, leukoanthocyanidins - there is a so-called ‘dilution’ due to an increase in cell volume (Richter, 2001, p. 37; Famiani et al., 2015; Walker et al., 2018; Famiani et al., 2020).

The total content of phenolic compounds in sweet cherry fruits was in the range of 3.4–89.3 mg of GA $(100\ g)^{-1}$ of raw mass under the conditions of bare fallow and significantly higher (by 23–56% during all stages of fruit ripening) under the conditions of living mulch - from 5.3 to 110.1 mg of GA $(100\ g)^{-1}$ of raw mass. Phenolic substances accumulated in the fruits during ripening, reaching a maximum in the stage of picking ripeness, during which in 2018 they were 47% higher under the conditions of living mulch, compared to the conditions of bare fallow, in 2019 - 23% higher. The content of phenolic substances correlated mostly with the content of anthocyanins. This is natural, as anthocyanins are the most represented class of phenolic compounds in red-coloured sweet cherry fruits (Martini et al., 2017). Compared to 2018, in 2019 the content of phenolic substances in the fruits increased by 33% under bare fallow conditions, and by 11% - under living mulch conditions. Phenolic substances, as a dietary component, play an important role in shaping the sensory characteristics of fruits, giving them specific tartness, as well as being responsible for their colour and firmness (Richter, 2001, p. 38). Phenolic substances are natural antioxidants with a strong ability to neutralize free radicals; they exhibit anti-cancer, anti-ulcer properties (Ballistreri et al., 2013). The composition and content of phenolic substances in fruits depends, not least, on cultivar, genotype, climatic and agronomic conditions (Vursavus et al., 2006; Średnicka-Tober et al., 2019).

Based on our results, living mulch promotes phenolic substance accumulation in sweet cherry fruits, which makes them especially valuable for therapeutic and prophylactic nutrition.

Anthocyanin content in sweet cherry fruits was in the range from 0 to 12.1 mg $(100\ g)^{-1}$ of raw mass under the conditions of bare fallow and from 0 to 15.2 mg $(100\ g)^{-1}$ of fresh mass under the conditions of living mulch. Anthocyanins accumulated in fruits starting from the stage of partial reddening, with significantly more anthocyanins contained in the fruits under living mulch conditions, both in 2018 (by 36%) and in 2019 (by 26%). In 2019, compared to 2018, more anthocyanins accumulated in the fruits: by 63% under the conditions of bare fallow, and by 51% under the conditions of living mulch. The increase in anthocyanin content in the fruits is due to their participation in the formation of the characteristic colour of the fruit.

Weather conditions significantly affect anthocyanin content in sweet cherry fruits: in dry and hot weather, the content of anthocyanins increases (Goncalves et al., 2004).

The content of ascorbic acid in the fruits reached a maximum in the stage of the hardening of the stone (15.4–16.8% under bare fallow and 17.6–19.4% under living mulch), and then decreased during fruit ripening, but in the stage of picking ripeness was significantly higher in living mulch conditions compared to bare fallow conditions (by 29% in 2018 and by 21% in 2019). The greatest correlation was observed between the content of ascorbate and the content of phenolic substances, glutathione and anthocyanins. In 2019, compared to 2018, ascorbate content increased by 15% in bare fallow conditions, by 9% - in living mulch conditions. Ascorbate (vitamin C) is the most well-known antioxidant for consumers, which plays a protective role against cardio-vascular diseases (Vilchèze et al., 2018; Wang et al., 2018). Therefore, it can be stated that sweet cherry fruits grown in living mulch conditions have a higher consumer quality compared to fruits grown under bare fallow.

Glutathione content in the fruits reached a maximum in the stage of hardening of the stone in 2018 and in the phase of partial reddening of the fruits in 2019. Under living mulch conditions, the content of glutathione in the fruits was significantly higher compared to the conditions of bare fallow in the stages of petal fall and hardening of the stone (in 2018, respectively, 18 and 14%, in 2019 – 19 and 11%). Glutathione content in 2019, compared to 2018, has not changed in both variants of the experiment. Vitamin C (l-ascorbate, l-ascorbic acid) and glutathione are the main hydrophilic antioxidants in plants, which play an important role in plant stress resistance and fruit quality (Davey & Keulemans, 2004; Díaz-Mula et al., 2009b; Foyer & Noctor, 2011). Therefore, the increase in the content of ascorbate and glutathione in sweet cherry fruits under the conditions of living mulch, compared with bare fallow, indicates increased stress resistance and higher functional quality of such fruits for consumption.

The overall reducing activity tended to increase under living mulch conditions and was significantly higher compared to bare fallow: in the stage of petal fall in 2018 (by 13%), in the phases of falling petals and hardening of the bone in 2019, respectively, by 17 and 11%. In 2019, compared to 2018, the overall reducing activity remained virtually unchanged in both versions of the experiment. The highest total reducing activity correlated with the content of glutathione, ascorbate and dry soluble substances (Table 4). Our data is consistent with the results of studies by Commisso (2017): phenolic compounds are the main source of antioxidant activity, and experiments with artificial simplified phytocomplexes have shown strong synergies between anthocyanins and ascorbic acid.

Table 4. Correlation coefficients (r^2) of total reducing activity (TRA) with the content of biologically active substances in sweet cherry fruits

Year of the research	Correlation coefficient of TRA with the content:						
	SS	Sug	TA	Phen	Ant	AsA	Glu
2018	0.52**	0.25^{ns}	0.34*	0.32*	0.53**	0.88**	0.90*
2019	0.64**	0.49*	0.20^{ns}	0.79*	0.32*	0.36*	0.95*

Notes: **Correlation is significant at 0.01 levels; *Correlation is significant at 0.05 levels; ns correlation is not significant. SS: Soluble solids; Sug: Sugars; TA: Titrated acids; Phen: Phenolic substances; Ant: Anthocyanins; AsA: Ascorbate, Glu: Glutathione.

It should be noted that sweet cherry fruits grown organically in our experiment, had high phytochemical characteristics compared to the fruits grown in intensive technology in the region (Tolstolik, 2016; Bondarenko, 2018). The data on biochemical composition of sweet cherry fruits obtained by us coincides with the average data obtained in the

South of Ukraine (Richter, 2001; Kishchak, 2012; Ivanova et al., 2021). Anthocyanins content in sweet cherry fruits in our research is generally consistent with the data obtained by Italian researchers (Ballistreri et al., 2013), but is significantly lower compared to Spanish studies (González-Gómez et al., 2010). The total content of polyphenols in fruits in our study (during the ripening stage) is significantly lower, and the sugar content is consistent with data obtained for old sweet cherry cultivars in Czech Republic (Nawirska-Olszańska et al., 2017). It should be noted that as of today, there are new sweet cherry cultivars that are characterized by high anthocyanins content (approximately three times more than 'Dilemma' cultivar we studied) (Antognoni et al., 2020). If we compare phytochemical composition of sweet cherry fruits grown in our research area (South of Ukraine) with fruits grown in Turkey (Kelebek & Selli, 2011), 'Dilemma' fruits contain significantly more sugars, significantly less titrated acids, and the total phenolic content in them is similar to the level in 'Van' cultivar. Content of sugars, titrated acids and anthocyanins in 'Dilemma' fruits during the ripening stage was similar to the content of these compounds in the fruits of 'Bianca' sweet cherry grown in southern Poland (Skrzyński et al., 2016).

In our study, the content of soluble solids, sugars, phenolic substances and anthocyanins increased in sweet cherry fruits as they ripened and was closely correlated with the overall reducing activity. Similar trends have already been noted in the works by O.A. Grebennikova (2011): the content of biologically active substances in plum fruits increased during ripening and was greatest in mature and even overripe fruits, while the correlation between antioxidant activity and the content of biologically active substances also increased as the fruits ripened; H. M. Díaz-Mula et al. notes (2009a): as sweet cherry fruits mature, the nutritional and functional quality of them increases (including the content of phenols, anthocyanins and antioxidant capacity).

In our study, both variants were grown using organic technology. But sweet cherry trees grown under living mulch orchard floor management system experienced additional stress from competing with grasses. This contributed to the accumulation of protective antioxidant and biologically active compounds in the fruits. Fruits enriched with endogenous antioxidants are in good demand from consumers and retain consumer and functional quality for longer (have a longer shelf life) (Serrano et al., 2009). Therefore, it can be stated that using living mulch in the orchard helps to improve the consumer and functional quality of sweet cherry fruits.

CONCLUSIONS

The content of ascorbate, phenolic substances and anthocyanins in sweet cherry fruits under living mulch conditions were consistently significantly higher compared to the fruits under bare fallow conditions. Identified patterns can be explained by the stressful conditions of competition with natural grasses, which activates the synthesis of anti-stress biologically active substances in plant tissues (including fruits).

Since it is the antioxidants of the fruits that have physiological value for human health, it can be stated that fruits grown in the conditions of living mulch have a higher therapeutic and prophylactic value than the fruits grown in the conditions of bare fallow.

The content of dry soluble substances, sugars, titrated acids, glutathione and total reducing activity in the fruits experienced significant fluctuations during fruit ripening, but during the stage of picking maturity there was no significant difference between the variants of the experimental both in 2018 and 2019.

REFERENCES

Anjum, N.A., Umar, S. & Chan, M.T. 2010. *Ascorbate-Glutathione Pathway and Stress Tolerance in Plants.* Springer Dordrecht Heidelberg, London New York. https://doi.org/10.1007/978-90-481-9404-9

Antognoni, F., Potente, G., Mandrioli, R., Angeloni, C., Freschi, M., Malaguti, M. & Tartarini, S. 2020. Fruit Quality Characterization of New Sweet Cherry Cultivars as a Good Source of Bioactive Phenolic Compounds with Antioxidant and Neuroprotective Potential. *Antioxidants* **9**, 677. https://doi.org/10.3390/antiox9080677

Atucha, A., Merwin, I.A. & Brown, M.G. 2011. Long-term effects of four groundcover management systems in an apple orchard. *HortSci* **46**(8), 1176–1183. https://doi.org/10.21273/HORTSCI.46.8.1176

Atucha, A., Merwin, I.A., Brown, M.G., Gardiazabal, F., Mena, F., Adriazola, C. & Lehmann, J. 2013. Soil erosion, runoff and nutrient losses in an avocado (Persea americana Mill) hillside orchard under different groundcover management systems. *Plant Soil* **368**(1–2), 393–406. https://doi.org/10.1007/s11104-012-1520-0

Ballistreri, G., Continella, A., Gentile, A., Amenta, M., Fabroni, S. & Rapisarda, P. 2013. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *J. Food Chem.* **140**, 630–638. https://doi.org/10.1016/j.foodchem.2012.11.024

Bondarenko, P. 2018. Influence of rootstock-scion combinations and conditions of the year on sweet cherry fruit quality in the conditions of the Southern Steppe of Ukraine. *Bulletin of Lviv National Agrarian University. Agronomy* **22**(2), 96–102. https://doi:10.31734/agronomy2018.02.096 (in Ukrainian)

Commisso, M. 2017. Multi-approach metabolomics analysis and artificial simplified phytocomplexes reveal cultivar-dependent synergy between polyphenols and ascorbic acid in fruits of the sweet cherry (Prunus avium L.). *PLoS ONE* **12**(7), e0180889. https://doi.org/10.1371/journal.pone.0180889

Corneanu, M., Iurea, E. & Sîrbu, S. 2020. Biological properties and fruit quality of sweet cherry (Prunus avium L.) cultivars from Romanian assortment. *Agronomy Research* **18**(4), 2353–2364. doi:10.15159/ar.20.231

Culumber, C.M., Reeve, J.R., Black, B.L., Ransom, C.V. & Alston, D.G. 2019. Organic orchard floor management impact on soil quality indicators: nutrient fluxes, microbial biomass and activity. *Nutr Cycl Agroecosyst* **115**, 101. https://doi.org/10.1007/s10705-019-10007-2

Davey, M.W. & Keulemans, J. 2004. Determining the potential to breed for enhanced antioxidant status in Malus: mean inter- and intravarietal fruit vitamin C and glutathione contents at harvest and their evolution during storage. *J. Agric. Food Chem.* **52**, 8031–8038. https://doi.org/10.1021/jf048531k

Díaz-Mula, H.M., Castillo, S., Martínez-Romero, D., Valero, D., Zapata, P.J., Guillén, F. & Serrano, M. 2009a. Sensory, Nutritive and Functional Properties of Sweet Cherry as Affected by Cultivar and Ripening Stage. *Food Science and Technology International* **15**(6), 535–543. https://doi.org/10.1177/1082013209351868

Díaz-Mula, H., Valero, D., Zapata, P., Guillén, F., Castillo, S., Martínez-Romero, D. & Serrano, M. 2009b. The functional properties of sweet cherry as a new criterion in a breeding program. *Acta Hort* **839**, 275–280. https://doi.org/10.17660/ActaHortic.2009.839.34

Famiani, F, Bonghi, C, Chen, Z.-H., Drincovich, M.F., Farinelli, D., Lara, M.V. & Walker, R.P. 2020. Stone Fruits: Growth and Nitrogen and Organic Acid Metabolism in the Fruits and Seeds. *Plant Sci.* **11**, 572601. https://doi.org/10.3389/fpls.2020.572601

Famiani, F., Battistelli, A., Moscatello, S., Cruz-Castillo, J.G. & Walker, R.P. 2015. The organic acids that are accumulated in the flesh of fruits: occurrence, metabolism and factors affecting their contents - a review. *Rev. Chapingo Ser. Hortic* **21**, 97–128. https://doi.org/10.5154/r.rchsh.2015.01.004

Fidalski, J., Tormena, C.A. & da Silva, A.P. 2010. Least limiting water range and physical quality of soil under groundcover management systems in citrus. *Sci. agric. (Piracicaba, Braz.)* **67**(4). https://doi.org/ 10.1590/S0103-90162010000400012

Fonseca, L.R.S., Silva, G.R., Luís, Â., Cardoso, H.J., Correia, S., Vaz, C.V. & Socorro, S. 2021. Sweet Cherries as Anti-Cancer Agents: From Bioactive Compounds to Function. *Molecules* **26**, 2941. https://doi.org/10.3390/molecules26102941

Foyer, C.H. & Noctor, G. 2011. Ascorbate and Glutathione: The Heart of the Redox Hub. *Plant Physiology* **155**(1), 2–18. https://doi.org/10.1104/pp.110.167569

Francis, F.J. 1982. Analysis of anthocyanins. In P. Markakis (Ed.). *Anthocyanins as food colors*. Academic Press, New York, NY, 181–207. https://doi.org/10.1016/B978-0-12-472550-8.50011-1

Ivanova, I., Serdyuk, M., Malkina, V., Priss, O., Herasko, T. & Tymoshchuk, T. 2021. Investigation into sugars accumulation in sweet cherry fruits under abiotic factors effects. *Agronomy Research* **19**(2), 444–457. doi:10.15159/ar.21.004

Gerasko, T.V. 2020. Influence of live mulch on physiological and biochemical parameters of cherry leaves and fruits by organic cultivation technology. *Collection of scientific works 'Agrobiology'* **1**, 20–28. https://doi.org/10.33245/2310-9270-2020-157-1-20-28 (in Ukrainian).

Gerasko, T., Pyda, S. & Ivanova, I. 2021. Effect of Living Mulch on Soil Conditions and Morphometrical Indices of Sweet Cherry Trees. *Inter J Appl Agric Sci* **7**(1), 50–56. https://doi.org/10.11648/j.ijaas.20210701.14

Gomez, J.A., Amato, M., Celano, G. & Koubouris, G. C. 2008. Organic olive orchards on sloping land: more than a specialty niche production system? *J. Environ Sci.* **89**(2), 99–109. https://doi.org/10.1016/j.jenvman.2007.04.025

Gonçalves, A.C., Bento, C., Silva, B., Simões, M. & Silva, L.R. 2019. Nutrients, Bioactive Compounds and Bioactivity: The Health Benefits of Sweet Cherries (*Prunus avium* L.) *Current Nutrition & Food Science* **15**(3), 208–227. https://doi.org/10.2174/1573401313666170925154707

Goncalves, B., Landbo, A.-K., Knudsen, D., Silva, A. P., Moutinho-Pereira, J., Rosa, E. & Meyer, A. S. 2004. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). *J. Agric. Food Chem.* **52**, 523–530. https://doi.org/10.1021/jf030595s

González-Gómez, D., Lozano, M., Fernández-León, M.F., Bernalte, M.J., Ayuso, M.C. & Rodríguez, A. B. 2010. Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). *Journal of Food Composition and Analisis* **23**(6), 533–539. https://doi.org/10.1016/j.jfca.2009.02.008

Gorodniy, M.M., Melnichuk, S.D. & Gonchar, O.M. 2006. *Applied biochemistry and quality management of crop products: Textbook*. Aristei, Kyiv, 484 pp., p. 442–443 (in Ukrainian).

Grebennikova, O.A. 2011. *Biologically active substances in fruits, leaves and products of processing of perspective varieties of plum*. PhD Thesis, Odessa, 22pp. (in Ukrainian).

Gu, C., Liu, Y., Mohamed, I., Zhang, R., Wang, X., Nie, X. & Li, Z. 2016. Dynamic Changes of Soil Surface Organic Carbon under Different Mulching Practices in Citrus Orchards on Sloping Land. *PLoS ONE* **11**(12), e0168384. https://doi.org/10.1371/journal.pone.0168384

Hallmann, E. & Rozpara, E. 2017. The estimation of bioactive compounds content in organic and conventional sweet cherry (Prunus avium L.). *J. Res. Appl. Agric. Eng.* **62**, 141–145. http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-584e09be-6f70-4b88-a129-5c0ba9ff7254

Holden, E., Linnerud, K., Banister, D., Schwanitz, V.J. & Wierling, A. 2017. *The Imperatives of Sustainable Development: Needs, Justice, Limits.* Routledge, London, UK. https://doi.org/10.4324/9780203022177

Kelebek, H. & Selli, S. 2011. Evaluation of chemical constituents and antioxidant activity of sweet cherry (Prunus avium L.) cultivars. *Int J Food Sci Technol* **46**, 2530–2537. https://doi.org/10.1111/j.1365-2621.2011.02777.x

Kishchak, O.A. 2012. Commodity quality and biochemical composition of cherry fruits depending on the type of plantings. *Bulletin of Agricultural Science* **4**, 37–41. Retrieved from http://nbuv.gov.ua/UJRN/vaan_2012_4_7 (in Ukrainian).

Martini, S., Conte, A. & Tagliazucchi, D. 2017. Phenolic compounds profile and antioxidant properties of six sweet cherry (Prunus avium) cultivars. *Food Research International* **97**, 15–26. https://doi.org/10.1016/j.foodres.2017.03.030

Merwin, I.A. 2010. Keeping Under Cover: The Ideal Look of an Orchard Floor. *Fruit Growers News.* Retrieved from http://fruitgrowersnews.com/article/keeping-under-cover-the-ideal-look-of-an-orchard-floor/

Methods of qualification examination of plant varieties for suitability for distribution in Ukraine. *Methods for determining the quality of crop products*. 2021. Retrieved from https://minagro.gov.ua/ua/napryamki/roslinnictvo/reyestr-sortiv-roslin-ukrayini/metodiki-provedennya-kvalifikacijnoyi-ekspertizi-sortiv-roslin (in Ukrainian).

Nawirska-Olszańska, A., Kolniak-Ostek, J., Oziembłowski, M., Ticha, A., Hyšpler, R., Zadak, Z. & Paprstein, F. 2017. Comparison of old cherry cultivars grown in Czech Republic by chemical composition and bioactive compounds. *Food Chem* **228**, 136–142. https://doi.org/10.1016/j.foodchem.2017.01.154

Neilsen, G.H., Neilsen, D., Kappel, F. & Forge, T. 2014. Interaction of Irrigation and Soil Management on Sweet Cherry Productivity and Fruit Quality at Different Crop Loads that Simulate Those Occurring by Environmental Extremes. *HortScience* **49**(2), 215–220. https://doi.org/10.21273/HORTSCI.49.2.215

Palese, A.M. 2014. Influence of soil management on soil physical characteristics and water storage in a mature rainfed olive orchard. *Soil Tillage Res.* **144**, 96–109. https://doi.org/10.1016/j.still.2014.07.010

Pangelova, J. 1970. Chemical composition of the fruits of some cherry and sour cherry varieties. *Fruit growing* **8**, 30–33 (in Bulgarian).

Richter, A.A. 2001. *Improving the quality of fruits of southern cultures*. Tavria, Simferopol. 426 pp. (in Russian).

Sánchez, E.E., Giayetto, A., Cichón, L., Fernández, D., Aruani, M.C. & Curetti, M. 2007. Cover crops influence soil properties and tree performance in an organic apple (*Malus domestica* Borkh) orchard in northern Patagonia. *Plant Soil* **292**(1–2), 193–203. https://doi.org/10.1007/s11104-007-9215-7

Serrano, M., Guillén, F., Martínez-Romero, D., Castillo, S. & Valero, D. 2005. Chemical Constituents and Antioxidant Activity of Sweet Cherry at Different Ripening Stages. *J. Agric. Food Chem* **53**(7), 2741–2745. https://doi.org/10.1021/jf0479160

Serrano, M., Diaz-Mula, H. M., Zapata, P.J., Castillo, S., Guillen, F., Martinez-Romero, D. & Valero, D. 2009. Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. *J. Agric. Food Chem.* **57**, 3240–3246. https://doi.org/10.1021/jf803949k

Simões, M.P., Belo, A., Cruz, C.P. & Pinheiro, A.C. 2014. Natural vegetation management to conserve biodiversity and soil water in olive orchards. *Spanish J. Agric. Res.* **12**(3), 633–643. https://doi.org/10.5424/sjar/2014123-5255

Skrzyński, J., Leja, M., Gonkiewicz, A. & Banach, P. 2016. Cultivar effect on the sweet cherry antioxidant and some chemical attributes. *Folia Hort* **28**(1), 95–102. https://doi.org/10.1515/fhort-2016-0011

Skvareninova, J. 1997. Premenlivost Kvality Populacii Cerasne Vtacej (Ctrasus avium (L.) Moench.) a jej vertikalne rozsirenie v niektorych oblastiach Slovenska. *Acta Fac Forest Zvolen* **39**, 21–31 (in Slovak).

Średnicka-Tober, D., Ponder, A., Hallmann, E., Głowacka, A. & Rozpara, E. 2019. The Profile and Content of Polyphenols and Carotenoids in Local and Commercial Sweet Cherry Fruits (Prunus avium L.) and Their Antioxidant Activity In Vitro. *Antioxidants* **8**(11), 534. https://doi.org/10.3390/antiox8110534

Tahir, M., Farooq, A., Bhatti, I.A. & Tahira, I. 2013. Effect of maturity on proximate composition, phenolics and antioxidant attributes of cherry fruit. *Pakistan Journal of Botany* **45**(3), 909–914. https:http://www.pakbs.org/.../25.pdf

Tolstolik, L. 2016. *Biochemical composition and technological properties of fruits of elite forms and varieties of cherries.* Retrieved from http://sophus.at.ua/publ/2016_10_28_kampodilsk/sekcija_section_1_2016_10_28/biokhimichnij_sklad_i_tekhnologichni_vlastivosti_plodiv_elitnikh_form_ta_sortiv_chereshni/129-1-0-2013 (in Ukrainian).

Vilchèze, C., Kim, J. & Jacobs, W.R., Jr. 2018. Vitamin C potentiates the killing of Mycobacterium tuberculosis by the first-line tuberculosis drugs isoniazid and rifampin in mice. *Antimicrob Agents Chemother* **62**, e02165-17. https://doi.org/10.1128/AAC.02165-17

Vursavus, K., Kelebek, H. & Selli, S. 2006. A study on some chemical and physico-mechanic properties of three sweet cherry varieties (Prunus avium L.) in Turkey. *J. Food Eng.* **74**, 568–575. https://doi.org/10.1016/j.jfoodeng.2005.03.059

Walker, R.P., Benincasa, P., Battistelli, A., Moscatello, S., Técsi, L., Leegood, R.C. & Famiani, F. 2018. Gluconeogenesis and nitrogen metabolism in maize. *Plant Physiol Biochem* **130**, 324–333. https://doi.org/10.1016/j.plaphy.2018.07.009

Wang, K., Jiang, H., Li, W., Qiang, M., Dong, T. & Li, H. 2018. Role of vitamin C in skin diseases. *Front Physiol* **9**, 819. https://doi.org/10.3389/fphys.2018.00819

Workshop on agrochemistry: *Textbook.* / Ed. V.G. Mineev. 2001. MSU Publishing House, Moscow, 689 pp., pp. 419–422. (in Russian).

Yaman, M. 2022a. Evaluation of genetic diversity by morphological, biochemical and molecular markers in sour cherry genotypes. *Molecular Biology Reports* **49**(6), 5293–5301. https://doi.org/10.1007/s11033-021-06941-6

Yaman, M. 2022b. Determination of genetic diversity in european cranberrybush (Viburnum opulus L.) genotypes based on morphological, phytochemical and ISSR markers. *Genetic Resources and Crop Evolution* **69**(5), 1889–1899. https://doi.org/10.1007/s10722-022-01351-4

Yao, S., Merwin, I.A., Bird, G.W., Abawi, G.S. & Thies, J.E. 2005. Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. *Plant Soil.* **271**(1–2), 377–389. https://doi.org/10.1007/s11104-004-3610-0

Zheng, W., Gong, Q., Zhao, Z., Liu, J., Zhai, B., Wang, Z. & Li, Z. 2018. Changes in the soil bacterial community structure and enzyme activities after intercrop mulch with cover crop for eight years in an orchard. *Eur J. Soil Biol.* **86**, 34–41. https://doi.org/10.1016/j.ejsobi.2018.01.009

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Effect of the predecessor and the nitrogen rate on productivity and essential oil content of coriander (*Coriandrum sativum* L.) in Southeast Bulgaria

A. Harizanova[1,*], V. Delibaltova[1], M. Shishkova[1], N. Neshev[1], M. Yanev[1], A. Mitkov[1], N. Yordanova[1], S. Manhart[1], M. Nesheva[2] and P. Chavdarov[3]

[1]Agricultural University of Plovdiv, Faculty of Agronomy, 12 Mendeleev Blvd, BG4000 Plovdiv, Bulgaria
[2]Fruit Growing Institute, Department of Breeding, Genetic Resources and Biotechnology, 12 Ostromila Str, BG4004 Plovdiv, Bulgaria
[3]K. Malkov Institute of Plant Genetic Resources, Department of Plant Genetic Resources, 2 Druzba Str., BG4122 Sadovo, Bulgaria
[*]Correspondence: aharizanova@yahoo.com

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Abstract. Coriander (*Coriandrum sativum* L.) is one of the most important essential oil crops on a global scale. Coriander productivity is determined by the genotype, the environmental factors, as well the agronomic practices. A field experiment was conducted in Southeast Bulgaria during three vegetation seasons (2015, 2016, and 2017). The present study aimed at analysing the influence of two crop predecessors (winter wheat and sunflower) and four nitrogen (N) levels (0, 40, 80, and 120 kg ha^{-1}). Productivity elements, seed yield, and seed essential oil content of coriander (cv. Mesten drebnoploden) were under evaluation. The results obtained showed that winter wheat was a more suitable predecessor of coriander in comparison to sunflower. The highest results regarding the number of umbels per plant, the umbel's diameter, the number of umbellets per umbel, the number of seeds per umbel, the seed weight per plant, the 1,000 seed mass, as well as the seed yield for the rate of 80 kg ha^{-1} of N were recorded. The highest essential oil content after applying 120 kg ha^{-1} of N was established. Increasing the N level from 0 to 120 kg ha^{-1} led to a positive and significant effect on essential oil yield. No significant differences between the N rates of 80 and 120 kg ha^{-1} were recorded. The received results contributed for the evaluatation of the optimum nitrogen level, as well as for the determination of a more suitable predecessor of coriander in order to obtain the highest yield of better quality in the region of Southeast Bulgaria.

Key words: *Coriandrum sativum*, crop rotation, fertilization, precursor, seed yield.

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is one of the most significant essential oil crops grown in the world. It is an annual herbaceous plant, belonging to the Apiaceae family. Coriander`s habitat is the southern part of Europe and the western Mediterranean region. The coriander plant is used as four main products: fresh green herb, fruit as spice, and herb and fruit essential oils as flavourings (Weiss, 2002). The whole seed is added in pickles, chutneys, and other similar products. It is also used to flavour alcoholic beverages including gin, vermouth and vodka, as well as to be crystallized in sugar as a sweetmeat. The main use of the ground spice worldwide is in flavourings or mixed spices. It is an important ingredient in curry powders - between 25% and 40%. It is extensively added in processed foods, meats, sausages, baked products including special breads, and sauces. The essential oil, obtained from coriander fruits at amounts from 0.5 to 2.5% approximately, is used both in the make of flavours and in the manufacture of perfumes and soaps (Carrubba et al., 2002). The oil is a pale or colourless liquid having a specific odour; the taste is sweet. The flavour is mild, sweet and spicy-aromatic, somewhat warm and slightly burning. Over 200 constituents have been determined, as linalool is the main component. The coriander essential oil is triglyceride oil containing petroselinic acid, which makes it a good source of lipids (Mandal & Mandal, 2015). It is also rich in linalool (60–80%), geraniol (1.2–4.6%), terpinen-4-ol (3%), α-pinene (0.2–8%), myrcene (0.2–2%) and other substances (Nadeem et al., 2013). The productivity of coriander is determined by genotype, environmental factors, and agrotechnical practices (Lenardis et al., 2000; Khalid, 2013).

Fertilization is one of the main agronomic factors for the expression of the variety's biological potential. Nitrogen is a plant nutrient that is effective not only in terms of growth and yield, but also in terms of seed quality (Kizil & Ipek, 2004; Skudra & Ruza, 2019). The latter plays an important role in the synthesis of plant components with enzyme activity (Marschner, 2011; Moosavi et al., 2013; Izgi, 2020). Khalid (2013) stated that after the nitrogen applications the obtained values were significantly higher than those of the control group. They found significant changes in plant growth characteristics. Several studies have shown that nitrogen fertilization increases plant growth, plant height, number of branches, number of umbels per plant, number of umbellets per umbel, seed yield per plant, test weight, and vigor index (Oliveira et al., 2003; Gujar et al., 2005; Kumar et al., 2007; Kumar et al., 2008; Rahimi et al., 2009; Ali et al., 2015). Moosavi et al. (2013) reported that nitrogen level had a significant influence on fruit yield, essential oil percent, and yield. On the contrary, with the increase of N rate from 0 to 80 kg ha^{-1}, plant height and fruit yields increased by 19.8 and 74.1%, respectively. In addition, the percentage of essential oil increased from 0.153 to 0.33%, and the yield of essential oil was 2.68 folds higher. Akbarinia et al. (2007) found that the highest seed and essential oil yield were obtained by using 60 kg ha^{-1} of N, while the highest essential oil content was obtained after the application of 90 kg N ha^{-1}. Erdoğdu & Esendal (2018) established that increasing the nitrogen level led to growth in plant height and seed yield. However, the number of umbels per plant, the number of seeds per umbel, the seed yield per plant, and 1,000 seed weight were not affected by the increase of N level. Lokhande et al. (2015) reported that the 1,000 seed weight and the seed yield differed between the studied N rates. Okut & Yildirim (2005) stated that the seed yield and 1,000 seed weight differed by the increase of N levels, while plant height, number of umbels, and harvest index were not influenced by N levels. Szempliński & Nowak (2015) reported that seed yield, number of umbels per plant, and 1,000 seed weight were not altered by N fertilizer rates.

Rotation of crop is one of the most important agronomic practices that may have a significant effect on crop quality and quantity. It has been reported that proper crop rotation leads to increase in crop yield (Berzsenyi & Gyorffy, 1997; Sieling et al., 2005;

Hilton et al., 2018; Neshev, 2022). It provides more sustainable crop production (Bullock, 1992; Bailey et al., 2001; Mamolos & Kalburtji, 2001). Another benefit of the appropriate crop rotation is the reduction of disease development and prevention of pesticide resistance (Stoddard, 2010). According to Zaitsev & Kovalenko (2020), in the case of winter wheat, the share of influence of predecessors on yield could reach 15–35%. With relation to this, Delibaltova et al. (2012) examined the influence of the predecessor and the sowing rate on seed yield and yield components of coriander.

There is limited information about the interaction between the predecessor and the nitrogen level and its effect on growth and productivity of coriander. Taking into account the above-mentioned facts, the present study aims to enrich the knowledge of coriander cultivation based on different predecessors and different N levels.

MATERIALS AND METHODS

Plant material, experimental design, and soil analyses

The field experiment was carried out in the period 2015–2017 on leached Smolnitsa soil type on the territory of the village of Zhrebino, Southeast Bulgaria. The experiment was conducted with Mesten drebnoploden coriander variety. Two predecessors (Wheat and Sunflower) and four nitrogen levels (0, 40, 80, and 120 kg ha^{-1}) were tested. Fertilization was performed with Portable Chest-mount Spreader (SOLO, model 421-S). The experiment was set by the block (split-plot) method in four replications with experimental plot size of 15 m^2. The soil pH, mobile N, P, and K, as well as the humus content, were analysed in the Accredited Laboratory Complex of the Agricultural University - Plovdiv. The soil pH was measured according to the BDS ISO 10390:2011 standard, the mobile N - according to a modified Kjeldahl method meeting the BDS ISO 11261:2002 standard, the P content was measured via spectrometer - according to BDS ISO 11263:2002 standard, and K was determined according to GOST 26209-91/01.07.93. Humus content was measured according to BDS ISO 14235:2002 standard. The analysis registered the following: pH - 7.77, mobile N - 28.6 mg kg^{-1}, P - 113 mg kg^{-1}, K - 365 mg kg^{-1}, and humus - 1.4%.

Study treatments

During the three experimental years sowing was done in the period 10^{th}–20^{th} February. The spacing between rows was 12–15 cm. The seeding rate was 250 germinating seeds m^{-2}, and the sowing depth was 3–4 cm. Soil cultivation included plowing of the stubble in July and plowing at a depth of 20–22 cm in September, double pre-sowing cultivation with harrowing, the last being at a depth of 5–6 cm. The phosphorus fertilizer was added before plowing at a rate of 80 kg ha^{-1}. The nitrogen fertilizer was added with the last pre-sowing soil cultivation in the form of ammonium nitrate (34.4% N). Weed control was achieved by treatment with the herbicide Linurex 45 SC - 2.00 L ha^{-1}, applied after sowing before crop germination. Harvesting was done at full crop maturity. Seed yield was determined at a standard grain moisture content of 9%.

Meteorological data

The average amount of precipitation and the average monthly air temperatures (from February to July) during the experimental years are presented in Fig. 1. Data showed that air temperatures during the experimental period were close to or slightly higher than temperatures established for a multiple-year period, with no significant deviations from the crop requirements. Differences between the three years of the study were found in the precipitation amount during vegetation. The lowest amount of precipitation was reported in 2017 - 215 mm versus 276 mm for a multiple-year period. The year 2017 was characterized by an uneven distribution of rainfall, which was not enough to meet the water requirements at the critical stages. In April, May, and June at the stages of buttoning, flowering, and fruit setting the amount of rainfall was 107.6 mm versus 156 mm for a multiple-year period, i.e. it was about 31% less. This determined the third experimental year as the less favourable one for the productivity of coriander compared to the other two years.

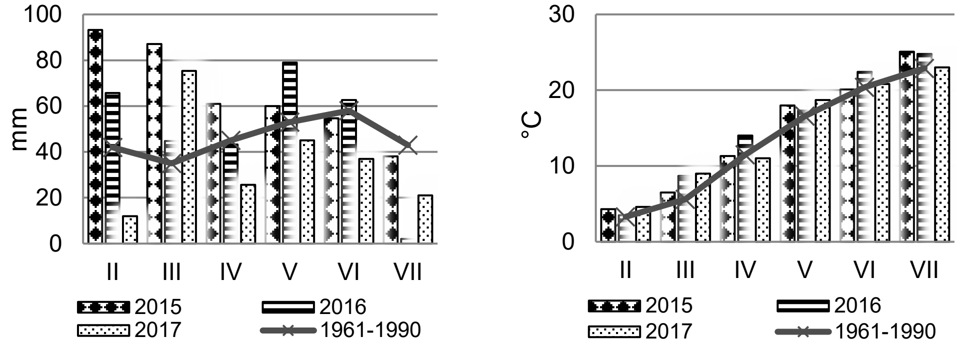

Figure 1. Precipitation, (mm) and average monthly air temperature, (°C) for the years of the experiment and average for the period 1961–1990.

The first year of study (2015) was characterized with the highest amount of precipitation during vegetation (383.9 mm). The registered amount was 107.9 mm above the climatic norm. Precipitation was evenly distributed during vegetation and in combination with the reported temperatures, it was considered to be the most favourable for coriander growth during the three experimental years. In 2016 the total amount of precipitation was 295.7 mm and exceeded the values for the period 1961–1991 by 19.7 mm, however it was not very well distributed. At the beginning of vegetation (February-March), precipitation was 33.5 mm above the norm. During the buttoning and flowering stages, it was 24.5 mm more than the amount reported in the multiple-year period, and during the ripening stage (July) precipitation was very little.

Essential oil extraction

Essential oil content was determined by the steam distillation method in a Clevenger-type apparatus. Samples with a mass of 50 g up to 200 g were set up in 800 mL water and air temperature of 20 °C ± 2. The distillation was carried out at the Institute of Roses, Essential and Medical Cultures - Kazanlak meeting the standard BDS ISO 6571:2010. To speed up the process and reveal the essential oil tanks, the raw material, mature coriander seeds, was crushed before technical processing. The distillation time was 2 hours, calculated from the appearance of the first drop of essential oil.

Yield structural elements were determined on 50 plants per square meter. The following parameters were evaluated: plant height, number of umbels per plant, umbel's diameter, number of umbellets per umbel, number of seeds per umbel, seed weight per plant, 1,000 seed weight, seed yield, essential oil content, and essential oil yield.

Statistical analyses

In order to determine the quantitative relationship between the studied indicators, the experimental data were processed by the Method of analysis of variance (ANOVA), and the differences between the variants were determined using Dunkan's Multiple Range Test.

RESULTS AND DISCUSSION

Yield structural elements averagely for the three years are presented in Table 1. According to the given data, the predecessor, as well as the nitrogen level, had a significant influence on plant height, number of umbels per plant, umbel diameter, number of umbellets per umbel, number of seeds per umbel, seed weight per plant, and 1,000 seed weight of coriander. The highest values of the evaluated parameters for the predecessor winter wheat and the N rate of 80 kg ha^{-1} were reported. The lowest parameters were established for the predecessor sunflower without the use of fertilization.

Table 1. Structural elements of the yield (Average for the period 2015–2017)

		Plants height (cm)	Umbels plant^{-1} (No)	Umbel's diameter (cm)	Umbellets umbel^{-1} (No)	Seeds umbel^{-1} (No)	Weight of seeds umbel^{-1} (g)	1,000 seed weight (g)
Predecessor (A)	Wheat	78.5 ^{a}	13.4 ^{a}	4.31 ^{a}	5.33 ^{a}	31.4 ^{a}	1.24 ^{a}	6.76 ^{a}
	Sunflower	69.0 ^{b}	11.9 ^{b}	4.14 ^{b}	4.92 ^{b}	29.0 ^{b}	1.11 ^{b}	6.43 ^{b}
Nitrogen rate (B)	0	68.8	9.6	3.92	4.58	26.3	1.05	6.49
	40	72.8	11.9	4.18	4.91	30.7	1.20	6.61
	80	75.6	16.5	4.65	6.05	32.7	1.31	6.72
	120	77.8	12.7	4.17	4.96	31.1	1.14	6.58
Wheat	0	74.2 ^{a}	10.5 ^{a}	3.95 ^{a}	4.75 ^{a}	27.7 ^{a}	1.14 ^{a}	6.67 ^{a}
	40	77.5 ^{b}	12.3 ^{b}	4.20 ^{b}	5.08 ^{b}	31.8 ^{b}	1.25 ^{b}	6.79 ^{b}
	80	80.1 ^{c}	17.7 ^{c}	4.85 ^{c}	6.27	33.9 ^{c}	1.39 ^{d}	6.89 ^{c}
	120	82.0 ^{c}	13.2 ^{b}	4.25 ^{b}	5.20 ^{b}	32.0 ^{b}	1.18 ^{c}	6.71 ^{b}
Sunflower	0	63.4 ^{a}	8.6 ^{a}	3.88 ^{a}	4.41 ^{a}	24.8 ^{a}	0.95 ^{a}	6.30 ^{a}
	40	68.0 ^{b}	11.4 ^{b}	4.16 ^{b}	4.74 ^{b}	29.5 ^{b}	1.15 ^{b}	6.43 ^{b}
	80	71.0 ^{c}	15.3 ^{c}	4.44 ^{c}	5.82 ^{c}	31.4 ^{c}	1.22 ^{c}	6.55 ^{c}
	120	73.5 ^{c}	12.2 ^{b}	4.08 ^{b}	4.71 ^{b}	30.2 ^{b}	1.10 ^{b}	6.45 ^{b}
Anova	A	**	*	*	*	*	*	*
	B	*	**	*	*	*	*	*
	AB	n.s	n.s	*	n.s	*	*	n.s

Means within columns followed by different lowercase letters are significantly different ($P < 0.05$) according to the *LSD test* * *F–test* significant at $P < 0.05$; ** *F–test* significant at $P < 0.01$; n.s – non-significant.

Coriander's height, being cultivated after winter wheat as a predecessor was 78.5 cm and 69.0 cm after sunflower predecessor, respectively. The values of plant height rose with the increse of the nitrogen levels from 3.3 to 7.8 cm after the winter wheat predecessor and 4.6 to 10.1 cm after the sunflower, respectively. The differences among the studied variants were statistically significant. These data regarding the influence of nitrogen on the increase of plant height of coriander were consistent with the results reported by other researchers (Khalid, 2013; Lokhande et al., 2015; Erdoğdu & Esendal, 2018). On one hand, according to Erdoğdu & Esendal (2018), plants supplied with 120 kg ha^{-1} of N were the highest compared to those supplied with 60 and 90 kg ha^{-1} of N, respectively. On the other hand, some researchers reported that plant height of coriander was not affected by the fertilization with nitrogen (Okut & Yildirim, 2005; Moosavi et al., 2013).

Analysis results showed the strongest influence of factor A (Predecessor), with a dominant effect on differences in plant height (significant at $P < 0.01$), followed by factor B (Nitrogen rate).

Number of umbels per plant varied from 10.5 to 17.7 for the plants grown after winter wheat, and it varied from 8.6 to 15.3 for the plants grown after sunflower predecessor depending on nitrogen rates. With the increase of nitrogen rate the values of the relevant parameter increased up to 7.2 umbels for the coriander sown after winter wheat, and up to 6.7 - after sunflower. The statistical processing of the obtained data showed significant differences. The effect of nitrogen on the increasing number of umbels per plant in coriander was also found by Erdoğdu & Esendal (2018), Khalid (2013), and Lokhande et al. (2015).

Analysis results related to the influence of the factors (A and B) and their interaction on the number of umbels per plant showed statistically significant variations and the interaction between the two factors was statistically insignificant.

The umbel's diameter varied from 3.88 to 4.85 cm depending on the predecessor and applied nitrogen levels. Coriander's umbel diameter after winter wheat predecessor (4.31 cm) was higher than coriander's umbel diameter grown after sunflower (4.14 cm). The increase of nitrogen levels led to an increase of umbel`s diameter. An increase of 6.3 to 22.8% (after winter wheat predecessor) and of 7.2 to 14.4% (after sunflower predecessor) was reported for the variants applied with N in comparison to the unfertilized control. The obtained results were statistically significant.

The analysis of variance reported for a statistically significant influence of both studied factors A (predecessor) and B (N level). An interaction between the two examined factors (A x B) was also established.

Both, the predecessor and the nitrogen level, influenced the number of umbellets per umbel. The values of the studied parameter varied from 4.75 to 6.27 for the coriander grown after the preceding winter wheat crop, and from 4.41 to 5.82 after the preceding sunflower depending on N rates. The lowest number of umbellets per umbel was reported for the unfertilized control, and the highest – for the plants applied with 80 N kg ha^{-1}.

The predecessor, the nitrogen rate, and their interaction significantly affected the number of seeds per umbel (Table 1). The highest results of this parameter was established after the application of 80 kg ha^{-1} of N, which was 22.3, 6.6, and 5.9% (after wheat predecessor), and 26.6, 6.4, and 3.9% (after sunflower predecessor), which was higher than those treated with 0, 40 and 120 kg ha^{-1} N, respectively.

An important characteristic determining the coriander's seed yield is the seed weight per plant. The different preceding crops, as well as the applied N rate, influenced the formation of seed yields of different weights (Table 1). The lowest values of the indicator for the unfertilized control were reported - 1.14 g after wheat predecessor, and 0.95 g after sunflower predecessor. The highest seed weight per plant was reported after applying 80 kg N ha^{-1}. The received results surpassed those of the unfertilized control with 21.9% and with 28.4% respectively.

Variance analysis related to the effect of the factors and their interaction on seed weight per plant showed a clear statistical significance in the changes of the parameter and the interaction between both factors, which was statistically significant.

With relation to the other structural elements of yield, the 1,000 seed weight also had the highest results for coriander grown after winter wheat having rate of 80 kg N ha^{-1}. Regarding the unfertilized variants, the 1,000 seed weight varied from 6.30 to 6.67 g depending on the predecessor crop. The nitrogen fertilization increased the value index up to 3.3% for the plants grown after winter wheat, and up to 4.0% for the plants grown after sunflower. The received results were statistically significant. Data related to the influence of nitrogen on the increase of 1,000 seed weight of coriander corresponded to the results reported by other authors (Okut & Yildirim, 2005; Patel et al., 2013; Lokhande et al., 2015). On the contrary, some researchers reported that 1,000 seed weight of coriander was not affected by nitrogen fertilization (Szempliński & Nowak, 2015; Erdoğdu & Esendal, 2018).

Seeds yield varied throughout the years being influenced by the predecessor crop and the nitrogen rates (Table 2). Seed yield obtained at different nitrogen rates after wheat predecessor varied from 1,536 to 2,208 kg ha^{-1}, from 1,131 to 1,660 kg ha^{-1}, and from 1,025 to 1,490 kg ha^{-1}, correspondingly for the three experimental years (2015, 2016, and 2017). After sunflower predecessor, seed yield varied from 1,452 to 1,940 kg ha^{-1}, from 990 to 1,375 kg ha^{-1} and from 750 to 880 kg ha^{-1}, respectively. Seed yield increased with statistical significance along with the nitrogen rate increase.

Table 2. Seeds yield (kg ha^{-1}) of coriander, cv. Mesten drebnoploden, depending on the crop predecessor and the N rate

Predecessor	Nitrogen rate	Years of study			Average for the period
		2015	2016	2017	
Wheat	0	1,536 ^{a}	1,131 ^{a}	1,025 ^{a}	1,231
	40	1,910 ^{b}	1,473 ^{b}	1,120 ^{b}	1,501
	80	2,208 ^{d}	1,660 ^{d}	1,490 ^{d}	1,786
	120	2,077 ^{c}	1,518 ^{c}	1,210 ^{c}	1,602
Sunflower	0	1,452 ^{a}	990 ^{a}	750 ^{a}	1,064
	40	1,620 ^{b}	1,110 ^{b}	768 ^{b}	1,166
	80	1,940 ^{c}	1,375 ^{d}	880 ^{c}	1,398
	120	1,755 ^{b}	1,200 ^{c}	770 ^{b}	1,208

Means within columns followed by different lowercase letters are significantly different ($P < 0.05$) according to the *LSD test*.

The yields obtained on average for the experimental period (2015–2017) varied from 1,231 to 1,786 kg ha^{-1} after the predecessor winter wheat and from 1,064 to 1,398 kg ha^{-1} for the sunflower predecessor depending on the applied nitrogen rates.

The highest yields were reported after applying 80 kg ha^{-1} of N. The results surpassed those without fertilization up to 45.1% and up to 31.4% for the predecessor winter wheat and the predecessor sunflower respectively. The effect of nitrogen on increasing seed yield has been also stated by various researchers (Gujar et al., 2005;

Oliveira et al., 2006; Akbarinia, et al., 2007; Carrubba, 2009; Moosavi et al., 2013; Ali et al., 2015; Lokhande et al., 2015; Erdoğdu & Esendal, 2018) while Szempliński & Nowak (2015) reported that seed yield was not affected by the different N rates.

The more favourable combination of the major meteorological conditions during vegetation led to the obtainment of higher yields in the first year of study compared to the second and the third one. The highest seed yield (2,208 kg ha^{-1}) was reported after wheat predecessor having nitrogen rate of 80 kg ha^{-1}, and the lowest (1,452 kg ha^{-1}) - after sunflower predecessor having nitrogen rate of 0 kg ha^{-1}. Differences between the examined variants were statistically significant. In the second experimental year (2016) yields varied from 1,660 to 900 kg ha^{-1}, i.e. they were by 29% lower in average in comparison to the previous year. In the last year of study (2017) yields were within the limits from 1,490 to 750 kg ha^{-1} i.e. they were by 38.7% and 12.5% lower in comparison to 2015 and 2016, respectively. The less amount of precipitation during the critical stages for the crop development led to a decrease in seed yields during the last year of the experiment. This is in line with the results reported by Dyulgerov & Dyulgerova (2016). According to the authors the reduced precipitation in the phases of stem elongation, budding and flowering of coriander leads to a decrease in seed yield.

The dispersion analysis results regarding the effect of the factors and their interaction on seed yield show a clear statistical significance in the changes of the characteristics and the interaction between both factors, which was statistically significant (Table 3).

Table 3. Two-way ANOVA analysis of the seed yield of coriander

Years of study	Source of variation	Sum of square (SS)	df	Mean square (MS)	F	*P*-value	F crit
2015	Predecessor	566,048	1	566,048	107.7981	0.00*	4.2597
	Nitrogen rate	1,394,342	3	464,780.66	88.5128	0.00*	3.0088
	Interactions	116,080	3	38,693.33	7.3688	0.00*	3.0088
2016	Predecessor	612,724.5	1	612,724.5	386.9228	0.00*	4.2597
	Nitrogen rate	864,133.5	3	288,044.5	181.8941	0.00*	3.0088
	Interactions	55,273.5	3	18,424.5	11.6347	0.00*	3.0088
2017	Predecessor	1,360,425	1	1,360,425	1,397.637	0.00*	4.2597
	Nitrogen rate	442,330.4	3	147,443.5	151.4765	0.00*	3.0088
	Interactions	104,445.4	3	34,815.13	35.76743	0.00*	3.0088

* *F-test* significant at $P < 0.05$.

Nitrogen is one of the elements, which are of grate importance for the essential oil synthesis and composition. According to Nurzyńska-Wierdak (2013), nitrogen contributes to the greatest extent to the increase in biosynthesis of essential oil and its composition in numerous aromatic plant species. Plants use nitrogen to synthesize many organic compounds including amino acids, proteins, enzymes, and nucleic acids. Amino acids and enzymes play a key role in the biosynthesis of numerous compounds, which are essential oil constituents (Koeduka et al., 2006). One of the hypotheses about the formation of these compounds links the process of formation of terpenes to the transformation of carbohydrates and makes it dependent on this transformation while the other one links it to the transformation of proteins (Dubey et al., 2003; Lattoo et al., 2006).

According to Aziz & El-Ashry (2009), the type of the N fertilizer plays a significant role in the formation and the essential oil contents in the aromatic plants. Ammonium nitrate gives the highest value of oil yield in coriander while urea is more effective for dill (Olle & Bender, 2010). The application rate also modifies the quantity and composition of oils for several species (Zheljazkov et al., 2010; Hendawy & Khalid, 2011). Some researchers have declared that levels of N from 50 to 200 kg ha^{-1} have no significant effect on yield (Reddy & Rolston, 1999), which is contrary to our results. On the other hand, many researchers are confident that higher nitrogen rates lead to a higher seed yield (Akbarinia et al., 2007). The authors examined three nitrogen doses including 30, 60, and 90 kg ha^{-1} and the highest one proved to be the best for gaining the highest seed yield.

The results of the present study showed that the nitrogen application significantly affected essential oil content but the predecessor and their interaction did not affect this trait (Table 4).

Table 4. Essential oil content (%) of coriander, cv. Mesten drebnoploden, depending on the crop predecessor and the N rate

		Years of study			Average for the period
		2015	2016	2017	
Predecessor (A)	Wheat	1.06 ^{a}	1.14 ^{a}	0.99 ^{a}	1.06
	Sunflower	1.05 ^{a}	1.13 ^{a}	0.96 ^{a}	1.05
Nitrogen rate (B)	0	0.89	0.99	0.86	0.91
	40	1.04	1.10	0.97	1.04
	80	1.11	1.19	1.00	1.10
	120	1.19	1.27	1.09	1.18
Wheat	0	0.90 ^{a}	0.97 ^{a}	0.88 ^{a}	0.92
	40	1.05 ^{b}	1.12 ^{b}	0.99 ^{b}	1.05
	80	1.10 ^{c}	1.20 ^{c}	1.00 ^{b}	1.10
	120	1.20 ^{d}	1.28 ^{d}	1.10 ^{c}	1.19
Sunflower	0	0.88 ^{a}	1.00 ^{a}	0.83 ^{a}	0.90
	40	1.03 ^{b}	1.08 ^{b}	0.95 ^{b}	1.02
	80	1.12 ^{c}	1.18 ^{c}	0.99 ^{bc}	1.10
	120	1.18 ^{d}	1.26 ^{d}	1.07 ^{cd}	1.17
Anova	A	n.s	n.s	n.s	
	B	*	*	*	
	AB	n.s	n.s	n.s	

Means within columns followed by different lowercase letters are significantly different *(P < 0.05)* according to the *LSD test* * *F–test* significant at P < 0.05; ** *F–test* significant at P < 0.01; n.s – non-significant.

The highest seed oil content was reported in 2016. Temperatures during the fruit ripening stage were 2.1 folds higher than those for a multiple-year period. The essential oil content compared with the control was 11.1 to 28.3% higher. In the other years of the experiment the N application increased the essential oil content from 1.04 to 1.19% and from 0.97 to 1.09% in the fertilized variants, and 0.89%–0.86% in the control in 2015 and 2017, respectively. Averagely for the period 2015–2017, the values of that trait in all fertilized variants exceeded the unfertilized control from 14.3 to 29.6%.

The results indicated that the increase in the applied N rate had a positive and significant effect on this trait and the highest essential oil content was obtained at 120 kg ha^{-1} of N. The received results corresponded to those of Akbarinia et al. (2007), Moosavi et al. (2013), and Izgi (2020).

The analysis of variance related to the effect of factors A (predecessor) and B (N rate) on the essential oil yield, as well as their interaction, showed a significant influence of the factors on the changes of the studied indicator and statistically insignificant effect of the interaction between them (Table 5).

Table 5. Essential oil yield (kg ha^{-1}) of coriander, cv. Mesten drebnoploden, depending on the crop predecessor and the N rate

		Years of study			Average for the period
		2015	2016	2017	
Predecessor (A)	Wheat	20.8 ^{b}	16.7 ^{b}	12.1 ^{b}	16.5
	Sunflower	18.0 ^{a}	13.3 ^{a}	7.7 ^{a}	13.0
Nitrogen rate (B)	0	13.3	10.5	7.6	10.5
	40	18.4	14.3	9.4	14.0
	80	23.0	18.1	11.9	17.7
	120	22.8	17.2	10.8	16.9
Wheat	0	13.8 ^{a}	11.0 ^{a}	9.0 ^{a}	11.3
	40	20.1 ^{b}	16.5 ^{b}	11.1 ^{b}	15.9
	80	24.3 ^{c}	19.9 ^{c}	14.9 ^{c}	19.7
	120	24.9 ^{c}	19.4 ^{c}	13.3 ^{c}	19.2
Sunflower	0	12.8 ^{a}	9.9 ^{a}	6.2 ^{a}	9.6
	40	16.7 ^{b}	12.0 ^{b}	7.6 ^{b}	12.1
	80	21.7 ^{c}	16.2 ^{c}	8.8 ^{c}	15.6
	120	20.7 ^{c}	15.0 ^{c}	8.2 ^{c}	14.6
Anova	A	*	*	*	
	B	*	*	*	
	AB	n.s	n.s	n.s	

Means within columns followed by different lowercase letters are significantly different *(P < 0.05)* according to the *LSD test* * *F–test* significant at P < 0.05; ** *F–test* significant at P < 0.01; n.s – non-significant.

The favourable combination of temperature and moisture during the vegetation period of coriander was a precondition for obtaining a higher essential oil yield in 2015 compared to 2016 and 2017.

During the first experimental year, the values of that indicator in the fertilized variants ranged from 20.1 to 24.9 kg ha^{-1} versus 13.8 kg ha^{-1} in the control after winter wheat predecessor, and from 16.7 to 21.7 kg ha^{-1} versus 12.8 kg ha^{-1} in the control after sunflower predecessor. In 2016 the coriander essential oil yield was approximately 24% lower than in the previous year. In the last year of experiment the essential oil yield ranged from 11.1 to 14.9 kg ha^{-1} in the fertilized variants, and in the control, it was 9.0 kg ha^{-1}. After winter wheat predecessor it was from 7.6 to 8.8 kg ha^{-1} versus 6.2 kg ha^{-1} in the control after sunflower predecessor.

Averagely for the study period (2015–2017), the increase in N rates from 0 to 80 kg ha^{-1} of N had a positive and significant effect on essential oil yield. The yield increased by 1.74 folds after winter wheat predecessor and 1.63 folds after sunflower predecessor. There was no significant difference between N rates of 80 and 120 kg ha^{-1}.

CONCLUSIONS

Coriander is an essential oil plant that is gaining more and more interest, as it is widely used both as a spice and as an ingredient in the cosmetic, pharmaceutical and other industries. For this reason, it is essential for farmers to study the most favourable conditions for achieving a large quantity of high quality production.

The present study aimed to investigate the more suitable predecessor and the optimum levels of nitrogen fertilization in order to achieve higher yields and more essential oil.

The examined factors (A predecessor and B nitrogen rate) had a significant influence on the productivity of coriander, grown in the conditions of Southeast Bulgaria.

The highest number of umbels per plant, umbel's diameter, the number of umbellets per umbel, the number of seeds per umbel, seed weight per plant, 1,000 seeds weight, and coriander seeds yield were in favor of the winter wheat predecessor and the N application rate of 80 kg ha^{-1}.

The highest essential oil content after applying 120 kg N ha^{-1} was found. The predecessor did not affect this trait.

The increase of N rate from 0 to 120 kg ha^{-1} had a positive and significant effect on essential oil yield. There was no significant difference between the rates of 80 and 120 kg N ha^{-1}.

REFERENCES

Akbarinia, A., Daneshian, J. & Mohmmadbiegi, F. 2007. Effect of nitrogen fertilizer and plant density on seed yield, essential oil and oil content of *Coriandrum sativum* L. *(Persian) Iranian Journal of Medicinal and Aromatic Plants* **22**(4), 10–19.

Ali, H., Ayub, G., Elahi, E., Shahab, M., Ahmed, S. & Ahmed, N. 2015. Response of coriander (Coriandrum sativum L.) to different nitrogen levels and sowing dates. *Asian Journal of Agriculture and Biology* **3**(4), 155–158.

Aziz, E. & El-Ashry, S. 2009. Efficiency of slow release urea fertilizer on herb yield and essential oil production of lemon balm (*Melissa officinalis* L.) plant. *American-Eurasian Journal of Agricultural and Environmental Science* **5**, 141–147.

Bailey, K., Gossen, B., Lafond, G., Watson, P. & Derksen, D. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: univariate and multivariate analyses. *Canadian Journal of Plant Science* **81**, 789–803. doi: 10.4141/P00-152

BDS EN ISO 6571. 2010. Spices, condiments and herbs – Determination of volatile oil content (hydrodistillation method) (ISO 6571:2008). European standards, Bulgaria.

BDS ISO 10390:2011. 2011. Soil quality - Determination of pH. International standards, Bulgaria.

BDS ISO 11261:2002. 2002. Soil quality - Determination of total nitrogen - Modified Kjeldahl method. International standards, Bulgaria.

BDS ISO 11263:2002. 2002. Soil quality - Determination of phosphorus - Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution. International standards, Bulgaria.

BDS ISO 14235:2002. 2002. `Soil quality – Determination of organic carbon by sulfochromic oxidation`. International standards, Bulgaria.

Berzsenyi, Z. & Gyorffy, B. 1997. Effect of crop–rotation and fertilization on wheat yields and yield stability in long-term experiments. *Novenytermeles* **46**, 145–161.

Bullock, D. 1992. Crop rotation. *Critical Reviews in Plant Sciences* **11**, 309–326. doi: 10.1080/07352689209382349

Carrubba, A. 2009. Nitrogen fertilisation in coriander (*Coriandrum sativum* L.): a review and meta analysis. *Journal of the Science of Food and Agriculture* **89**(6), 921–926. doi: 10.1002/jsfa.3535

Carrubba, A., la Torre, R., Prima, A.D., Saiano, F. & Alonzo, G. 2002. Statistical analyses on the essential oil of Italian coriander (*Coriandrum sativum* L.) fruits of different ages and origins. *Journal of Essential Oil Research* **14**(6), 389–396.

Delibaltova, V., Kirchev, Hr., Zheliazkov, I. & Yanchev, I. 2012. Influence of predecessor and sowing rate on seed yield and yield components of coriander (*Coriandrum sativum* L.) in Southeast Bulgaria. *Bulgarian Journal of Agricultural Science* **18**, 315–319.

Dubey, V., Bhalla, R. & Luthra, R. 2003. Sucrose mobilization in relation to essential oil biogenesis during palmarosa (*Cymbopogon martinii* Roxb. Wats. var. motia) inflorescence development. *Journal of Biosciences* **28**(4), 479–487.

Dyulgerov, N. & Dyulgerova, B. 2016. Effect of some meteorological factors on main breeding traits in coriander. *Bulgarian Journal of Crop Science* **53**(5/6), 6066.

Erdoğdu, Y. & Esendal, E. 2018. The effects of nitrogen doses on the seed yield and some agronomic characteristics of coriander cultivars. *Journal of Tekirdag Agricultural Faculty* **15**(1), 95–101.

GOST 26209-91. 1993. Determination of mobile compounds of phosphorus and potassium by Egner-Riem method (DL-method). Technical standards GOST. (in Russian).

Gujar, S., Warade, A., Mohariya, A. & Paithankar, D. 2005. Effect of dates of sowing and nitrogen levels on growth, seed yield and quality of coriander. Crop Research (Hisar) 29(2), 288–291.

Hendawy, S. & Khalid, K. 2011. Effect of chemical and organic fertilizers on yield and essential oil of chamomile flower heads. *Medicinal and Aromatic Plant Science and Biotechnology* **5**(1), 43–48.

Hilton, S., Bennett, A., Chandler, D., Mills, P. & Bending, G. 2018. Preceding crop and seasonal effects influence fungal, bacterial and nematode diversity in wheat and oilseed rape rhizosphere and soil. *Applied Soil Ecology*, 1–13.

Izgi, M. 2020. Effects of Nitrogen fertilization on coriander (*Coriandrum sativum* L.): Yield and quality characteristics. *Applied Ecology and Environmental Research* **18**(5), 7323–7336. doi:10.15666/aeer/1805_73237336

Khalid, A. 2013. Effect of nitrogen fertilization on morphological and biochemical traits of some Apiaceae crops under arid region conditions in Egypt. *Bioscience* **5**(1), 15–21. doi: 10.13057/nusbiosci/n050103

Kizil, S. & Ipek, A. 2004. The effects of different row spacing and nitrogen on yield, yield components and essential oil content of some coriander (*Coriandrum sativum* L.) lines. *Journal of Agricultural Science* **10**(3), 237–240.

Koeduka, T., Fridman, E., Gang, D., Vassao, D., Jackson, B., Kosh, Ch., Orlova, I., Spassova, S., Lewis, N., Noel, J., Baiga, T., Dudareva, N. & Pichersky, E. 2006. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *PNAS* **103**(26), 10128–10133.

Kumar, A., Singh, R. & Chhillar, R. 2008. Influence of omitting irrigation and nitrogen levels on growth, yield and water use efficiency of coriander (*Coriandrum sativum* L.). *Acta Agronomica Hungarica* **56**(1), 69–74. doi: 10.1556/AAgr.56.2008.1.7

Kumar, K., Singh, G.P., Singh, N., Bhatla, A. & Nehra, B. 2007. Performance of seed crop of coriander under different levels of row spacing, nitrogen and cycocel. *Haryana Journal of Horticultural Sciences* **36**(1 and 2), 127–128.

Lattoo, S., Dhar, R., Dhar, A., Sharma, P. & Agarwal, S. 2006. Dynamics of essential oil biosynthesis in relation to inflorescence and glandular ontogeny in *Salvia sclarea*. *Flavour and Fragrance Journal* **21**, 817–821.

Lenardis, A., Fuente, E., Gil, A. & Tubra, A. 2000. Response of coriander (*Coriandrum sativum* L.) to nitrogen availability. *Journal of Herbs, Spices and Medicinal Plants* **7**(4) 47–58. doi: 10.1300/J044v07n04_06

Lokhande, S., Jogdande, N. & Thakare, S. 2015. Effect of varying levels of nitrogen and hosphorus on growth and seed yield of coriander (*Coriandrum sativum*). *Plant Archives* **15**(1), 57–59.

Mamolos, A. & Kalburtji, K. 2001. Significance of allelopathy in crop rotation. *Journal of Crop Production* **4**, 197–218. doi: 10.1300/J144v04n02_06

Mandal, S. & Mandal, M., 2015. Coriander (*Coriandrum sativum* L.) essential oil: Chemistry and biological activity. *Asian Pacific Journal of Tropical Biomedicine* **5**(6), 421–428.

Marschner, H. 2011. *Marschner's mineral nutrition of higher plants*. Academic press. London, 651 pp.

Moosavi, G., Seghatoleslami, M., Ebrahimi, A., Fazeli, M. & Jouban, Z. 2013. The effect of nitrogen rate and plant density on morphological traits and essential oil yield of coriander. *Journal of Ornamental and Horticultural Plants* **3**(2), 95–103.

Nadeem, M., Anjum, F., Khan, M., Tehseen, S., El-Ghorab, A. & Sultan, J. 2013. Nutritional and medicinal aspects of coriander (*Coriandrum sativum* L.): A review. *British Food Journal* **115**(5), 743–755. doi: 10.1108/00070701311331526

Neshev, N. 2022. Preceding crop influences on the development and yields of the winter oilseed rape (*Brassica napus* L.). *Agricultural Sciences* **14**(32), 56–62. doi: 10.22620/agrisci.2022.32.009

Nurzyńska-Wierdak, R. 2013. Does mineral fertilization modify essential oil content and chemical composition in medicinal plants? Acta Scientarum Polonorum, Hortorum Cultus 12(5), 3–16.

Okut, N. & Yildirim, B. 2005. Effect of different row spacing and nitrogen doses on certain agronomic characteristics of Coriander (*Coriandrum sativum* L.). *Pakistan Journal of Biological Sciences* **8**(6), 901–904. doi: 10.3923/pjbs.2005.901.904

Oliveira, A., Alves, E., Bruno, R., Sader, R. & Alves, A. 2006. Yield and quality of coriander seeds in function of nitrogen levels. *Revista Brasileira de Sementes* **28**(1), 193–198. doi: 10.1590/s0101-31222006000100027

Oliveira, A., Paiva Sobrinho, S., Barbosa, J., Ramalho, C. & Oliveira, A. 2003. Rendimento de coentro cultivado com doses crescentes de N. *Horticultura Brasileira* **21**(1), 81–83. doi: 10.1590/S0102-05362003000100017

Olle, M. & Bender, I. 2010. The content of oils in umbelliferous crops and its formation. *Agronomy Research* **8**(Special Issue III), 687–696.

Patel, C., Amin, A. & Patel, A. 2013. Effect of varying levels of nitrogen and sulphur on growth and yield of coriander (*Coriandrum sativum* L.). *The Bioscan* **8**(4), 1285–1289.

Rahimi, A., Mashayeki, K., Hemati, K. & Dordipoor, A. 2009. Effect of salicylic acid and mineral nutrition on fruit yield and yield components of coriander (*Coriandrum sativum* L.). *Journal of Plant Production* **16**(4), 149–156.

Reddy, K. & Rolston, M. 1999. Coriander (*Coriandrum sativum* L.) seed production: nitrogen, row spacing, sowing rate and time of sowing. *Journal of Applied Seed Production* **17**, 49–53.

Sieling, K., Stahl, C., Winkelmann, C. & Christen, O. 2005. Growth and yield of winter wheat in the first 3 years of a monoculture under varying N fertilization in NW Germany. *European Journal of Agronomy* **22**, 71–84. doi: 10.1016/j.eja.2003.12.004

Skudra, I. & Ruza, A. 2019. Effect of nitrogen fertilization management on mineral nitrogen content in soil and winter wheat productivity. *Agronomy Research* **17**(3), 822–832.

Stoddard, F. 2010. Improving food and feed security in the Nordic and Baltic region by using appropriate crop rotations. *Agronomy Research* **8**(Special Issue II), 311–316.

Szempliński, W. & Nowak, J. 2015. Nitrogen fertilization versus the yield and quality of coriander fruit (*Coriandrum sativum* L.). *Acta Scientiarum Polonorum Hortorum Cultus* **14**(3).

Weiss, E.A. 2002. Coriander. In Weiss, E.A. (eds). *Spice Crops*, CABI Publishing, Wallingford, 243–261.

Zaitsev, A. & Kovalenko, I. 2020. The influence of crop predecessor and chemicalization levels on grain quality and spring wheat productivity. *IOP Conference Series: Earth and Environmental Science* **548,** 072014. doi: 10.1088/1755-1315/548/7/072014

Zheljazkov, V., Cantrell, C., Astatkie, T. & Ebelhar, M. 2010. Peppermint productivity and oil composition as a function of nitrogen, growth stage, and harvest time. *Agronomy Journal* **102**(1), 124–128.

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The system of soil protection and general balance of main nutrient elements in perennial plantations of semi-desert natural soil zone of Armenia

S.S. Harutyunyan*, L.G. Matevosyan, A.G. Ghukasyan and M.H. Galstyan

Scientific Centre of Agriculture, Isi-le-Mulino 1, AM1101 Ejmiatsin, Armavir region, Armenia
*Correspondence: ss_harutyunyan@mail.ru

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Abstract. The aim of the research is to study the biological removal of the main nutrient elements from the most common technical grape varieties, as well as from apricot and peach plantations in the farms, situated on semi-desert natural soils of Armenia, to identify the extent of their input and losses due to natural factors and to calculate the balance associated with the soil conservation system in the absence of comprehensive fertilization. In the inter-row spaces of all fruit plantations and even vineyards of the republic, grass cover of productive significance has been established (4.5–6.5 t ha^{-1} yield of air-dry grass), through which the removal of nutrient elements is 2–3 times higher than the biological removal through trees and vines. The research was conducted in 2015–2020, in the grape, apricot and peach plantations of the semi-desert natural zone of Armenia (Armavir region), where the irrigation norm is 5,000 m^3 ha^{-1}, and the atmospheric precipitation is 256 mm, through which 40 kg ha^{-1} N, 2 kg ha^{-1} P_2O_5, and 44 kg ha^{-1} K_2O enter the soil. The losses due to erosion and washing away are N - 12 kg ha^{-1}, P_2O_5 - 7 kg ha^{-1}, K_2O - 75 kg ha^{-1}. The balance of nutrient elements in all plantations is negative, nitrogen in plantations with industrial grass cover is 154, P_2O_5 - 52, K_2O - 311, and in the system of black fallow - 15, 16 and 85 kg ha^{-1}, respectively. The negative balance of nitrogen in apricot and peach plantations is 121, P_2O_5 - 44, K_2O - 296 kg ha^{-1}.

Key words: grass stand, black fallow, grape vine, apricot tree, peach tree, balance.

INTRODUCTION

The nutrient balance in soils reflects their general agrochemical characteristics, being the primary condition for the rational use of organic fertilizers and for increasing soil fertility, and is one of the important principles for ensuring the ecological safety of plant products and the environment. In agrochemical research, the balance approach has intensified, especially with the intensification of agriculture and with the addition of fertilizers per unit area, which resulted in a positive nitrogen-phosphorus balance in Russia, Ukraine, Belarus, Moldova, and Eastern Europe in the 1980s (Dasberg et al., 1984; Donos, 1988; Shafran & Korchaghina, 1990; Ladonin & Velichko, 1994; Mineyev, 2000). Studies conducted in Armenia have shown that in 1976–1983 nitrogen-phosphorus balance in arable land and perennial plantations was +61.8 and +38.2 and K_2O was negative (-22.5 kg ha^{-1}) (Avagyan, 1980; Babayan, 1980, 1985).

After the break-up of the Soviet Union, unprecedented changes took place in the agro-complex of Armenia: privatization of lands and agricultural objects, as a result of which 340 thousand farms were formed, natural plots (about 150 thousand) were divided into 1200 thousand small plots, where crop rotation, irrigation and application of mechanisms became very difficult. The use of organic fertilizers has been sharply reduced, as a result of which the deficit of nitrogen, phosphorus and potassium in all agricultural soils is increasing year by year, the yield of crops has been significantly reduced. Prior to land privatization (1991), the agricultural system in Armenia was intensive. The annual use of mineral fertilizers was 350 thousand tons and 1.8 million tons of local organic fertilizers, which contained 57,000 tons of N, 33,300 tons of P_2O_5 and 24,300 tons of K_2O (per 100% of active substance), providing arable lands and perennial plantations with $N_{100}P_{58}K_{43}$ kg ha^{-1} doses (Harutyunyan & Sabeti, 2012). After the privatization of the land the use of organic fertilizers became spontaneous. Every year an average of 30–40 thousand tons of ammonium nitrate (in physical weight) is used, but not by all farmers, and phosphorus-potassium fertilizers are imported in very limited quantities by large landowners for their needs.

The deficit of nutrient elements is also deepening in Russia, where the largest amount of fertilizers was used in the 1980s - about 13 million tons of NPK, which was only 60% of the real demand. Since 1990, the use of mineral fertilizers in the Russian Federation has been reduced thrice, and of organic fertilizers 2.5 times, as a result of which the balance of nutrient elements in the soil has become negative, leading to a sharp decline in arable land efficiency (Milashchenko, 1999; Mineyev, 2000).

The negative balance of nutrients in Russia's arable land continues to grow. If in 2011 the negative nitrogen balance was 34–50 per year, P_2O_5 was 9–16, K_2O was 38–64 kg ha^{-1} (Kudeyarov & Semenov, 2014), then in 2016 the deficit was 63.5 kg, 23 and 62.2 kg ha^{-1}, respectively, for 18 kg of nitrogen, 6 kg of phosphorus and 5 kg of potassium per hectare of active substance (Kudeyarov, 2018). Almost the same result is recorded in the lands of the non-chernozem zone of Russia in 2011–2013, where the balance of basic nutrients was negative. nitrogen - 22, phosphorus - 6, potassium - 31 kg ha^{-1}, and the compensation was 40, 45 and 21%, respectively (Shafran, 2016). According to the author, in the arable lands of Russia in 2018 16 kg ha^{-1} of nitrogen was used, and the removal through harvest was 42 kg ha^{-1}, deepening the negative balance to - 26 kg ha^{-1} (Shafran, 2020).

At present, Armenia pays no attention to soil protection systems, to the ecological situation of the environment and the consumption of soil humus and nutrient elements. During the last 30 years, detailed studies have been conducted in different regions of Armenia on different varieties concerning the removal of the main nutrient elements from grape and fruit plantations, as well as from the fields of clean-cultivated crops. As a result, large variations in the removal of nutrient elements were detected, depending on the doses of fertilizers, irrigation factor, and the size of the vegetative mass (Harutyunyan & Harutyunyan, 1985; Harutyunyan, 1995; Harutyunyan, 1996; Harutyunyan & Harutyunyan, 2002; Harutyunyan & Grigoryan, 2005, Harutyunyan & Sabeti, 2012; & Miqaelyan, 2021).

Purpose of the research and problems

The aim of the research is to study the reasons of decrease in soil fertility and crop yield in perennial plantations of Armenia, depending on soil protection system. For this reason it is necessary to specify the extent of biological removal of main nutrients from the most common technical varieties of grapes, as well as apricot and peach plantations. It is necessary to calculate the entrance and losses of nitrogen, phosphorus and potassium, as well as their balance due to natural factors, in the absence of fertilization.

The scientific novelty of the work is directly connected with the protection of the balance of main nutrients and soil fertility in perennial plantations. The results of the research will enable us to rely on the balance of main nutrients in different phytocenoses and to work out an optimal system of fertilization, which will prevent the decline of soil fertility and dehumification. This is the utilitarian goal of the study.

Materials and methods

Field experiments were conducted in 2015–2020 in the semi-desert zone (236.2 thousand hectares), one of the most favorable natural areas for grape, apricot and peach plantations cultivated in Armenia, which occupies the lowlands of the foothills of Ararat valley, Ararat, Armavir, Aragatsotn, Kotayk districts (800–1,250 m above sea level), where the climate is very dry, with cold winters and hot summers. The average annual air temperature is 10–12 °C, the sum of temperatures above 10 °C is 3,800–4,200 °C, the average annual precipitation is 230–300 mm, the humidity coefficient is 0.3. Four soil types have been formed in this zone: semi-desert gray (152.2 thousand ha), irrigated meadow gray (53 thousand ha), saline-alkaline (29 thousand ha), paleohydromorphic (2 thousand ha). Perennial plantations are based exclusively on semi-desert gray soils, located in the communities of Armavir region, where the irrigation norm is 5,000 m^3 ha^{-1}. Irrigation is provided by Nerkin Hrazdan and Talin canals.

The vineyards are 10–25 years old with a feeding area of 1.5: 2.5 m. The white grape variety ‘Rkatsiteli’ is cultivated in Aragats community, the red variety ‘Anahit’ and the black variety ‘Haghtanak’ are cultivated in Aghavnatun community, the white variety ‘Kangun’ and the black variety ‘Haghtanak’ are cultivated in Talvorik community. The vines are trained in multi-trunk fan system, raised on the supporting wires. The vines are buried in winter, and the spring pruning is done according to the strength. The mixed plantation of apricots (Yerevan and Sateni varieties) is about 25–30 years old, the feeding area of the trees is 8 : 6 m, the mixed peach plantation (varieties Mijahas Narinj and Limoni) is 12 years old, the feeding area of the trees is 5 : 4 m. The apricot and peach plantations are situated in the community (village) Mrgashat (All the communities are in the Armavir region). The founding of mixed plantations is associated with tree pollination. The yield from the vines and trees, pruned and leaf masses were calculated on 45 vines and 30 trees, and the grass was calculated on about 500 m^2 (the hay was removed with the help of a hand scythe, as hay-cutting machines are not available in these farms).

Soil sampling in all plantations was done according to genetic horizons (up to the depth of active root propagation), irrigation water samples were taken from the canals entering the plantations. The total data of the last 6 years of atmospheric precipitation were taken from the agrometeorological station of Armavir city. The yield of vines and trees, mass of spring prunings, mass of green cuttings, leaf mass and the mass of harvested hay was calculated by the weight method. The samples were made air-dry in the laboratory. Soil, water and plant analyses were performed in the agrochemistry laboratory of the Agricultural Science Center. The balance of main nutrient elements was calculated by the method accepted in agronomy (Yurkin, 1974; Guidelines for compiling the balance..., 1975).

Physico-mechanical and agrochemical analyses of soils were performed by well-known methods (Alexandrova & Naidionova, 1976; Workshop on agricultural chemistry by Yagodin, 1987). The data on the pH of the water content of the dry matter, as well as total nitrogen, were not included in Table 1, so as not to overload it, but their values are presented in the text. Soil analyses were performed on samples sifted with a 1 mm sieve. The mechanical composition was determined by the classical pipette method and evaluated according to the N.A. Kachinsky classification scale. The pH of the water extract which characterizes the actual acidity of the soil, was determined by the potentiometric method, and the dry residue by the weight method. Carbonates were determined by calcimeter using 10% HCl. Humus was determined by the I.V. Turin method, total nitrogen by the Kjeldal method. The easily hydrolyzable nitrogen in the soil was determined by I.V. Turin and M.M. Kononova methods, which is considered to be an indicator of mobile nitrogen compounds, and the mobile forms of phosphorus and potassium by the Machigin method, which is based on the principle of removing them by a 1% $(NH_4)_2SO_4$ solution.

Total nitrogen, P_2O_5 and K_2O in plant samples were determined by K. Ginsburg wet ash method, after which nitrogen was determined by the Kjeldal micromethod, P_2O_5 by photoelectric calorimeter, and K_2O by flame photometer (Workshop on agricultural chemistry by Yagodin, 1987). The content of nitrogen, phosphorus and potassium in atmospheric precipitation and irrigation water was determined according to the instruction developed by O.B. Gasparyan (1970).

RESULTS AND DISCUSSION

The soils of the test site belong to the semi-desert gray soil type. The data in Table 1 show that the mechanical composition in these soils (the sum of < 0.01 mm particles or physical clay) in different genetic horizons according to N.A. Kachinsky classification (Soil science edited by Kaurichev, 1982) is rated as light-clay-sandy (20–30%), medium-clay-sandy (30–45%), heavy-clay-sandy (45–60%), and only in the horizon C_1 of the peach plantation (107–140 cm) it is considered light-clayey (63.5%). In all the mentioned soils, the pH of the water extract is alkaline and is in the range of 8.1–8.6, and the dry residue of the water extract in the upper humus horizons A and B is 0.09–0.21%, i.e. the content of harmful salts (Na_2CO_3, $NaHCO_3$, NaCl, Na_2SO_4) does not represent any danger to the plants. The total nitrogen content in the soil profile is also low, ranging from 0.02 to 0.097%, which is primarily due to the low humus content. As for carbonates, their content in the horizons A and B of the studied lands (except for the Talvorik test field: 14.5–15.4%) is low, ranging from 1.1 to 5.4%. The humus content in the soil horizons A and B is rather low due to the active mineralization of organic matter at high temperatures.

The soils of the test field according to Turin and Kononova scale have very low (< 3) and low (4 mg per 100 g soil) nitrogen content (mobile or available to plants). The upper horizons of soils, studied according to the Machigin grouping, are also assessed at the same levels by their content of P_2O_5: very low (< 1), low (1–1.5) and medium

(1.5–3 mg per 100 g in soil). The content of mobile forms of nitrogen and phosphorus in the soil depends on both the degree of mineralization of organic matter and the hydrolysis and oxidation of mineral compounds, where the microbiological activity of the soil plays an important role. As for K_2O, test soils have high (40–60) and very high (> 60 mg per 100 g soil) levels according to the Machigin scale (Workshop on agricultural chemistry..., 1987).

Table 1. Physico-mechanical and agrochemical characteristics of test site soils

Community, plantation and variety	Genetic horizon and depth, cm		< 0.01 mm - sum of particles (phys. clay), %	$CaCO_3$ according to CO_2, %	Humus, %	Mobile forms mg per 100 g in the soil		
						N	P_2O_5	K_2O
Aragats, grape (Rkatsiteli, white variety)	A	0–36	46.1	1.5	1.57	4.20	1.95	85
	B_1	37–67	35.7	2.3	0.98	2.52	2.10	70
	B_2	68–85	24.3	5.4	0.83	2.24	1.57	68
	C	86–120	21.3	8.2	0.77	1.40	0.82	60
Aghavnatun, grape (Anahit - red), (Haghtanak - black)	A	0–35	44.4	2.7	1.58	3.92	1.68	75
	B	36–62	37.0	4.9	0.54	3.36	1.64	71
	C	63–130	27.9	8.7	0.50	2.24	1.33	57
Talvorik, grape (Kangun - white), (Haghtanak - black)	A	0–46	35.4	14.5	1.68	3.84	2.81	53
	B	47–65	37.2	15.4	1.52	3.61	1.73	46
	C	66–107	38.6	10.9	1.08	2.29	1.02	48
Mrgashat, apricot tree (Yerevani, Sateni) mixed plantation	A	0–24	47.5	1.1	1.26	3.61	2.10	80
	B_1	25–46	43.8	-	0.79	3.18	1.04	88
	B_2	47–71	35.4	-	0.34	3.00	0.62	73
	C_1	72–102	34.4	1.5	0.33	2.82	0.56	73
Mrgashat, peach tree (Narinj mijahas, Limoni) mixed plantation	A	0–33	41.9	1.5	1.54	3.84	1.90	67
	B	34–70	45.3	2.1	0.89	3.40	1.56	64
	C_1	71–107	59.3	-	0.78	3.15	0.56	65
	C_2	108–140	63.5	1.1	0.70	2.62	0.50	67

The average yield of 3 years from grape plantations of different varieties varies considerably in different communities, which in our observations depends not only on the characteristics of the variety, but also on the individual approaches of the owner farmer, the effectiveness of disease and pest control, as well as the irrigation factor (Table 2). From this point of view, Aghavnatun community is well supplied with irrigation water, as it is located at the bottom of Nerkin Hrazdan canal. From the data in Table 2 it can be seen that despite the rather large differences in the yields of the studied grape varieties, in case of recalculation of 10.0 t ha^{-1}, the weight of grape juice fluctuates in the approximate range 8.8. t ha^{-1} (Hakhtanak) - 9.57 t ha^{-1} (Kangun). In case of recalculation of 10.0 t ha^{-1} yield, there are bigger differences between grape stems (65.8 Kangun - 149.9 Hakhtanak kg ha^{-1} air-dry) and seeds (239.9 Hakhtanak - 293.1 Rkatsiteli kg ha^{-1} air-dry), which is definitely conditioned with varietal features. However, it should also be noted that the masses of the yield of the variety Hakhtanak, as well as the masses of their stems and seeds cultivated in two different places (Aghavnatun and Talvorik) are quite different, which in our opinion is conditioned by the level of agro-technical measures and violations in the irrigation water regime. The mass of the spring prunings

(1,292–1,452 kg ha^{-1}, air-dry) leaving the above-mentioned grape plantations, as well as the mass of green cuttings (275–398 kg ha^{-1}, air-dry) are quite close in different varieties, which is due to their almost identical growth intensity. It can also be seen from the table that at the end of the vegetation the leaf mass of the black variety Hakhtanak exceeds the leaf mass of the white varieties.

Table 2. Annual biomass removed from grape plantations (average of 2018–2020)

Community, variety and soil conservation system	Commodity yield and its structural elements				Air-dry, kg ha^{-1}			
	Yield, t ha^{-1}	Juice with peels, t ha^{-1}	Dry stem (air-dry), kg ha^{-1}	Seed (air-dry), kg ha^{-1}	Mass of spring prunings	Mass of green cuttings	Leaf mass	Mown hay
Aragats (Rkatsiteli), natural grass	5.48	5.20	43.5	160.6	1,452	275	741	6,580
Aghavnatun (Anahit), natural grass	14.89	13.77	162.7	375.4	1,448	367	798	6,667
Aghavnatun (Haghtanak), natural grass	21.18	19.36	249.8	508.2	1,292	295	854	6,533
Talvorik (Kangun), black fallow	6.69	6.40	44.0	163.5	1,352	345	814	-
Talvorik (Haghtanak), black fallow	10.04	8.84	150.5	290.2	1,350	398	1,029	-

The data in Table 3 show that the three-year average yield from the apricot plantations was 13.0 t ha^{-1} and from the peach plantation it was 10.2 t ha^{-1}. It should also be noted that with proper implementation of agro-technical measures, the yield from apricot and peach plantations in Armenia can reach 20–25 t ha^{-1}, which was often recorded in the 1980s. Nitrogen is the first limiting nutrient element for almost all crops in the soils of Armenia. It is not surprising that the N + manure combination provides the highest quality and high yield of peaches in other countries, too (Chatzitheodorou, et al., 2004). In case of recalculation on 10 t ha^{-1} of apricot, the pulp is 9.45 t ha^{-1}, the average mass of the kernel is 118.0 kg ha^{-1}, the shell of the kernel is 233.0 kg ha^{-1} (air-dry), while in the case of peaches these indices are respectively 9.08 t ha^{-1}, 30.6 and 389.0 kg ha^{-1} (air-dry). The pruned mass removed from the apricot plantation was about 200 kg more than the mass leaving the peach plantation in the spring, and the leaf mass in the peach plantation was about 110 kg more, which is due to both the size of the trees and the differences in their feeding surface. In addition, the wood of apricot trees is heavier than that of peach trees.

The differences in the masses of hay removed from grape and fruit plantations (Tables 2 and 3) are mainly due to the amount of harvested hay. In the inter-row spaces of grape plantations, hay is harvested 3 times: in late May, mid-July and mid-September (before harvest), and about 6.5 t ha^{-1} of dry grass is obtained. In apricot and peach plantations, hay is harvested twice (in mid-June, before the apricot harvest, and in late August, before the peach harvest), because the trees have more shading, and the resulting grass is about 4.5 t ha^{-1}.

Table 3. Annual biomass removed from apricot and peach plantations (average of 2015–2017)

Community and fruit type	Commodity yield and its structural elements				Air-dry, kg ha^{-1}		
	Yield, t ha^{-1}	Pulp, t ha^{-1}	Stone kernel (air-dry), kg ha^{-1}	Stone shell (air-dry), kg ha^{-1}	Mass of spring prunings	Leaf mass at the end of vegetation	Mown hay
Mrgashat, apricot tree (mixed plantation)	13.0	12.29	154.3	302.9	1,690	2,426	4,666
Mrgashat, peach tree (mixed plantation)	10.2	9.26	31.2	396.8	1,480	2,540	4,673

Removal of nitrogen and ash elements from the soil by means of crop and vegetative mass is considered to be the main article in the loss of nutrient balance.

Numerous studies show that the amounts of nitrogen, phosphorus and potassium removed from the soil depend on the crop variety characteristics, selective properties, yield level, soil-climatic conditions, soil moisture and agrotechnics, fertilizer doses, their ratio and other factors. The amount of nitrogen, phosphorus and potassium removed through 1 t of commodity and corresponding non-commodity products of different agricultural crops varies in a wide range, thus: autumn wheat: N - 24–27, P_2O_5 - 10–13, K_2O - 18–23 kg ha^{-1}, barley - 22–24, 9–10, 17–23, corn - 2–3, 1–2, 2–3, clover (grass) - 18–22, 5–6, 14–18, sugar beet - 4–6, 1–1.5, 7, potatoes - 4–6, 1.5–1.6, 4–7, raw cotton - 45, 15, 45, tomatoes - 2.1, 1.19, 3.21, pepper - 2.39, 1.65, 3.33, respectively (Harutyunyan & Miqaelyan, 2021; Krivenya, 1977; Marinchik & Zakharova, 1987; Peterburgsky, 1979).

54–110 kg ha^{-1} of nitrogen, 17–40 kg ha^{-1} phosphorus, 70–130 kg ha^{-1} potassium are removed from the soil annually through the vegetative mass of 10.0 t ha^{-1} of different varieties of grapes (Bondarenko, 1986; Malakhova, 1980; Merzhanyan, 1951; Serpukhovitina, 1982; Walessky & Kononikhina, 1983). In a number of European countries, grape and fruit plantations are founded on poor, rocky, sloping lands with mandatory grass cover in the intercropping space, which is a significant land conservation measure. However, the amount of nutrient elements removed from plantations with industrial grass cover is 2–5 times higher than the amount of nutrient elements removed from black fallow. And if the harvested grass remains in its place, the fertilizers are applied superficially, which preserves the quality of the grapes (Batukayev, 1999; Novosadyuk, 1988).

The content of main nutrient elements in different organs of grapes varies to a large extent, while the concentration of nitrogen in the same organ is 2–5, and that of K_2O is 2–10 times higher than the concentration of P_2O_5, due to which nitrogen and potassium are removed in larger quantities (Table 4). The data from Table 4 show that the lowest content of nutrient elements is present in the grape juice, where nitrogen content fluctuates between 0.076–0.102%, that of P_2O_5 - 0.029–0.045% and K_2O - 0.23–0.39%, which causes the largest removal of potassium by the clusters. The nitrogen content in the mass of spring prunings in different varieties varies in the range of 0.55–0.72%, P_2O_5 - 0.17–0.21%, K_2O - 0.58–1.22%, and the removal is definitely conditioned by the size of that mass. The content of nutrient elements in the green cuttings and leaf mass is significantly higher than their content in the trimmed mass, which leads to greater removal of these elements. As for natural grass, it has the highest content of nutrient elements, so their removal by this mass is 2–3 times greater than the amount removed by the vine. The data in Table 4 show that the nitrogen removed by the vines (taking into account the yield level) ranges from 29.1 to 55.4 kg ha^{-1}, P_2O_5 - 8.6–19.2 and K_2O - 34.4–110.0 kg ha^{-1}, while with the grass, respectively, 138.2–143.3, 31.4–36.0 and 209.7–216.5 kg ha^{-1} of nutrient elements are removed. In case of recalculation of 10.0 t ha^{-1} grape harvest and the corresponding vegetative mass, nitrogen removal (average of five field experiments) is 43.2, P_2O_5 - 12.9 and K_2O - 67.8 kg ha^{-1}, which can be compensated by organic mineral fertilizers in the conditions of black fallow.

Table 4. Biological removal of main nutrient elements from grape plantations (according to the initial data of Table 2)

Commu-nity, variety and soil conservation system	Nutrient elements	Biomass (numerator %, denominator kg ha^{-1})							Removed, sum, kg ha^{-1}
		Juice with pulp	Dry stem (air-dry)	Seed (air-dry)	Mass of spring pru-nings (air-dry)	Mass of green cut-tings (air-dry)	Leaf mass (air-dry)	Mass of mown hay (air-dry)	
Aragats (Rkatsi-teli), natural grass	N	0.078 / 4.06	1.05 / 0.46	1.59 / 2.55	0.60 / 8.71	1.38 / 3.80	1.28 / 9.48	2.10 / 138.2	167.3
	P_2O_5	0.045 / 2.34	0.28 / 0.12	0.82 / 1.31	0.21 / 3.04	0.28 / 0.77	0.32 / 2.37	0.50 / 32.9	42.8
	K_2O	0.32 / 16.64	2.25 / 0.98	0.56 / 0.90	0.58 / 8.42	1.32 / 3.63	1.35 / 10.0	3.29 / 216.5	257.1
Aghav-natun (Anahit), natural grass	N	0.076 / 10.47	0.92 / 1.50	1.31 / 4.92	0.61 / 8.83	1.27 / 4.66	2.05 / 16.36	2.15 / 143.3	190.0
	P_2O_5	0.030 / 4.13	0.29 / 0.47	0.65 / 2.44	0.17 / 2.46	0.25 / 0.92	0.37 / 2.95	0.54 / 36.0	49.4
	K_2O	0.36 / 49.57	2.48 / 4.03	0.61 / 2.29	1.22 / 17.67	1.33 / 4.88	1.10 / 8.78	3.19 / 212.7	299.9
Aghav-natun (Haghta-nak), natural grass	N	0.095 / 18.39	1.07 / 2.67	1.77 / 9.00	0.55 / 7.11	1.39 / 4.10	1.65 / 14.09	2.14 / 139.8	195.2
	P_2O_5	0.040 / 7.74	0.36 / 0.90	0.71 / 3.61	0.17 / 2.20	0.35 / 1.03	0.44 / 3.76	0.48 / 31.4	50.6
	K_2O	0.37 / 71.63	4.22 / 10.54	0.57 / 2.90	0.67 / 8.66	1.62 / 4.78	1.37 / 11.70	3.21 / 209.7	319.9
Talvorik (Kangun), black fallow	N	0.082 / 5.25	0.76 / 0.33	1.52 / 2.49	0.72 / 9.73	1.53 / 5.28	1.51 / 12.29	_	35.4
	P_2O_5	0.029 / 1.86	0.24 / 0.11	0.55 / 0.90	0.21 / 2.84	0.31 / 1.07	0.22 / 1.79	_	8.6
	K_2O	0.23 / 14.70	2.49 / 1.10	0.56 / 0.92	0.59 / 7.98	1.29 / 4.45	0.64 / 5.21	_	34.4
Talvorik (Haghta-nak), black fallow	N	0.102 / 9.02	0.88 / 1.32	1.64 / 4.76	0.69 / 9.32	1.80 / 7.16	1.72 / 17.70	_	49.3
	P_2O_5	0.038 / 3.36	0.28 / 0.42	0.65 / 1,89	0.21 / 2.84	0.49 / 1.95	0.31 / 3.19	_	13.6
	K_2O	0.39 / 34.48	2.56 / 3.85	0.60 / 1.74	0.74 / 9.99	1.46 / 5.81	1.05 / 10.80	_	66.7

In Armenia, in apricot, peach, plum and other fruit plantations, including intensive apple plantations, alfalfa or natural mixed grass is also grown, which increases the removal of nutrient elements by about 50–70%. Table 5 presents the data on the biological removal of nutrient elements from apricot and peach plantations, which are widespread in Armavir region, according to which most of the nutrient elements are removed by the harvested hay, which makes 63% of nitrogen and 58% of phosphorus and potassium. In grassy vineyards and fruit plantations the stubble is regularly tilled into the ground. Studies in Russia during 2005–2010 show that in one year 24 kg ha^{-1} of nitrogen enters the soil through the stubble of a papilionaceous plants, 8.9 kg ha^{-1} from annual grasses and 21 kg ha^{-1} from perennial grasses (Zavalin & Blagoveshchenskaya, 2012).

Table 5. Biological removal of main nutrient elements from apricot and peach plantations (according to initial data in Table 3)

Community and fruit type	Nutrient elements	Biomass (numerator %, denominator kg ha^{-1}) Pulp	Stone kernel (air-dry)	Stone shell (air-dry)	Mass of spring prunings (air-dry)	Leaf mass at the end of vegeta-tion (air-dry)	Mown hay (air-dry)	Removed sum, kg ha^{-1}
Mrgashat, apricot tree (mixed plantation)	N	0.093 / 11.43	2.87 / 4.40	0.23 / 0.70	0.47 / 7.94	1.40 / 33.96	2.08 / 97.05	155.5
	P_2O_5	0.037 / 4.55	0.54 / 0.83	0.024 / 0.07	0.17 / 2.87	0.31 / 7.52	0.50 / 23.33	39.2
	K_2O	0.38 / 46.70	0.78 / 1.19	0.23 / 0.70	0.31 / 5.24	3.43 / 83.21	3.29 / 153.51	290.6
Mrgashat, peach tree (mixed plantation)	N	0.049 / 4.54	2.97 / 0.93	0.25 / 0.99	0.50 / 7.40	1.44 / 36.58	1.91 / 89.25	139.7
	P_2O_5	0.037 / 3.43	0.54 / 0.17	0.025 / 0.10	0.17 / 2.51	0.40 / 10.16	0.49 / 22.89	39.3
	K_2O	0.26 / 24.07	1.11 / 0.35	0.21 / 0.83	0.30 / 4.44	2.06 / 52.32	3.17 / 148.13	230.1

The removal of about 24% of the nitrogen, 22% of P_2O_5 and 26% of K_2O occurs on the average through the leaf mass, the nutrient elements removed by the cuttings and the harvest are 5, 7, 2 and 7, 12, 14%, respectively. In case of recalculation of 10.0 t ha^{-1} of the yield and corresponding vegetative mass in the plantations, the nitrogen removed from the apricot plantation is 120, P_2O_5 - 30, K_2O - 224 kg ha^{-1}, and from the peach plantation 137, 38 and 226 kg ha^{-1}, respectively (accepting the same levels of vegetative mass of trees and the removed grass).

The calculation of nutrient elements removed from grape and fruit plantations grown in Armenia is directly or indirectly related to irrigation water, as this factor is mandatory and is always present in any plant cultivation system. Irrigation not only determines the final amount of nutrient balance, but is also the most active source of plant nutrition, so in the balance calculations the amount of nutrient elements entering the plantations through atmospheric precipitation should be taken into account. According to the studies, 44 kg ha^{-1} of N, 0.45 kg ha^{-1} of P_2O_5 and 14.7 kg ha^{-1} of K_2O enter annually into the perennial plantations of the semi-desert natural soil zone of Armenia, and through irrigation water 3.64 kg ha^{-1}, 7.5 kg ha^{-1} and 50.2 kg ha^{-1}, respectively. In addition, 5.2 kg ha^{-1} of nitrogen is detected on the average by the bacteria living freely in the soils of the mentioned zone (Babayan, 1980).

During the years of our research (2015–2020) the average amount of atmospheric precipitation in Armavir region was 256 mm or 2,560 m^3 ha^{-1} (mainly in the form of rain), in which the total nitrogen was 2.74 mg L^{-1} (7.02 kg ha^{-1}), P_2O_5 0.30 mg L^{-1} (0.77 kg ha^{-1}) and K_2O 2.30 mg L^{-1} (5.88 kg ha^{-1}). The plantations of the test fields are irrigated by the waters of Nerkin Hrazdan (Aragats, Aghavnatun) and Talin (Talvorik, Mrgashat) canals. Nitrogen content in the waters of Nerkin Hrazdan was 6.09 mg L^{-1} (30.45 kg ha^{-1}), P_2O_5 - 0.331 mg L^{-1} (1.66 kg ha^{-1}), K_2O - 10.04 mg L^{-1} (50.20 kg ha^{-1}), and in the waters of Talin canal these indices were 5.40 (27.0), 0.150 (0.75) and 6.70 (33.5), respectively. The recalculation was done in kg ha^{-1} according to the irrigation norm of 5,000 m^3 ha^{-1}.

The loss of main nutrient elements from perennial plantations by erosion and washing in semi-desert soils is N - 9.2; P_2O_5 - 6.8; K_2O - 68.4 kg ha^{-1} and N - 3.2; P_2O_5 - none, K_2O - 6.3 kg ha^{-1} annually (Babayan, 1980): According to studies, the highest nitrogen loss through water erosion occurs from clean-cultivated crops and makes 13.2 kg ha^{-1} per year, and about 0.3 kg ha^{-1} from perennial grasses (Tsybylka et al., 2013).

Nitrogen gas losses from the soil are based on the use of nitrogen fertilizers, which have not been taken into account, as there is no comprehensive fertilization in the plantations.

Table 6 shows the balance sheet calculations of the main nutrient elements in the studied plantations, according to which the average annual nitrogen loss from grape plantations with grass cover is 196.6, P_2O_5: 54.5, K_2O - 367.0 kg ha^{-1}, and in the soil conservation system of black fallow it is 54.7, 17.9 and 125.3 kg ha^{-1}. The balance of main nutrients in non-fertilized grape plantations in Ganja-Kazakh region of Azerbaijan is negative (Mammadov, 2015). The total nutrient losses in the apricot and peach plantations are less (nitrogen: 152.1–167.9, P_2O_5: 46.0–46.1 and K_2O: 304.8–365.3 kg ha^{-1}), which is mainly due to the amount of grass removed from these plantations. As for the nutrient inflow, the main difference here is related to the irrigation water.

The data in Table 6 show that in the absence of annual use of organic-mineral fertilizers in grape and fruit plantations, the balance of all nutrient elements is negative, especially if the plantations are placed under the grass of industrial significance. Balance losses include nutrient elements in the leaf mass which do not leave the plant and compensates for their deficiency to some extent. Corresponding nutrient losses and inflows in perennial plantations determine the intensity and volume of the balance, which is a unique key for understanding their overall displacement.

CONCLUSIONS

1. The soils under the vineyards and fruit plantations studied in the Ararat Valley are considered strong soils (A + B = 62–85 cm), but low levels of humus (0.34–1.68%), available nitrogen (2.24–4.20 mg per 100 g in soil) and phosphorus (0.62–2.81 mg per 100 g in soil) are a limiting factor for plant growth and yield, which can be overcome by annual fertilization. The soils have a high supply of K_2O.

2. The annual amount of nitrogen released through the grass of industrial importance (about 6.5 t ha-1 air-dry) grown in the inter-row spaces of grapevines is 76.2%, P2O5 - 70.2%, K2O - 72.9%, and through 4.5 t ha-1 of air-dry grass leaving apricot and peach plantations the annual amount of nitrogen is 63.1%, P2O5 - 58.8%, K2O - 58.6%. That is, the main factor in deepening the nutrient deficiency is the grass grown in the mid-rows of plantations.

Table 6. Total annual balance of main nutrient elements in perennial plantations of semi-desert natural soil zone of Armenia (according to generalized and averaged data of tables 4 and 5)

Irrigation source and norm, $m^3\ ha^{-1}$	Community, plantation, soil conservation system and average yield ($t\ ha^{-1}$)	Nutrient elements	Losses, $kg\ ha^{-1}$			Entrance, $kg\ ha^{-1}$			Balance, $kg\ ha^{-1}$ + -	Balance intensity, %	Balance volume, $kg\ ha^{-1}$
			Total removal	Erosion and washing	Total	Atmospheric precipitation	Irrigation water	Total*			
Nerkin Hrazdan canal, 5,000	Aragats, Aghavnatun, grape (natural grass), 13.85	N	184.2	12.4	196.6	7.0	30.5	42.7	-153.9	21.7	239.3
		P_2O_5	47.6	6.8	54.4	0.8	1.7	2.5	-51.9	4.6	56.9
		K_2O	292.3	74.7	367.0	5.9	50.2	56.1	-310.9	15.3	423.1
Talin canal, 5,000	Talvorik, grape (black fallow), 8.36	N	42.3	12.4	54.7	7.0	27.0	39.2	-15.5	71.7	93.9
		P_2O_5	11.1	6.8	17.9	0.8	0.8	1.6	-16.3	8.9	19.5
		K_2O	50.6	74.7	125.3	5.9	33.5	39.4	-85.5	31.4	164.7
	Mrgashat, apricot tree (natural grass), 13.0	N	155.5	12.4	167.9	7.0	27.0	39.2	-128.7	23.3	207.1
		P_2O_5	39.2	6.8	46.0	0.8	0.8	1.6	-44.4	3.5	47.6
		K_2O	290.6	74.7	365.3	5.9	33.5	39.4	-325.9	10.8	404.7
	Mrgashat, peach tree (natural grass), 10.2	N	139.7	12.4	152.1	7.0	27.0	39.2	-112.9	25.8	191.3
		P_2O_5	39.3	6.8	46.1	0.8	0.8	1.6	-44.5	3.5	47.7
		K_2O	230.1	74.7	304.8	5.9	33.5	39.4	-265.4	12.9	344.2

* 5.2 $kg\ ha^{-1}$ of nitrogen accumulated in the soil by asymbiotic nitrogen fixation was added to the total input.

3. Regardless of the soil conservation system, the amount of nitrogen entering perennial plantations in the Ararat Valley through natural factors (atmospheric precipitation, irrigation water, asymbiotic fixation of nitrogen) exceeds the losses (erosion and washing away), while the losses of P2O5 and K2O are much greater.

4. In order to prevent further deepening of the deficiency of main nutrient elements in the vineyards and fruit orchards of Armenia, it is necessary to fertilize the grassy orchards of production significance with N150P80, K200 and in the black fallow system with doses of N80P60 K100 kg ha^{-1}, taking into account the high content of potassium in the soil, losses of nitrogen gas (about 20%) and the immobilization of phosphorus fertilizers in the form of hard to reach compounds.

REFERENCES

Alexandrova, L.N. & Naidionova, O.A. 1976. *Laboratory-practical trainings on soil science,* 3rd edition, Kolos, Leningrad, 280 pp. (in Russian).

Avagyan, N.O. 1980. Balance of nutrient elements in the agriculture of Armenian SSR. *Biological journal of Armenia* **33**(5), 574–582 (in Russian).

Babayan, G.B. 1980. *Balance of nitrogen, phosphorus and potassium in the agriculture of the Armenian SSR.* Yerevan, 13–180 (in Russian).

Babayan, G.B. 1985. Balance of nutrient elements in the agriculture of the Armenian SSR. *Biological journal of Armenia* **38**(5), 415–42 (in Russian).

Batukayev, A.A. 1999. Viticulture and wine-making in Spain. *International agricultural journal* **4**, 60–62 (in Russian).

Bondarenko, S.G. 1986. *Fertilization of vineyards in Moldova*. Kishinev, 232 pp. (in Russian).

Chatzitheodorou, I.T., Sotiropoulos, T.E. & Moutharidou, G.I. 2004. Effect of nitrogen, phosphorus, potassium fertilisation and manure on fruit yield and fruit quality of the peach cultivars 'Spring Time' and 'Red Haven'. *Agronomy Research* **2**(2), 135–143.

Dasberg, S., Erner, V. & Bielorari, H. 1984. Nitrogen balance in a citrus orchard. *J. Environ Quel* **13**(3), 353–356.

Donos, A.I. 1988. Balance of nutrient elements in common chernozem at regular use of fertilizers. *Effectiveness of application of fertilizers in Moldova*, 97–107 (in Russian).

Gasparyan, O.B. 1970. Chemical analysis of irrigational waters. *Agrochemical characteristics of atmospheric precipitation and irrigational waters.* Proceedings of Academy of Sciences of Armenian SSR, pp. 61–81 (in Russian).

Guidelines for compiling the balance of nitrogen and ash elements in agriculture of the USSR. 1975. *VASKHNIL, Institute of soil named after V.V. Dokuchayev,* 3–31 (in Russian).

Harutyunyan, S.S. & Harutyunyan, A.S. 1985. Removal of main nutrient elements by grapes after fertilization and irrigation. *Agrochemistry* **10**, 68–72 (in Russian).

Harutyunyan, S.S. 1995. Biological removal of main nutrient elements by grapevines depending on norms of mineral fertilizers. Anthropogenetic factor of soil formation and fertility of soils in the Republic of Armenia. *Works of Scientific-research Institute of Soil Sciense and Agrochemistry* **28–29**, 177–184 (in Russian).

Harutyunyan, S.S. 1996. Balance of nitrogen, phosphorus and potassium in apple and pear plantations of Ararat valley. *Agroscience* **3–4**, 150–159 (in Armenian).

Harutyunyan, S.S. & Harutyunyan, A.F. 2002. Biological Removal of Nitrogen, Phosphorus and Potassium in the Vineyards of the Vaik district of Armenia. *Problems of the stable development of the agroindustrial system in the South Caucasus region*. Inter. Scient. Confer. Mater., part I, Yerevan, pp. 298–303 (in Armenian).

Harutyunyan, S.S. & Grigoryan, A.M. 2005. Productive removal of basic nutrient elements in fruit plantations in Kotayk marz. *Bulletin of State Agrarian University of Armenia* **61**, 8–11 (in Russian).

Harutyunyan, S.S. & Sabeti, Amirhandeh, M. 2012. Productive output of main nutrients depending on the source of nitrogen feeding. *Agroscience* (**5–6**), 339–343 (in Russian).
Harutyunyan, S.S. & Miqaelyan, H.A. 2021. Biological Removal of main nutrient elements by tomato and pepper on the background of organic-mineral fertilizers and microbiological concentrates. *Biological journal of Armenia* **73**(3), 18–26 (in Russian).
Krivenya, N.I. 1977. Removal of nitrogen, phosphorus and potassium by row crops depending on fertilizers. In the digest *Ways to increase the yield of field crops*. Harvest 8, Minsk, 126–133 (in Russian).
Kudeyarov, V.N. & Semenov, V.M. 2014. Agrochemical problems and the current state of agricultural chemicalization in the Russian federation. *Agrochemistry* **10**, 3–17 (in Russian).
Kudeyarov, V.N. 2018. The balance of nitrogen, phosphorus and potassium in agriculture of Russia. *Agrochemistry* **10**, 3–11 (in Russian).
Ladonin, V.F. & Velichko, V.A. 1994. All-Russian forums of agrochemists on modern problems of agrochemistry and chemicalization of agriculture. *Agrochemistry* **6**, 119–123 (in Russian).
Malakhova, N.P. 1980. Removal of mineral nutrient elements by the grapevine in the conditions of light brown soils in the south-east of Kazakhstan. *Agrochemistry* **10**, 100–106 (in Russian).
Mammadov, M.I. 2015. The balance of nutrients in grey-brown (chestnut) soil under vineyard in Azerbaijan. *Agrochemistry* **6**, 11–18 (in Russian).
Marinchik, A.F. & Zakharova, V.V. 1987. Removal of nutrient elements by sugar beet taking into account dead leaves, depending on the mineral nutrition. *Bulletin of agricultural science* **5**, 64–68 (in Russian).
Merzhanyan, A.S. 1951. Viticulture. *Pishchepromizdat*, 455 (in Russian).
Milashchenko, N.Z. 1999. Fertility of soils is the central issue of agriculture. *Agriculture* **5**, 15–16 (in Russian).
Mineyev, V.G. 2000. Ecological functions of agrochemistry in modern agriculture. *Agrochemistry* **5**, 5–13 (in Russian).
Novosadyuk, Yu.N. 1988. Influence of various agricultural plants on grapes. *The biology of grapes and the development of elements of the progress technology of its reproduction and cultivation,* pp. 33–38 (in Russian).
Peterburgsky, A.V. 1979. Cycle and balance of nutrients in agriculture. *Science*, 168 pp. (in Russian).
Serpukhovitina, K.A. 1982. *Fertilization and productivity of grapes.* Krasnodar, 3–174 (in Russian).
Shafran, S.A. & Korchaghina, Yu.I. 1990. Nitrogen balance and nitrogen regime of soils in the Non-chernozem zone. *Nitrogen problems and farming intensity*, Abstracts of All-union conference, Novosibirsk, 90–92 (in Russian).
Shafran, S.A. 2016. The dynamics of soil fertility in the non-chernozem zone and its reserves. *Agrochemistry* **8**, pp. 3–10 (in Russian).
Shafran, S.A. 2020. Nitrogen balance in agriculture of Russia and its regulation in modern conditions. *Agrochemistry* **6**, 14–21 (in Russian).
Soil science edited by I.S. Kauricheva, Kolos, 1982, Moscow, 34–35 (in Russian).
Tsybylka, N.N., Chernysh, A.F., Zhokova, I.I. & Punchenko, S.S. 2013. Nitrogen pool in eroded coddy-podzolic soils and nitrogen loss due to water erosion. *Agrochemistry* **2**, 3–10 (in Russian).
Walessky, V.M. & Kononikhina, O.N 1983. Removal of nutrients by a grape plant on sandy medium humus soil. *Improving the efficiency of grapes*, Novocherkassk, 59–62 (in Russian).
Yagodin, B.A. 1987. *Workshop on agricultural chemistry*, Agropromizdat, Moscow, 512 pp. (in Russian).
Yurkin, S.N. 1974. Methodical issues of balance development in agriculture, *Bulletin of VIUA*, **20**, 12–37 (in Russian).
Zavalin, A.A. & Blagoveshchenskaya, G.G. 2012. Contribution of leguminous biological nitrogen to the nitrogen budget of Russian agriculture. *Agrochemistry* **6**, 32–37 (in Russian).

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Cultivar features of polyphenolic compounds and ascorbic acid accumulation in the cherry fruits (*Prunus cerasus* L.) in the Southern Steppe of Ukraine

I. Ivanova[1], M. Serdyuk[1], V. Malkina[1], O. Tonkha[2], O. Tsyz[2], B. Mazur[2], A. Shkinder-Barmina[3], T. Herasko[1] and O. Havryliuk[2,*]

[1]Tavriya State Agrotechnological University named after Dmytro Motorny, Khmelnytsky Ave., 18 B, UA72312 Melitopol, Ukraine
[2]National University of Life and Environmental Sciences of Ukraine, Heroiv Oborony Str., 12 A, 13 UA03041 Kyiv, Ukraine
[3]Melitopol Research Station of Horticulture, named after M.F. Sydorenko of IH NAAS, Vakulenchuka Str., 99, UA72311 Melitopol, Ukraine
[*]Correspondence: o.havryliuk@nubip.edu.ua

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Abstract. Cherry is a popular and widespread fruit crop in many European countries. Significant areas of its plantation are located in the Southern Steppe subzone of Ukraine. Modern biochemical research aims to determine the amplitude of cherries varietal difference within the studied species and determine selection possibilities for the most important chemical components. In this regard, the study of fruits biochemical composition of different cherries cultivars is relevant. The aim of the research was to build a mathematical model based on Multiple linear regression method, which reveals the degree of weather factors influence on the dynamics of polyphenolic compounds and ascorbic acid accumulation in cherries fruits in the Southern Steppe subzone of Ukraine and in regions with similar hydrothermal parameters. The cultivar 'Ihrushka' was characterized by the lowest variability in the concentration of polyphenolic compounds with the value of the variation coefficient of 9.9%. The optimal average concentration of polyphenolic compounds at the level of 224.6 mg (100 g)$^{-1}$ had fruits of the cherry cultivar 'Siianets Turovtsevoi' (Vp – 12.8%). Fruits of the 'Vstrecha' cultivar were characterized by the optimal average concentration of ascorbic acid at the level of 9.6 mg (100 g)$^{-1}$ and variability of the indicator 14.0%. The dominant influence of varietal characteristics on the polyphenolic compounds accumulation in cherry fruits has been established. The share of the factor impact B was 41.3%. It was determined that weather conditions with a share of influence of 69.2% are crucial for the formation of the ascorbic acid. The correlation analysis showed the presence of a linear correlation between seven weather factors (X_i, i = 1..7) and the concentration of polyphenolic compounds (Y_1) and ascorbic acid (Y_2) in cherry fruits. The values of the pairwise correlation coefficients $r_{Y_1X_i}, r_{Y_2X_i}$ $i = 1..7$ were within the interval [-0.55; 0.55], which showed the presence of an impact between these weather factors and the studied indicators. The average monthly precipitation in June became decisive for the accumulation of polypolyphenolic compounds ($\Delta_{X2} = 35.2\%$). The average monthly amount of precipitation in May was determined to be the most important for the formation of the ascorbic acid level ($\Delta_{X1} = 37.1\%$).

Key words: *Prunus cerasus* L., climate, cherry, phenolic compounds, ascorbic acid, variability, stone fruits, ridge-regression.

INTRODUCTION

Cherry belongs to the popular and widespread fruit crops of Ukraine. This is due to its biological characteristics, namely undemanding for soil conditions, high winter hardiness, and the fruits are suitable for consumption in fresh, frozen, and in the form of various high quality processed products (Bak et al., 2010; Alrgei et al., 2016).

Ukraine is one of the countries producing cherries. It is noted that in the total world production of cherries about 70% belong to European countries, followed by Asia - 20% and North America - 10% (Bouhadidaa et al., 2009; Keserović et al., 2014; Barac et al., 2014; Mézes et al., 2017; Gaudet et al., 2019; Sanderson et al., 2019).

The increase in fruit production is due to a number of reasons: cultivar renewal and the introduction into production of new high-quality fruit cultivars (Mezhenskyj et al., 2020; Shevchuk et al., 2021a, 2021b), development of agriculture and fruit processing technology (Mir et al., 2021). The popularity of fruit consumption is supported by numerous studies of the biologically active compounds concentration in fruits and their therapeutic and prophylactic effects on health (Miloševic & Miloševic, 2012; Bell et al., 2014; Mezhenskyj, 2019; Eslami et al., 2022). Blando & Oomah (2019) noted that the consumption of cherries has a positive effect on human health: counteracts oxidative stress, reduces inflammation, regulates blood glucose and improves cognitive function, promotes faster recovery from muscle damage, caused by physical exertion.

Clinical studies related to the consumption of cherries and products of their processing, conducted by Kelley et al. (2018), stated a high concentration of polyphenols and ascorbic acid, as well as the presence of antioxidant and anti-inflammatory properties. Similar studies of the biochemical composition of cherry fruits, the functional effect of their use, and the resulting health effects were described in the works of Keane et al. (2016) and Alba et al. (2019). Much research has been devoted to the study of cherries biochemical composition in terms of species and varietal characteristics. Studies of the fruit characteristics of four species of the genus *Prunus* were conducted by Cao et al. (2015) in China and noted the suitability of *Prunus cerasus* for processing due to the high concentration of ascorbic acid and anthocyanins.

A group of Serbian researchers studied the total phenols, anthocyanins, antioxidant activity, and polyphenolic profile of 39 ‘Oblačinska’ cherry clones in Serbia and concluded that the most common polyphenols were rutin and chlorogenic acid. They were the first who detect compounds such as pinobaxin, hesperetin and galangin in the cherry fruits of some clones (Alrgei et al., 2016; Guffa et al. 2016).

Grafe & Schuster (2014) investigated the physicochemical characteristics of fruits in 78 cherry genotypes from the genetic collection of the Dresden Institute, Germany, and noted a more significant influence of cultivar factor on the fluctuations of the studied traits than the year factor.

The chemical composition of fruit crops, in addition to varietal characteristics, largely depends on ripeness degree and growing conditions including soil and climatic conditions (Gavryliuk et al., 2019; Vasylenko et al., 2021; Havryliuk et al., 2022a, 2022b). Ganopoulos et al. (2016) also studied 27 cultivars of cherries located at the Institute of Fruit Pomology of the Genetic Bank, Naoussa, Greece. In Croatia, Viljevac-Vuletic et al. (2017) investigated influence of different locations, years and cultivars on the concentration of polyphenols and anthocyanins in the fruits of five cultivars of cherries (*Prunus cerasus* L.). They observed that the greatest source of variability among the studied factors (location-year-cultivar) is the cultivar factor. The 'Maraska' had the highest concentration of polyphenols and anthocyanins, and 'Oblačinska' had the lowest concentration of polyphenols and anthocyanins, regardless the year and place of cultivation.

Khoo et al. (2011) studied 34 cherry cultivars for total phenols, anthocyanins, total antioxidant capacity, and cancer cell proliferation inhibitory activity. They found that 'Birgitte × Böttermö' and 'Fanal' had the highest concentration of total polyphenolic compounds (754 ± 13.4 and 596 ± 5.7 mg of gallic acid equivalent/100 g, respectively), the highest total antioxidant capacity (63.0 ± 7.5 and 52.0 ± 6.9 Mmol of trolox equivalent/g, respectively for each cultivar) and the highest cancer cell proliferation inhibition activity (63.0 ± 1.7 and 70.0 ± 1.6%, respectively for each cultivar).

Numerous publications were devoted to the study of cherries and the biochemical composition of their fruits by scientists from Poland. Sokół-Łętowska et al. (2020) determined the concentration of polyphenolic compounds in fruits of 21 cultivars and genotype *Prunus cerasus L.* and found that the highest concentration of polyphenolic compounds was in fruits of cultivars 'Wieluń 17', 'Sokówka Nowotomyska', 'Grosenkirch', 'Grookkirch', 'Sokówka Nowotsenysk' (anthocyanins, flavanols, and common phenolic compounds) and 'Meteor' (phenolic acids). Cultivars with medium and late maturity had more flavonols and anthocyanins and were identified as higher antioxidant capacity than cultivars with early and early-medium maturity. Wojdyło et al. (2014) and Borowy et al. (2018) conducted a comparative study of the biochemical composition (polyphenols, antioxidant properties, and nutrients) of *Prunus cerasus L.* fruit cultivars 'Kelleris 16', 'Nefris' and 'Łutówka' in the central-eastern regions of Poland.

Studies of the antioxidant properties of cherries in cultivars, such as griotte and amarelle, depending on the color of the flesh were conducted in Hungary. Papp et al. (2010) found that the anthocyanin concentration ranged from 11.3 to 93.5 mg $(100\ g)^{-1}$, and the 'Pipacs 1' cultivar showed the highest antioxidant capacity (21.85 Mmol AA/l) and ascorbic acid concentration (8.98 mg $(100\ g)^{-1}$).

Picariello et al. (2016) studied the biochemical composition of five cultivars of cherries. The researchers found that the most common anthocyanins were cyanidin derivatives. The authors developed anthocyanin profiles and verified to be specific both for a certain type and cultivar.

The literature review shows that modern biochemical research is aimed primarily at determining the amplitude of varietal differences within the studied species and determining the possibilities of selection for the most important chemical components. In this regard, it is worth noting the study of the biochemical composition of fruits of new domestic cherry cultivars and hybrids, obtained through selection work conducted in Melitopol Research Station of Horticulture, named after M.F. Sydorenko of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine, where both interspecific *Prunus cerasus L.* and interspecific crosses within *Prunus cerasus L.* × *Prunus avium Moench* (Turovtseva et al., 2016) were used to create new cultivars. This study of the biochemical composition of the existing and promising range of cherries will allow using the data for further selection work for the improved biochemical composition of cherries.

MATERIALS AND METHODS

The research was conducted during 2007–2019 in the orchards of the Southern Steppe subzone of Ukraine on the basis of the laboratory of primary processing and storage of plant products of the Research Institute of Agrotechnology and Ecology of Tavriya State Agrotechnological University named after Dmytro Motorny, Ukraine, Melitopol.

The aim of the research was to build a mathematical model, the analysis of which will reveal the degree of weather factors influence in the Southern Steppe subzone of Ukraine and regions with similar hydrothermal parameters on the formation of polyphenolic compounds and ascorbic acid (vitamin C) in cherries.

The tasks were set to achieve this goal:

– to analyze the weather conditions during the phenological phases of cherries growth and development;

– to determine the concentration of polyphenolic compounds, and ascorbic acid in fruits and select the best cultivars;

– to study the relationship between polyphenols, ascorbic acid accumulation, and weather factors;

– to determine the degree of each weather factor influence on the concentration of polyphenolic compounds and ascorbic acid in the studied cultivars of cherries.

The study site is characterized by insufficient precipitation. The climate is Atlantic-continental, arid, with high air temperatures during vegetation period. The direction of dry winds is northeast. According to the set of climatic indicators, the studied region is suitable for cherries growing (Table 1).

The data of the Melitopol meteorological station of the South of Ukraine were used to calculate the model for predicting the polyphenolic compounds and ascorbic acid concentration in cherry fruits (46° 49’N, 35° 22’E) (Tonkha et al., 2020).

Table 1. Meteorological conditions of the Southern Steppe subzone of Ukraine

Indicator	Value
Average annual air temperatures, °C	9.1–9.9
Average monthly air temperatures in the warmest months, °C	20.5–23.1
The sum of active temperatures above 10 °C from April to October, °C ($\Sigma_{akt}t \geq 10$ °C)	3,316
Average annual amount of precipitation, mm	475
Average annual relative humidity, %	73
Average annual wind speed, m s^{-1}	3
Hydrothermal coefficient	0.22–0.77

The fruits of registered cherry cultivars selected for the study were: ‘Vstriecha’, ‘Ozhydaniie’, ‘Shalunia’, ‘Siianets Turovtsevoi’, ‘Hriot Melitopolskyi’, ‘Solidarnist’, ‘Ihrushka’; and promising in Ukraine - ‘Melitopolska Purpurna’, ‘Modnytsia’, ‘Ekspromt’ (Promising is a variety that is widespread in Ukraine, but is not included in the ‘State Register of Plant Varieties Suitable for Distribution in Ukraine’).

The soil of the research areas is chernozem southern light loamy. Agrochemical characteristics of the soil are given in Table 2.

The fruits of 10 experimental cultivars were selected in the cherry plantations of the Melitopol Research Station of Horticulture named after M.F. Sydorenko of IH NAAS, located in the Melitopol district of Zaporizhzhia region, which belongs to the horticulture zone of the Southern Steppe.

Trees of the studied cultivars were grafted on seedlings of Magaleb cherry, planted in 2001 according to the 6×4 m scheme. Growing conditions are rainfed. Fruits were harvested from typical trees of a certain pomological cultivar and the same age. The agricultural technology in the research areas during all research years was uniform.

Table 2. Agrochemical characteristics of tested soil

Depth of arable layer, cm	Humus content, %	pH_{KCl}	Available nutrients content, mg (100 g)$^{-1}$ of soil		
			N	P_2O_5	K_2O
40.0	1.38	6.9	27.0	90.0	154.0

To study the concentration of polyphenolic compounds and ascorbic acid, 100 fruits were selected from 6 trees of each cherry cultivar when samples have reached full maturity, in 3 repetitions. The fruits of each pomological cultivar were harvested by hand in 4 different places of the cherry crown. After harvesting, the cherries were weighed separately and counted. Cherry fruits were transported to the laboratory within 2–3 hours after harvesting to determine the studied indicators.

During the harvesting period, the consumer ripeness of cherries was determined visually and organoleptically. The flesh of the fruit was quite dense, the color and taste were special characteristic of each pomological cultivar. Cherry fruits were harvested with stalks. Transportation and storage of fruits were carried out under the condition of preserving the appearance and taste characteristics of each cultivar.

Ascorbic acid (AsA) was quantitively determined by titrimetric method (Ivanova et al., 2019), with Tillman's reagent (2.6-dichlorophenol-indophenol dye). According to the amount of reagent spent on titration, the concentration of AsA in the extracts was calculated, results were expressed in mg (100 g)$^{-1}$ fresh weight.

The polyphenolic compounds concentration was determined by the Folin-Denis' reagent. The method involves the reaction of polyphenols complexation with Folin-Denis' reagent and the formation of dyes, followed by the determination of optical density. As a standard, rutin was used to recalculate the polyphenolic compounds concentration in cherries. The optical density of the obtained experimental solutions and the solution of the rutin standard sample was determined on a spectrophotometer at a wavelength of 670 nm in a cuvette with a distance between the working faces of 10 mm. Results were expressed in mg (100 g)$^{-1}$ fresh weight.

The fraction of polyphenols was calculated by the formula:

$$X = (c \times V \times a \times 100) / (m \times V_1)$$

c – concentration of rutin, which is determined by the calibration curve, mL g cm^{-3}; V – extract volume, cm^{-3}; V_1 – volume of extract for analysis, cm^3; m – weight of the product, g (cherries); 100 – the coefficient of the polyphenols concentration determination per 100 g^{-1} of the product; a – the coefficient of the sample extract dilution (Ivanova et al., 2019).

The model of the dependence of polyphenolic compounds and ascorbic acid concentration in cherry fruits on weather factors was formed according to the scheme proposed in the works of Ivanova et al. (2021a, 2021b):

– quantitave determination of polypolyphenolic compounds and ascorbic acid;
– analysis of meteorological indicators for the years of research;
– calculation of the Hydrothermal Coefficient (HTC) of G.T. Selyaninov, the sum of effective temperatures, the sum of active temperatures, and temperature differences for certain growing seasons of research years;
– selection of meteorological factors that show a significant correlation with polyphenolic compounds and AsA concentration in fruits, based on correlation analysis;
– construction of a regression model of the dependence of polyphenolic compounds and AsA concentration in the fruits of cherry cultivars on meteorological indicators;
– determination and ranking of weather factors according to the degree of their influence on the studied indicator of fruit quality on the basis of the built regression model.

As a rule, the Least Squares Approach is used for a regression model building. However, in order for the calculated coefficients of the regression model to be effective and unbiased estimates of the parameters of the generalized regression model, the conditions of the Gauss-Markov theorem (Damodar, 2009) must be met.

If the influencing factors correlate with each other, then the multicollinearity effect appears which is a violation of the Gauss-Markov theorem. In this case, the estimates of parameters of the constructed model coefficients by the Least Squares Approach are not unbiased. On the basis of this model, the results of the analysis of the degree of influence of each factor separately are not valid.

One of the recommended methods for building a regression model under multicollinearity conditions is regularization method. Ivanova et al. (2020) and Malkina et al. (2019) proposed an algorithm for analyzing the influence of correlating factors on the resulting indicator based on a regression model built in this way - the LASSO method. It is proposed in this paper to build a regression model based on ridge-regression.

According to the Ridge-regression method, the minimum of the function is found to determine the model parameters:

$$L = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2 + \lambda \sum_{i=1}^{n} \beta_i{}^2 \quad (1)$$

where y_i – the experimental values of the indicator; $\hat{y}_i$ – theoretical values of the indicator, calculated on the basis of the constructed regression equation; λ – the set parameter (penalty); β_i – regression model coefficients.

Thus, the study is proposed to be conducted according to the following algorithm:

Based on experimental data x_{ij}, ($i = 1 \dots n$ – weather factor number, $j = 1 \dots m$ – study year number), we build a Ridge-regression model in the form

$$\hat{Y} = a_0 + \sum_{j=1}^{n} a_j \cdot X_j \quad (2)$$

where X_j – factors; a_j – model parameters; $\hat{Y}$ – dependent variable.

Calculate the values of the coefficients of the corresponding regression model in normalized factors by the formula

$$\tilde{a}_i = a_i \frac{\bar{S}_{X_i}}{\bar{S}_Y}, \quad (3)$$

where a_i – calculated coefficients of the regression model (2); $\bar{S}_{X_i}$ – standard deviation of factors X_i; $\bar{S}_Y$ – standard deviation of the studied indicator Y.

We analyze the constructed regression (2) to determine the degree each of the climatic factors influences the studied indicator. To determine the share of weather factors in the total impact of all factors, we calculate the coefficient Δ_i by the formula:

$$\Delta_i = \left|\frac{\tilde{a}_i \cdot r_{YX_i}}{R^2}\right|, \qquad (4)$$

where $\tilde{a}_i$ – parameters of the regression model in normalized factors $\tilde{X}_i$; r_{YX_i} – correlation coefficients; R^2 – determination coefficient.

RESULTS AND DISCUSSION

According to the results of thirteen years of research, it was determined that the average concentration of polyphenolic compounds in cherry fruits grown in the Southern Steppe subzone of Ukraine was 199.137 mg (100 g)$^{-1}$ (Table 3). The minimum concentration of polyphenolic compounds was recorded in the fruits of 'Vstrecha' cherries - 129.17 mg (100 g)$^{-1}$ in 2018. It was lower than the average varietal value by 35.1%. The maximum concentration of polyphenolic compounds, at the level of 305.9 mg (100 g)$^{-1}$, was found in the fruits of the cultivar 'Melitopolska Purpurna' of the 2010 harvest. At the same time, the exceeding over the average varietal value was 53.6%. The highest average polyphenolic compounds level was in 'Melitopolska Purpurna' cultivar, and the lowest was in 'Ekspromt'cultuvar, according to thirteen years of research.

Table 3. The concentration of polyphenolic compounds in cherry fruits, mg (100 g)$^{-1}$ (2007–2019), $\bar{x} \pm s\bar{x}$, n = 5

Pomological cultivar	Average concentration, mg (100 g)$^{-1}$	Min concentration, mg (100 g)$^{-1}$	Max concentration, mg (100 g)$^{-1}$	Variation by the years, Vp, %
'Vstrecha'	198.7 ± 28.8^{d}	129.2	249.8	14.4
'Ozhydaniie'	218.8 ± 39.2^{c}	178.0	289.7	17.9
'Shalunia'	197.4 ± 22.5^{de}	171.3	241.1	11.3
'Siianets Turovtsevoi'	224.6 ± 28.7^{b}	188.8	265.8	12.8
'Hriot Melitopolskyi'	193.3 ± 25.7^{f}	161.3	234.1	13.2
'Melitopolska Purpurna'	243.1 ± 45.7^{a}	189.1	305.9	18.8
'Modnytsia'	168.3 ± 19.5^{h}	147.9	147.7	11.5
'Ekspromt'	165.0 ± 20.7^{i}	136.2	211.6	12.5
'Solidarnist'	196.6 ± 24.1^{e}	158.1	236.1	12.2
'Ihrushka'	185.5 ± 18.4^{g}	158.2	211.3	9.9
Average value	199.1 ± 36.0^{d}	136.5	219.3	12.5

Means in column with the different letter are highly significantly different according to the Fisher's test ($P \leq 0.05$).

To compare the variability of the studied characteristics, namely to assess the stability of the cultivar in relation to weather factors, the coefficient of variation Vp was calculated. It is known that the value of the indicator can be divided into 3 groups with the aim to analyze research results. If the coefficient of variation is less than 10%, the variability of the indicators is considered insignificant or low, for values from 10 to 20% - medium, above 20% - significant or strong (Ivanova et al., 2021b).

The research results show the average variability of the content of polyphenolic substances over the years of research for almost all cherry varieties. The exception was the cultivar 'Ihrushka' - Vp = 9.9% (Table 3).

The maximum influence of weather factors on the concentration of the polyphenolic compounds in fruits with medium variability was observed for cultivars 'Melitopolska Purpurna' and 'Ozhydaniie' with variation coefficients of 18.8 and 17.9%, respectively. Fruits of 'Siianets Turovtsevoi' cultivar (Vp = 12.8%) were characterized by the optimal average concentration of polyphenolic compounds at the level of 224.615 mg (100 g)$^{-1}$.

The average ascorbic acid concentration for studied cultivars in research years was 9.17% (Table 4).

Table 4. The concentration of ascorbic acid in cherry fruits, mg (100 g)$^{-1}$ (2007–2019), $\bar{x} \pm s\bar{x}$, $n = 5$

Pomological cultivar	Average concentration, mg (100 g)$^{-1}$	Min concentration, mg (100 g)$^{-1}$	Max concentration, mg (100 g)$^{-1}$	Variation by the years, Vp, %
'Vstrecha'	9.59 ± 1.34^{b}	7.99	12.01	14.0
'Ozhydaniie'	8.97 ± 1.68^{bcd}	6.21	11.54	18.7
'Shalunia'	10.44 ± 2.11^{a}	8.22	13.80	20.2
'Siianets Turovtsevoi'	10.10 ± 2.29^{ab}	7.84	13.87	22.6
'Hriot Melitopolskyi'	8.23 ± 1.28^{dc}	6.39	10.09	15.6
'Melitopolska Purpurna'	8.44 ± 1.20^{cd}	6.18	10.17	14.3
'Modnytsia'	9.00 ± 1.65^{bcd}	7.08	11.45	18.4
'Ekspromt'	9.00 ± 2.18^{bcd}	6.24	12.36	24.2
'Solidarnist'	9.13 ± 1.67^{bc}	7.14	12.05	18.3
'Ihrushka'	8.75 ± 1.21^{cd}	7.16	10.97	13.8
Average value	9.17 ± 1.77	7.04	11.83	18.01

Means in column with the different letter are highly significantly different according to the Fisher's test ($P \leq 0.05$).

The minimum concentration of ascorbic acid within the studied cultivars was recorded in the fruits of 'Melitopol Purpurna' cultivar - 6.18 mg (100 g)$^{-1}$ in 2008. The obtained value of the indicator was 32.6% lower than the average varietal value. The fruits of the 'Siianets Turovtsevoi' of the 2010 harvest had a maximum ascorbic acid concentration of 13.87 mg (100 g)$^{-1}$. At the same time, the exceeding over the average varietal value was 51.2%. According to thirteen years of research, 'Shalunia' and 'Siianets Turovtsevoi' cultivars were characterized by the highest average concentration of the studied indicator, and 'Hriot Melitopolskyi' cultivar - by the lowest (Table 4).

The variability of ascorbic acid concentration according to the years of research in cherry fruits was medium and high with a range of Vp = 14.0–24.2%. The most stable concentration of ascorbic acid was in fruits of cultivar Vstrecha (Vp = 14.0%), and the most variable - in the cultivar 'Ekspromt' (Vp = 24.2%). 'Vstrecha' cultivar fruits were characterized by optimal average ascorbic acid concentration of 9.6 mg (100 g)$^{-1}$. The dominant influence of varietal characteristics (factor B) on the level of polyphenolic compounds formation of cherry fruits was confirmed by the results of a two-factor analysis of variance (Table 5). The share of the impact of factor B (cultivar) was - 41.3% against the share of the impact of factor A (year) - 32.2%.

Weather conditions (factor A) also had an influence of 69.2% on the ascorbic acid concentration (Table 5). The influence of varietal characteristics (factor B) was less significant - 13.2%.

Table 5. The results of the two-factor analysis of variance in the polyphenolic compounds and ascorbic acid concentrations in cherries

Source of variation	Sum of squares	Degree of freedom	Dispersion	F_{fact}	$F_{0.95}$	Influence, %
Phenolic compounds						
Factor A (year)	161,933.2	12	13,494.4	11,312.9	1.8	32.2
Factor B (cultivars)	207,608.3	9	23,067.5	19,338.4	1.9	41.3
Interaction AB	132,466.0	108	1,226.5	1,028.2	1.3	26.3
Vitamin C						
Factor A (year)	889.2	12	74.1	325.8	1.8	69.2
Factor B (cultivars)	169.6	9	18.8	82.8	1.9	13.2
Interaction AB	164.0	108	1.5	6.67	1.3	12.7

According to the test of hypothesis H_0 about the significance of correlation coefficients according to Student's criterion at the significance level of 0.05, significant even correlation coefficients are $\left|r_{Y_j X_i}\right| > 0.55,\ i = 1..7, j = 1..2$. Therefore, weather factors that have values of paired correlation coefficients from the specified interval were selected.

According to the research results, seven weather factors were selected. The presented weather factors significantly correlate with the levels of polyphenolic compounds and AsA in cherry fruits. The indicators Y_1 – AsA accumulation and Y_2 – the concentrations of polyphenolic compounds, also significantly correlate with each other, the correlation coefficient between them is $r_{Y_1 Y_2} = 0.9274$. This explains the fact that the indicators Y_1 – AsA accumulation and Y_2 – the concentrations of polyphenolic compounds are significantly affected by the same set of weather factors.

To perform correlation analysis, a matrix of paired correlation coefficients was constructed, which is shown in Table 6.

Table 6. Correlation matrix for weather factors

	X_1	X_2	X_3	X_4	X_5	X_6	X_7
X_1	1.0000	0.2952	0.7970	0.0630	-0.3847	0.6950	0.0473
X_2	0.2952	1.0000	0.2561	0.8385	-0.2531	0.5967	0.8346
X_3	0.7970	0.2561	1.0000	0.0736	-0.2477	0.6020	0.0792
X_4	0.0630	0.8385	0.0736	1.0000	-0.3386	0.6337	0.9342
X_5	-0.3847	-0.2531	-0.2477	-0.3386	1.0000	-0.6438	-0.0554
X_6	0.6950	0.5967	0.6020	0.6337	-0.6438	1.0000	0.5304
X_7	0.0473	0.8346	0.0792	0.9342	-0.0554	0.5304	1.0000

X_1 – the average monthly precipitation in May; X_2 – the average monthly precipitation in June; X_3 – the number of days with precipitation of more than 1 mm in May; X_4 – the number of days with precipitation of more than 1 mm in June; X_5 – duration of the frost-free period during the year; X_6 – the amount of precipitation in the period from flowering to fruit ripening; X_7 – total number of days with precipitation in June.

According to Table 6, the analysis of the correlation tightness revealed six weather indicators of humidity: the average monthly precipitation in May (X_1) and June (X_2); the number of days with precipitation more than 1 mm in May (X_3) and June (X_4); the amount of precipitation in the period from flowering to fruit ripening (X_6); the total number of days with precipitation in June (X_7), and accumulation of polyphenolic compounds and AsA was also influenced by the temperature indicator - the duration of the frost-free period during the year (X_5).

The values of correlation coefficients between factors close to 1.0 indicate close correlations between factors X_1 (average monthly precipitation in May) and X_3 (number of days with precipitation more than 1.0 mm in May), X_2 average monthly (precipitation in June) and X_4 (number of days with precipitation more than 1.0 mm in June), X_2 (average monthly precipitation in June) and X_7 (total number of days with precipitation in June), X_4 (number of days with precipitation more than 1.0 mm in June) and X_7 (the total number of days with precipitation in June). This states the presence of the effect of multicollinearity.

It was proposed to use the Redge-regression method to build a regression model in multicollinearity. The Redge-regression method (1) regularizes the parameters and allows to build a regression model, the parameters of which are unbiased estimates of the parameters of the corresponding generalized model. To determine the parameter λ cross-validation was performed and the optimal values of the parameter λ were determined. For the regression model of dependence of indicator Y_1 – AsA accumulation on weather factors as a result of cross-validation the parameter $\lambda = 10{,}000\ is\ defined.$ For the regression model of dependence of indicator Y_2 – the concentration of polyphenolic compounds on weather factors parameter $\lambda = 3792.6902$ is defined.

The regression model of the form (2) of the dependence of the indicator Y_1 – AsA accumulation on weather factors (in the normalized factors, which are calculated by the formula (3)) is:

$$\hat{Y}_1 = 0.0126X_1 + 0.0087X_2 + 0.0012X_3 + 0.0004X_4 - 0.0042X_5 + 0.0101X_6 + 0.0006X_7$$

where $\hat{Y}_1$ – the predicted value of AsA accumulation; X_1 – the average monthly precipitation in May; X_2 – the average monthly precipitation in June; X_3 – the number of days with precipitation of more than 1 mm in May; X_4 - the number of days with precipitation of more than 1 mm in June; X_5 – duration of the frost-free period during the year; X_6 – the amount of precipitation in the period from flowering to fruit ripening; X_7 – total number of days with precipitation in June.

The coefficient of determination, calculated on the basis of the constructed model $R^2 = 0.6928$, indicates a significant influence of weather factors on the AsA accumulation in comparison with random errors.

Regression model (2) of the dependence of the indicator Y_2 – the concentration of polyphenolic compounds from weather factors (in normalized factors, which are calculated by the model (3)) is:

$$\hat{Y}_2 = 0.1120X_1 + 0.2194X_2 + 0.0263X_3 + 0.0166X_4 - 0.1075X_5 + 0.1628X_6 + 0.0202X_7$$

where $\hat{Y}_2$ – the predicted value of polyphenolic compounds concentration; X_1 – the average monthly precipitation in May; X_2 – the average monthly precipitation in June; X_3 – the number of days with precipitation of more than 1 mm in May; X_4 - the number of days with precipitation of more than 1 mm in June; X_5 – duration of the frost-free period during the year; X_6 – the amount of precipitation in the period from flowering to fruit ripening; X_7 – total number of days with precipitation in June.

The coefficient of determination, calculated on the basis of the constructed model $R^2 = 0.7928$, indicates a significant influence of weather factors on polyphenolic compounds concentration in comparison with random errors.

Based on the constructed models, the indicators Δx_i $(i = 1..7)$ were calculated assorting to model (4), which characterizes the degree of factors' influence on the studied indicator, and the factors were ranked according to their degree of importance. Table 7 shows the calculated indicators and ranks of factors.

The share of Δx_i influence in the studied cherry cultivars for polyphenolic compounds varied within 2.15–35.23%. The share of the influence of the studied factors on AsA accumulation in cherry cultivars was in the range of 0.69–37.11% (Table 7).

Table 7. Coefficients of pair correlation between weather factors (X_i,) and biochemical indicators, shares of influence of weather factors Δx_i,% on the level of polypoliphenolic compounds and ascorbic acid accumulation in cherry fruits and their rank

Symbol of the factor (X_i)	Factors	Polyphenolic compounds			Ascorbic acid		
		Paired correlation coefficients $r_{Y_1 x_i}$	Coefficients of factors influence (Δ_{xi}, %) and indicators of factors rank		Paired correlation coefficients $r_{Y_2 x_i}$	Coefficients of factors influence (Δx_i, %) and indicators of factors rank	
			Rank	Δx_i, %		Rank	Δx_i, %
X_1	The average monthly precipitation in May, mm	0.618	3	14.91	0.751	1	37.11
X_2	The average monthly precipitation in June, mm	0.746	1	35.23	0.595	3	20.18
X_3	The number of days with precipitation of more than 1 mm in May, days	0.594	5	3.36	0.649	5	2.94
X_4	The number of days with precipitation of more than 1 mm in June, days	0.679	7	2.15	0.469	7	0.69
X_5	Duration of the frost-free period during the year, days	-0.568	4	13.15	-0.518	4	8.41
X_6	The amount of precipitation in the period from flowering to fruit ripening, mm	0.808	2	28.30	0.753	2	29.72
X_7	Total number of days with precipitation in June, days	0.599	6	2.59	0.427	6	0.95

Depending on the values of the coefficients $\Delta_{X\mathrm{i}}(i = 1\ldots7)$ of influence on two indicators of cherry fruits quality, weather factors were distributed by ranks of influence on polyphenolic compounds and AsA accumulation in cherry fruits.

According to the Table 7, the humidity indicator - the average monthly precipitation - had the maximum impact on studied indicators level in cherry fruits, thus the 1st rank was assigned in terms of the factors share. Namely, June was the decisive month for the accumulation of the polyphenolic compounds level (X2) - $\Delta_{X2} - 35.23\%$. The average monthly amount of precipitation in May was determined to be the most important for the formation of the AsA level (X1) - $\Delta_{X1} - 37.11\%$.

The 2nd rank was assigned to the amount of precipitation in the period from flowering to ripening of cherry fruits, mm (X_6) that influenced both formation of polyphenolic compounds and AsA. The value of the share of factor influence Δ_{X6} for studied biochemical parameters ranged from 28.30% to 29.72%.

The following weather factors had a significant impact on the studied indicators in cherry fruits at the level of the 3rd rank: for the accumulation of polyphenol compounds - the average monthly precipitation in May ($\Delta_{X1} = 14.91\%$); for AsA accumulation level - the average monthly amount of precipitation in June ($\Delta_{X2} = 20.18\%$).

Other weather indicators (X_3, X_4, X_5, X_7) had less significant effect on biochemical compounds accumulation in cherry fruits. The share of factors influence of 4–7 ranks for polyphenolic compounds $\Delta_{X3,4,5,7} = 2.15\%–13.15\%$, for AsA $\Delta_{X3,4,5,7} = 0.69\%–8.41\%$.

CONCLUSIONS

The 'Ihrushka' cultivar was characterized by the lowest variability in the concentration of polyphenol compounds with a variation coefficient of 9.9%. The optimal average concentration of polyphenolic compounds at the level of 224.615 mg (100 g)$^{-1}$ had fruits of the cherry cultivar 'Siianets Turovtsevoi' (Vp – 12.8%). Fruits of the 'Vstrecha' cultivar were characterized by the optimal average concentration of ascorbic acid at the level of 9.59 mg (100 g)$^{-1}$ and variability of the indicator 14.0%. The dominant influence of varietal characteristics on the polyphenolic compounds formation of cherry fruits has been established. The share of the impact of cultivar was 41.3%. It was determined that weather conditions were crucial for the ascorbic acid concentration with a share of influence of 69.2%. Correlation analysis of weather factors influence on the concentration of polyphenolic compounds and ascorbic acid in cherry fruits was performed. The average and strong correlation between 7 weather factors (X_i, i = 1..7) and the concentration of polyphenolic compounds, ascorbic acid was proved for 10 cultivars of cherries ($\left|r_{Y_jX_i}\right| \geq 0.55$, $i = 1..7, j = 1$). Based on the constructed regression models, the analysis of the share of each weather factor influence on the concentration of polyphenolic compounds and ascorbic acid was performed. The average monthly precipitation, the humidity indicator, was determined to be the most important for biochemical compounds accumulation in cherry fruits. The average monthly precipitation in June (X_2) had the highest influence on pilyphenolic compounds - $\Delta_{X2} - 35.23\%$. The average monthly amount of precipitation in May (X_1) was determined to be the most important for the ascorbic acid formation: $\Delta_{X1} - 37.11\%$.

REFERENCES

Alba, C.M.A., Daya, M. & Franck, C. 2019. Tart Cherries and health: Current knowledge and need for a better understanding of the fate of phytochemicals in the human gastrointestinal tract. *Critical Reviews in Food Science and Nutrition* **59**(4), 626–638. https://doi.org/10.1080/10408398.2017.1384918

Alrgei, HO.S., Dabic, D.C., Natic, M.M., Rakonjac, VS., Milojkovic-Opsenica, D. & Aksic, MM.F. 2016. Chemical profile of major taste- and health-related compounds of Oblacinska sour cherry. *Journal of the Science of Food and Agriculture* **96**(4), 1241–1251. https://doi.org/10.1002/jsfa.7212

Bak, I., Lekli, I., Juhasz, B., Varga, E., Varga, B., Gesztelyi, R., Szendrei, L. & Tosaki, A. 2010. Isolation and analysis of bioactive constituents of sour cherry (*Prunus cerasus*) seed kernel: an emerging functional food. *Journal of medicinal food* **13**(4), 905–910. https://doi.org/10.1089/jmf.2009.0188

Barac, G., Ognjanov, V., Obreht, D., Ljubojevic, M., Bosnjakovic, D., Pejic, I. & Gasic, K. 2014. Genotypic and Phenotypic Diversity of Cherry Species Collected in Serbia. *Plant Molecular Biology Reporter* **32**, 92–108 https://doi.org/10.1007/s11105-013-0601-4

Bell, P.G., McHugh, M.P., Stevenson, E. & Howatson, G. 2014. The role of cherries in exercise and health. *Scandinavian journal of medicine & science in sports* **24**(3), 477–490. https://doi.org/10.1111/sms.12085

Blando, F. & Oomah, B.D. 2019. Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits. *Trends in Food Science & Technology* **86**, 517–529. https://doi.org/10.1016/j.tifs.2019.02.052

Borowy, A., Chrzanowska, E. & Kapłan, M. 2018. Comparison of three sour cherry cultivars grown in central-eastern Poland. *Acta Scientiarum Polonorum Hortorum Cultus* **17**(1), 63–73. https://doi.org/10.24326/asphc.2018.1.6

Bouhadidaa, M., Casas, A.M., Gonzaloa, M.J., Arúsc, P., Morenoa, M.A. & Gogorcenaa, Y. 2009. Molecular characterization and genetic diversity of *Prunus* rootstocks. *Scientia horticulturae* **120**(2), 237–245. https://doi.org/10.1016/j.scienta.2008.11.015

Cao, J., Jiang, Q., Lin, J., Li, X., Sun, C. & Chen, K. 2015. Physical and chemical characteristics of four types of cherries (*Prunus spp.)* grown in China. *Food Chemistry* **173**, 855–863. https://doi.org/10.1016/j.foodchem.2014.10.094

Damodar, N. 2009. *Basic Econometrics Fifth Edition*. McGraw-Hill. 946 pp.

Eslami, O., Khorramrouz, F., Ghavami, A., Khaniki, S.H. & Shidfar, F. 2022. Effect of cherry consumption on blood pressure: a systematic review and meta-analysis of randomized controlled trials. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* **18**(2), 102409. https://doi.org/10.1016/j.dsx.2022.102409

Ganopoulos, I., Moysiadis, T., Xanthopoulou, A., Osathanunkul, M., Madesis, P., Zambounis, A., Avramidou, E., Aravanopoulos, F.A., Tsaftaris, A., Sotiropoulos, T., Chatzicharisis, I. & Kazantzis, K. 2016. Morpho-physiological diversity in the collection of sour cherry (*Prunus cerasus*) cultivars of the Fruit Genebank in Naoussa, Greece using multivariate analysis. *Scientia Horticulturae* **207**, 225–232. https://doi.org/10.1016/j.scienta.2016.05.018

Gaudet, M., Villani, F., Cherubini, M., Beritognolo, I., Dalla Ragione, I., Proietti, S. & Mattioni, C. 2019. Genetic diversity and molecular fingerprinting of *Prunus cerasus var.* austera from central Italy. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* **153**(4), 491–497. https://doi.org/10.1080/11263504.2018.1498403

Gavryliuk, O.S., Kondratenko, T.Je. & Goncharuk, Ju.D. 2019. Features of formation of productivity of columnar apple-tree. *Bulletin of Agricultural Science* **97**(6), 27–34. doi: https://doi.org/10.31073/agrovisnyk201906-04

Grafe, C. & Schuster, M. 2014. Physicochemical characterization of fruit quality traits in a German sour cherry collection. *Scientia Horticulturae* **180**, 24–31. https://doi.org/10.1016/j.scienta.2014.09.047

Guffa, B., Alrgei, H., Fotirić-Aksić, M., Rakonjac, V., Nikolić, D., Čolić, S. & Ličina, V. 2016. Influence of fruiting twig type to fruit and leaf traits in 'Oblacinska' sour cherry clones. *Acta Hortic.* **1139**, 237–242. https://doi.org/10.17660/ActaHortic.2016.1139.41

Havryliuk, O., Kondratenko, T., Mazur, B., Kutovenko, V., Mazurenko, B., Voitsekhivska, O. & Dmytrenko, Y. 2022a. Morphophysiological peculiarities of productivity formation in columnar apple cultivars. *Agronomy research* **20**(1), 148–160. https://doi.org/10.15159/AR.22.007

Havryliuk, O., Kondratenko, T., Mazur, B., Tonkha, O., Andrusyk, Y. Kutovenko, V., Yakovlev, R., Kryvoshapka, V., Trokhymchuk, A. & Dmytrenko, Y. 2022b. Efficiency of productivity potential realization of different-age sites of a trunk of grades of columnar type apple-trees. *Agronomy research* **20**(2), 241–260. https://doi.org/10.15159/AR.22.031

Ivanova, I., Kryvonos, I., Shleina, L., Taranenko, G. & Gerasko, T. 2019. Multicriteria Optimization of Quality Indicators of Sweet Cherry Fruits of Ukrainian Selection During Freezing and Storage. In: Nadykto V. (eds) Modern Development Paths of Agricultural Production. *Springer*, Cham, 707–717. https://doi.org/10.1007/978-3-030-14918-5_69

Ivanova, I.Ie., Serdiuk, M.Ie., Shkinder-Barmina, A.N. & Kryvonos, I.A. 2020. The influence of abiotic factors on formation of taste qualities of chtrry fruits. *Collected Works of Uman National University of Horticulture* **96**(1), 416–432. https://doi.org/10.31395/2415-8240-2020-96-1-416-432

Ivanova, I., Serdyuk, M., Malkina, V., Priss, O., Herasko, T. & Tymoshchuk, T. 2021b. Investigation into sugars accumulation in sweet cherry fruits under abiotic factors effects. *Agronomy Research* **19**(2), 444–457. https://doi.org/10.15159/ar.21.004

Ivanova, I., Serdyuk, M., Malkina, V., Tymoshchuk, T., Kotelnytska, A. & Moisiienko, V. 2021a. The forecasting of polyphenolic compounds in sweet cherry fruits under the impact of weather factors. *Journal of Agricultural Scienc* **32**(2), 239–250. https://dx.doi.org/10.15159/jas.21.27

Keane, K.M., Bell, P.G., Lodge, J.K., Constantinou, C.L., Jenkinson, S.E., Bass, R. & Howatsonm, G. 2016. Phytochemical uptake following human consumption of Montmorency tart cherry (*L. Prunus cerasus*) and influence of phenolic acids on vascular smooth muscle cells in vitro. *European Journal of Nutrition* **55**, 1695–1705. https://doi.org/10.1007/s00394-015-0988-9

Kelley, S.D., Adkins, Y. & Laugero, D.K. 2018. A Review of the health benefits of cherries. *Nutrients* **10**(2), 368. https://doi.org/10.3390/nu10030368

Keserović, Z., Ognjanov, V., Magazin, N. & Dorić, M. 2014. Current situation and perspectives in sour cherry production. *Sour cherry breeding cost action FA1104 Sustainable production of highquality cherries for the European market Novi Sad*, Serbia, 1–25. Available at https://www.bordeaux.inra.fr/cherry//docs/dossiers/Activities/Meetings/2014%2009%2015-17%20WG1%20Meeting_Novi%20Sad/Abstract%20book_Novi-Sad2014.pdf

Khoo, G.M., Clausen, M.R., Pedersen, B.H. & Larsen, E. 2011. Bioactivity and total phenolic content of 34 sour cherry cultivars. *J. Food Compos. Anal.* **24**(6), 772–776. https://doi.org/10.1016/j.jfca.2011.03.004

Malkina, V., Ivanova, I., Serdiuk, M., Kryvonos, I. & Bilous, E. 2019. Regression analysis of the dependence of the cherry yield from hydro-thermal factors in the conditions of multicollinearity. *Scientific Horizons* **11**(84), 51–60. https://doi.org/10.33249/2663-2144-2019-84-11-51-60

Mézes, Z., Szenteleki, K. & Gaál, M. 2017. Simulation model for sour cherry product lines. *Computers and Electronics in Agriculture* **140**, 190–202. https://doi.org/10.1016/j.compag.2017.04.023

Mezhenskyj, V.M. 2019. Collecting sorboid plants for their horticultural merit and use in breeding work in Ukraine. *Acta Hortic* **1259**, 25–30. https://doi.org/10.17660/ActaHortic.2019.1259.5

Mezhenskyj, V., Kondratenko, T., Mazur, B., Shevchuk, N., Andrusyk, Y. & Kuzminets, O. 2020. Results of Ribes breeding at the National University of Life and Environmental Sciences of Ukraine. *Research for Rural Development* **35**, 22–26. https://doi.org/10.22616/rrd.26.2020.003

Miloševic, T. & Miloševic, N. 2012. Fruit quality attributes of sour cherry cultivars. *International Scholarly Research Notices* **2012**, 1–6. http://dx.doi.org/10.5402/2012/593981

Mir, M.M., Iqbal, U. & Mir, S.A. 2021. *Production Technology of Stone Fruits*. Springer Singapore, 436 pp. https://doi.org/10.1007/978-981-15-8920-1

Papp, N., Szilvássy, B., Abrankó, L., Szabó, T., Pfeiffer, P., Szabó, Z., Nyéki, J., Ercisli, S., Stefanovits-Bányai, É. & Hegedűs, A. 2010. Main quality attributes and antioxidants in Hungarian sour cherries: Identification of genotypes with enhanced functional properties. *Int. J. Food Sci. Technol* **45**(2), 395–402. https://doi.org/10.1111/j.1365-2621.2009.02168.x

Picariello, G., De Vito, V., Ferranti, P., Paolucci, M. & Volpe, M.G. 2016. Species- and cultivar-dependent traits of *Prunus avium* and *Prunus cerasus* polyphenols. *J. Food Compos. Anal.* **45**, 50–57. https://doi.org/10.1016/j.jfca.2015.10.002

Sanderson, V., Bamber, N. & Pelletier, N. 2019. Cradle-to-market life cycle assessment of Okanagan (Canada) cherries: helicopters, seasonal migrant labour and flying fruit. *Journal of Cleaner Production* **229**, 1283–1293. https://doi.org/10.1016/j.jclepro.2019.04.398

Shevchuk, L., Grynyk, I., Levchuk, L., Babenko, S., Podpriatov, H. & Kondratenko, P. 2021a. Fruit Quality Indicators of Apple (*Malus domestica Borkh*.) Cultivars Bred in Ukraine. *Journal of Horticultural Research* **29**(2), 95–106. https://doi.org/10.2478/johr-2021-0019

Shevchuk, L.M., Grynyk, I.V., Levchuk, L.M., Yareshcenko, O.M., Tereshcenko, Y. & Babenko, S.M. 2021b. Biochemical contents of highbush blueberry fruits grown in the Western Forest-Steppe of Ukraine. *Agronomy research* **19**(1), 232–249. https://doi.org/10.15159/ar.21.012

Sokół-Łętowska, A., Kucharska, A.Z., Hodunm, G. & Gołba, M. 2020. Chemical Composition of 21 Cultivars of Sour Cherry (*Prunus cerasus*) Fruit Cultivated in Poland. *Molecules* **25**(19), 4587. https://doi.org/10.3390/molecules25194587

Tonkha, O., Menshov, O., Bykova, O., Pikovska, O. & Fedosiy, I. 2020, November. Magnetic methods application for the physical and chemical properties assessment of Ukraine soil. In *XIV International Scientific Conference "Monitoring of Geological Processes and Ecological Condition of the Environment"* (Vol. 2020, No. **1**, pp. 1–5). European Association of Geoscientists & Engineers. https://doi.org/10.3997/2214-4609.202056027

Turovtseva, V.A., Turovtseva, N.N. & Shkinder-Barmina, A.N. 2016. Results of selection work with cherry and dukes at Melitopol Research Fruit Growing Station named after M.F. Sydorenko of the Institute of Horticulture of National Academy of Agrarian Sciences. *Bulletin of the Ukrainian Society of Genetics and Breeders* **2**(14), 227–238 (in Ukrainian).

Vasylenko, O., Kondratenko, T., Havryliuk, O., Andrusyk, Y., Kutovenko, V., Dmytrenko, Y., Grevtseva, N. & Marchyshyna, Y. 2021. The study of the productivity potential of grape cultivars according to the indicators of functional activity of leaves. *Potravinarstvo Slovak Journal of Food Sciences* **15**, 639–647. https://doi.org/10.5219/1638

Viljevac-Vuleti´c, M., Dugali´c, K., Mihaljevi´c, I., Tomaš, V., Vukovi´c, D., Zduni´c, Z., Puškar, B. & Jurkovi´c, Z. 2017. Season, location and cultivar influence on bioactive compounds of sour cherry fruits. *Plant Soil Environ* **63**(9), 389–395. https://doi.org/10.17221/472/2017-PSE

Wojdyło, A., Nowicka, P., Laskowski, P. & Oszmia´nski, J. 2014. Evaluation of sour cherry (*Prunus cerasus L*.) fruits for their polyphenol content, antioxidant properties, and nutritional components. *J. Agric. Food Chem* **62**(51), 12332–12345. https://doi.org/10.1021/jf504023z

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Wheat-Faba bean intercrops improve plant nutrition, yield, and availability of nitrogen (N) and phosphorus (P) in soil

G. Kaci[1,*], W. Ouaret[2] and B. Rahmoune[3]

[1]M'hamed Bougara University, Faculty of Sciences, Department of agronomy, DZ35000 Boumerdès, Algeria
[2]Miami University, Geospatioal Analysis Center, US-OH 45056 Ohio, USA
[3]High National School of Agronomy, Genetics Ressources and Biotechnology Laboratory, DZ16000 El Harrach, Algeria
[*]Correspondance: kaci.ghiles@gmail.com

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Abstract. In order to promote agroecological practices, this study compares two cropping systems, i.e., intercropping versus sole cropping of a cereal - durum wheat (*Triticum durum Desf.*; and a nitrogen-fixing legume - faba bean (*Vicia faba L.*) on plant growth, Efficiency in the use of rhizobial symbiosis (EURS), grain yield and phosphorus (P) and nitrogen (N) accumulation in soil and plant. This study conducted during two cropping seasons in a field trial in the region of Tizi Ouzou, Algeria, shows that shoot dry weight (SDW), nitrogen nutrition index (NNI), phosphorus use efficiency (PUE), land use efficiency (LER), and grain yield were significantly higher for intercropped than for the sole cropped wheat. Furthermore, there was a considerable increase in soil P and N content across the two years of intercropping and sole cropping compared to the unseeded weeded fallow. Intercropping, it is claimed, improves wheat N nutrition by increasing the availability of soil-N for wheat. This increase might be due to reduced interspecific competition between legumes and wheat plants than intraspecific competition between wheat plants due to the legume's ability to compensate by atmospheric nitrogen fixation.

Key words: legume, cereal, eurs, yield, soil fertility.

INTRODUCTION

By 2050, the global human population will reach 9.8 billion people (Layek et al., 2018). As a result of this population expansion, new urban agglomerations and industrial infrastructures will develop, often encroaching on agricultural lands reducing the land available for cultivation and increasing greenhouse gas emissions that are harmful to the environment and human health. By consequence, food security has become a severe concern for most governments, particularly emerging countries.

Intensive agriculture can help achieve food security by increasing crop yields and meeting the requirements of the world's population. This agricultural intensification requires many chemical inputs, which causes environmental issues (Chen et al., 2019).

Wang et al. (2015) report that nitrogen fertilizers improve crop yields while the efficiency of rhizobial symbiosis (EURS) decreases, and the massive nitrogen remains in the soil profile. However, the N that remains in the soil can pollute groundwater through leaching and contaminate the air by promoting the volatilization of NO, N_2O, and NH_3 (Zhu & Chen, 2002).

Integrating legumes into cropping systems, either as intercrops or as relay crops, might be an alternative agronomic approach for better utilization of growth resources by utilizing their agroecological services (Scalise et al., 2015; Kaci et al., 2018; Chen et al., 2019).

Growing two or more crop species on the same farmland is known as intercropping (Willey, 1979). Intercropping systems, including legumes, can help the intercropped species by increasing the efficiency of soil resource utilization (particularly phosphate and nitrogen) and suppressing weeds, pests, and diseases (Peoples et al., 2018; Kherif et al., 2021).

Several authors have reported the benefits of cereal-vegetable intercropping on grain yield, nitrogen nutrition, Land Equivalent Ration (LER), and P uptake (Betencourt et al., 2012; Cong et al., 2015; Kaci et al., 2018; Tang et al., 2020; Kherif et al., 2021).

In a three-year experiment, Kaci et al. (2018) showed that intercropping wheat and faba bean boosts aboveground productivity, grain yield, and Nitrogen Nutrition Index (NNI) for intercropped wheat by improving the effectiveness of faba bean's rhizobial symbiosis (EURS). According to these authors, this rise might be attributable to reduced interspecific competition between legumes and wheat plants than intraspecific competition between wheat plants, thanks to the legume's adaptation by fixing atmospheric nitrogen.

Otherwise, phosphorus (P) is a nutrient that strongly limits plant growth in many soils (Raghothama, 1999; Hinsinger, 2001; Betencourt et al., 2012). For several decades, the extensive use of fertilizers has been the primary practice to overcome this limitation and maintain high-yielding agroecosystems. The mined phosphate rock used to produce P fertilizer remains a finite resource (Cordell et al., 2009; Dawson & Hilton, 2011; Betencourt et al., 2012). Therefore, increasing phosphate fertilizer use to improve agricultural production in increasing global food demand is no more a suitable alternative (Vance, 2001; Hinsinger et al., 2011; Betencourt et al., 2012). New techniques are needed to better exploit soil P resources through efficient cultivar selection or alternative agroecosystem management strategies to optimize P bioavailability (Vance, 2001; Lambers et al., 2006; Betencourt et al., 2012; Tang et al., 2020).

In this context, cereal/legume intercropping benefits on P acquisition have been reported (Betencourt et al., 2012; Tang et al., 2016; Tang et al., 2020). These benefits may be due to belowground complementarity or facilitation effects (Li et al., 2014). Complementarity is defined as less competition for soil nutrients between two different species grown in the same area compared to their respective monoculture. Regarding complementarity, the amount of P available to plants is primarily affected by the geometry and volume of the rhizosphere. Thus, increasing root surface area in intercropping systems induces better soil exploration (Hinsinger et al., 2011). Moreover, Cahill et al. (2010) report that environmental stimuli perceived by plants generate a modification in their root distribution according to nutrient availability and competition with root systems of other species.

Finally, the cereal/legume intercrop could improve its P uptake capacity by depleting specific inorganic pools. Without mentioning the transfer of nutrients or the improvement of P availability by the action of one species, white lupin intercropped with wheat preferentially use either citric acid-leachable P or a water-leachable soil P pool (Cu et al., 2005), supporting Turner (2008) hypothesis regarding resource sharing for soil phosphorus. However, similar to other cereal-legume intercropping models, these finds suggest that this strict complementarity is difficult to disentangle from other changes in the rhizosphere induced by intercropping, such as soil pH or changes in the enzymatic activity involved in solubilizing inorganic P (Li et al., 2003 and 2007; Alkama et al., 2009). However, even if the limits of complementarity are not clear, a general partitioning process likely shortens the period of competitive relationships between plants (Vance, 2001).

As intensive agriculture directly causes global environmental changes, agroecology has emerged as one of several options for reducing agriculture's environmental effect, particularly in smallholder farming systems. Investigating agroecological principles is necessary to comprehend the multiple practices for developing this cropping system, hence lowering agriculture's negative environmental impact and augmenting its resiliency. To this end, our study investigates the influence of the wheat/faba bean intercropping system on symbiotic root nodulation, critical for enhancing soil's nutrient bioavailability. Moreover, we examined how this system affects rhizobial symbiosis activity for plant nitrogen nutrition and P use efficiency compared to the mono-cropping system in a smallholder agriculture context under Mediterranean climatic conditions.

MATERIAL AND METHODS

Experimental site

The field experiments were conducted over two growing seasons: 2019 and 2020. They were located in a farmer's plot in the province of Tizi Ouzou, 100 km east of Algiers, Algeria. The main sites of the experiments were located at 36°41'51.4 "N 4°04'58.6"E.

The region has irregular rainfall, with an annual mean of 733 mm and 660 mm respectively in 2019 and 2020. The driest month was June (with rainfall of about 1 mm) in 2019 and July (with rainfall of about 0 mm) in 2020, while the highest rainfall was in November 2019 and December 2020 with 220 mm and 179 mm, respectively. The warmest month was July over the two growing seasons with a mean of 30 °C and 37 °C respectively in 2019 and 2020, while the coldest month was January (mean of 6 °C and 7 °C respectively in 2019 and 2020).

Within the study plots, there were mainly Calcisols (IUSS Working Group, 2015), which are typical soils of the Mediterranean part of the Magreb (e.g. Baraket et al., 2021). The average texture of investigated soils was clay loam with 47% of silt, 29% of clay and 24% of sand. Regarding soil chemical properties, the topsoil was alkaline (pH 8.7), with 17 g kg^{-1} $CaCO^3$ and relatively low organic matter content (2.4%). The agricultural conditions at this experimental soil correspond to an N deficiency (total N: 1.2 g kg^{-1} and inorganic N ($N\text{-}NH_4^+ + NO_3^-$): 9 mg N kg^{-1}) and P sufficiency (total P: 289 mg kg^{-1} and P Olsen: 23.5 mg P kg^{-1}).

Cropping systems and plant growth conditions

The field experiment was carried out with one durum wheat cultivar (*Triticum durum Desf.* cv. Vitron) and one faba bean cultivar (*Vicia faba L.* cv. Diva). The experimental design was a randomized complete block design with four blocks (four replicates); each block was divided into four sub-plots. Each subplot was composed of the following cropping systems: (1) durum wheat-faba bean intercropping system; (2) durum wheat sole cropping system; (3) faba bean sole cropping system; (4) unseeded weeded fallow surface. Over the two growing seasons, the experiments covered an area of 1,000 m^2, each sub-plot being 6 m × 10 m. According to the current farming practice, the seeding density was 350 plants per m^2 for durum wheat as a sole crop, 60 plants per m^2 for faba bean as a sole crop, 175 plants for durum wheat, and 30 per m^2 for faba bean as intercrops. Both species were sown in the same row for the intercropping system to maximize root proximity and rhizospheric interactions between durum wheat and faba bean. Before crop planting, the first 20 cm of soil were mechanically plowed using a rotary spading machine, then a passage with a rotary harrow was carried out to prepare the seedbed. Finally, the seeding was sown manually at 5 cm depth, followed by a smooth roller pass to promote the contact between soil and seeds. No chemical or fertilizer treatment was applied to the crops.

Plant and soil sampling and measurements

Throughout two growing seasons, soil and plants were sampled during the entire flowering stage of faba beans. With each cropping system, four sampling points were chosen for each subplot. A sample of 8–10 plants was taken with soil around the roots at each sampling point. To not destroy the roots, the soil surrounding the roots was carefully scraped to a depth of 30 cm, and soil samples were mixed into a single sample for each subplot. After that, this soil was sieved to less than 4 mm to remove the coarse fraction and then dried in ambient air for at least 48 h. Shoots were separated from the roots at the cotyledonary nodes for sampled plants. In addition, nodules were carefully separated from the roots of faba beans. Shoots, roots, and nodules were dried for 48 h at 65 °C and then weighted.

The total yield of both crops was determined following a manual harvest; for each cropping system and sub-plot, the crop was collected separately and weighed in the laboratory. In addition, after each harvest, the crop residues of each sub-plot were crushed mechanically using the stubble cutter and then incorporated into the soil. The N concentration in the soil was determined using the Kjeldahl method (Lynch et al. 1999), and the total P concentration in the plants (shoots, roots, and grains) and soil was determined using the malachite green method after digestion by nitric and perchloric acid (Petibon et al., 2000). The soil P availability was determined by extraction with NaHCO3 (Olsen method).

Efficiency in the use of rhizobial symbiosis (EURS)

Drevon et al. (2011) defined the relationship between changes in symbiotic nitrogen-fixing nodule biomass and plant biomass as an estimator of efficiency in using rhizobial symbiosis. This simple relationship can also be considered an indicator of symbiotic nodules' ability to fix atmospheric nitrogen. In addition, in practice, shoot biomass is often used instead of the total plant biomass to avoid the possible underestimation of root biomass during field sampling.

Nitrogen nutrition index (NNI)

NNI is defined as the ratio of actual crop N uptake (N_a) to the critical N uptake (N_c) (Eq. (1)), which is the minimum N uptake without deficiency to allow for the maximum growth rate at any time of plant growth (Eq. (2)) (Lemaire et al. 2008).

$$NNI = \%N_a \times \%N_c^{-1} \tag{1}$$

$\%N_c$ is determined with empirical dilution curve such as:

$$\%N_c = ac \times W^{-b} \tag{2}$$

where W – actual crop mass (t ha^{-1}); ac – critical plant N concentration for 1 t W ha^{-1}, and b – constant (Lemaire et al., 2008). For wheat, the values of ac and b are 5.3% and 0.44 (Lemaire et al., 2008).

P use efficiency (PUE)

PUE stands for plant P usage efficiency; numerous methods conceptualize and measure physiological and agronomic PUE. In our study, we used physiological PUE, which is defined as the ratio of whole plant dry weight (DWp) to plant phosphorus concentration (Pc) (Eq. (3)). (Vadez & Drevon, 2001; Benlahrech et al., 2018).

$$PUE = DW_p \times P_c^{-1} \tag{3}$$

Land Equivalent Ratio for Yield (LER_Y)

Willey & Osiru (1972) have defined the relative land area required when growing sole crops to produce yield obtained in an intercrop as the land equivalence ratio for yield (LER_Y). LER_Y indicates the yield advantage of the intercrop over the sole cropping system for a similar unit area between the two systems (Eq. (4)).

$$LER_Y = (Y_{W\text{-}IC} \times Y_{W\text{-}SC}^{-1}) + (Y_{F\text{-}IC} \times Y_{F\text{-}SC}^{-1}) \tag{4}$$

where $Y_{W\text{-}IC}$ and $Y_{F\text{-}IC}$ are the yields of wheat and faba bean intercropped. On the other hand, $Y_{W\text{-}Sc}$ and $Y_{F\text{-}SC}$ yield wheat and faba bean yield in the sole crop, respectively.

Statistical analysis and calculations

Before statistical analysis, the data were checked for homogeneity of variance. The R software was used to run one- and two-way analyses of variance (ANOVA) (R Core team 2016). Tukey's multiple comparison tests at $P = 0.05$ were used to select the means. A linear regression relationship between shoot (SDW) and nodule (NDW) dry weights were determined for the cropping systems with faba bean each year. In order to compare the biomass stocks per equivalent area units (g m^{-2}), SDW and, for faba bean, NDW per plant (g plant^{-1}) were converted into stocks as follows:

$$\text{SDW (or NDW) stocks} = \text{SDW (or NDW)} \times SD_{corr} \tag{5}$$

where SD_{corr} – sowing density (SD) of a given species corrected by the area actually occupied by this species. Thus, SD_{corr} = SD for sole crops, and SD_{corr} = SD / 0.5 = SD × 2 in intercropping since the area was halved in intercropping for each species.

RESULTS

Plant growth and nodulation

Wheat and faba bean shoot dry weight was significantly affected by cropping system as shown in Table 1; the table also shows that only wheat SDW was significantly affected by growing season. Fig. 1, A, shows that SDW per equivalent unit area of durum wheat (SDW g m^{-2}) was significantly higher in intercropping than in sole cropping (38% in 2019 and 47% in 2020). However, faba bean shoot dry weight decreased significantly under the intercropping system by 29% in 2019 and 24% in 2020 compared to sole cropping (Fig. 1, B). In the same way, the cropping system had a considerable impact on faba bean nodulation (Table 1).

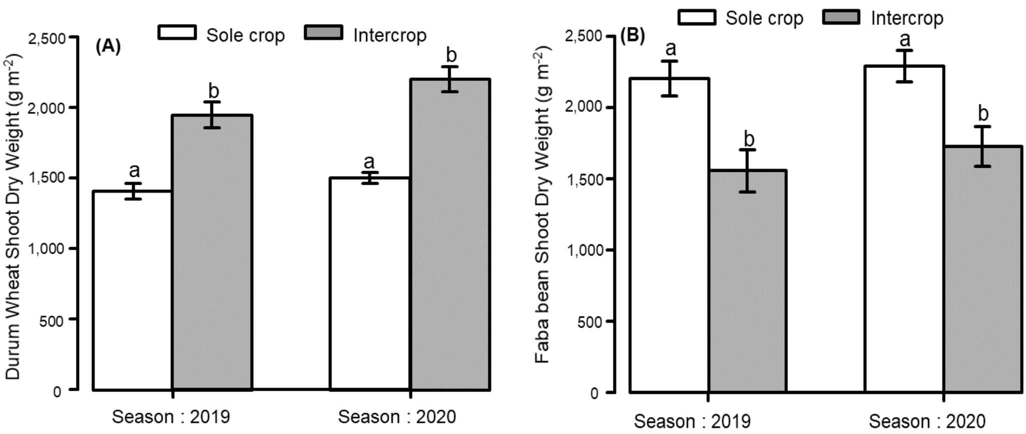

Figure 1. Durum wheat (A) and Faba bean (B) shoot dry weight for each year in sole cropping versus intercropping. Data are means and standard errors of 16 replicates harvested at 130 days after sowing. Within a given year, mean values labeled with different letters are significantly different at $P < 0.05$.

On another hand, when compared to sole cropping, intercropping significantly reduced nodule dry weight (NDW) and the number of nodules (NN) (Table 1). Regarding NDW, the loss was estimated to 28% in 2019 and 36% in 2020 (Fig. 2, A); for the number of nodules (NN), the diminution was estimated to 45% and 40% in 2019 and 2020, respectively (Fig. 2, B).

Table 1. *P*-values of two-way ANOVAs with factors growing season and cropping system on plants and soil variables

	Shoot dry weight (g m^{-2})		Nodule dry weight (g m^{-2})	Nodules number (Nod pl^{-1})	Soil N	Soil P
	Wheat	Faba bean	Faba bean		%	
Growing season	$1.73\times10^{-2*}$	0.32^{ns}	0.38^{ns}	0.33^{ns}	0.43^{ns}	0.39^{ns}
Cropping system	$9.33\times10^{-15***}$	$7.50\times10^{-6***}$	$2.94\times10^{-5***}$	$< 2\times10^{-16***}$	$1.55\times10^{-8***}$	$9.53\times10^{-11***}$
Growing season × Cropping system	0.26^{ns}	0.74^{ns}	0.42^{ns}	0.73^{ns}	0.98^{ns}	0.96^{ns}

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{ns} not significant ($P > 0.05$).

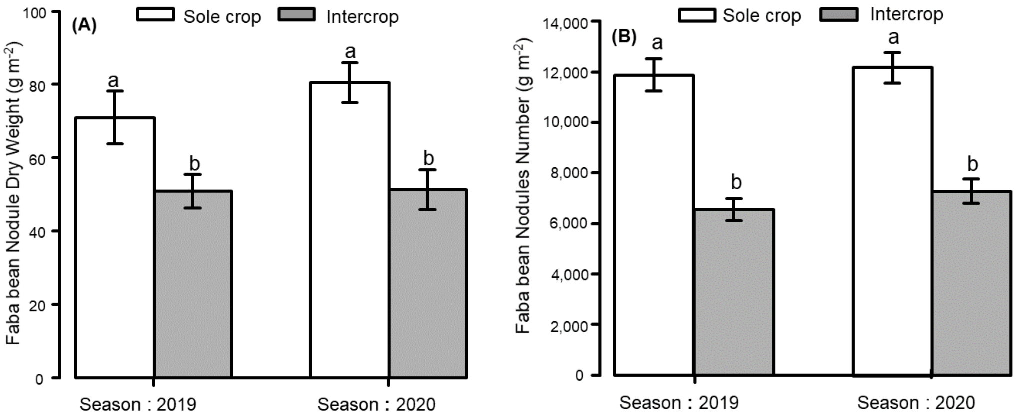

Figure 2. Faba bean nodule dry weight (A) and nodules number (B) for each year in sole cropping versus intercropping. Data are means and standard errors of 16 replicates harvested at 130 days after sowing. Within a given year, mean values labeled with different letters are significantly different at $P < 0.05$.

Efficiency in the use of rhizobial symbiosis (EURS)

EURS (calculated as the slope of the linear regression between plant and nodule biomass) were significantly different in Faba bean grown in intercrop and sole crop systems (Fig. 3). The shoot dry weight (SDW) of each faba bean plant was linked to the nodule dry weight (NDW). In both growing seasons, the EURS for intercropping was significantly higher than for sole cropping; in 2019, the EURS for intercropped faba bean (27.5 g SDW g^{-1} NDW) was 87% higher than sole cropped faba bean (14.7 g SDW g^{-1} NDW) (Fig. 3, A). In 2020, intercropped faba bean had a EURS of 26 g SDW g^{-1} NDW which was 47% higher than sole cropped faba bean (17.7 g SDW g^{-1} NDW) (Fig. 3, B).

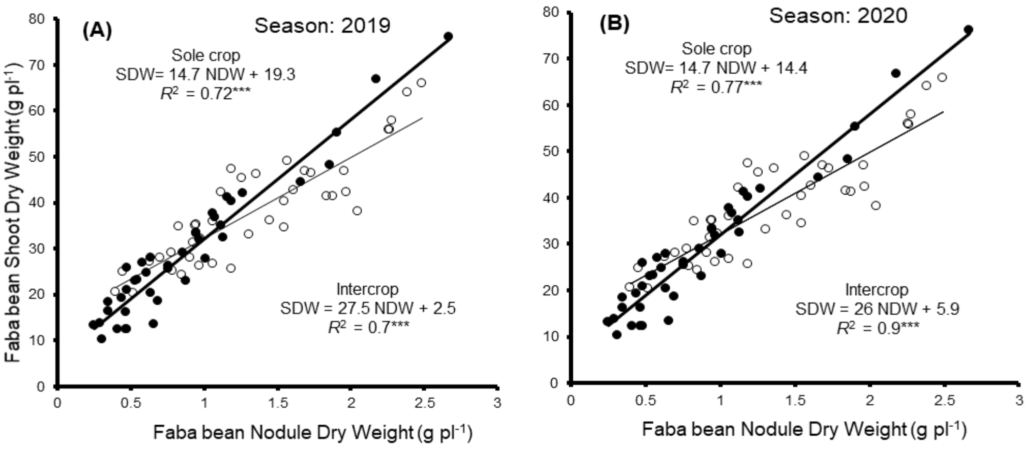

Figure 3. Efficiency in use of the rhizobial symbiosis for faba bean in sole cropping (white dots) versus intercrop ping (black dots) for (A) 2019; (B) 2020. The equations on the charts are the regressions for sole crops (light grey text) and intercrops (dark grey text). All regressions were established from sixteen replicates (sixteen plants). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Wheat Nitrogen Nutrition Index (NNI) and P Use Efficiency (PUE)

The two-way ANOVA in Table 2 shows that the growing season and cropping system significantly impact NNI and PUE. Fig. 4, A, indicates that the NNI for intercropping was higher than 1, but the NNI for sole cropping was less than 1. In 2019 and 2020, the NNI for intercropping was 64 and 91 percent higher than sole cropping, respectively. Furthermore, Fig. 4, B shows that PUE for intercropped wheat was much higher than sole cropped wheat by 97 percent and 124 percent, respectively, in 2019 and 2020.

Table 2. *P*-values of two-way ANOVAs with factors growing season and cropping system on grain yield, INN and PUE

	Grain yield (t ha^{-1})		Nitrogen nutrition index (NNI)	P use efficiency (PUE)
	Wheat	Faba bean	Wheat	Wheat
Growing season	0.62 ^{ns}	0.45 ns	$3.39\times10^{-5***}$	0.023^{*}
Cropping system	$1.95\times10^{-5***}$	0.30 ns	$< 2.2\times10^{-16***}$	$< 2\times10^{-16***}$
Growing season × Cropping system	0.75 ^{ns}	0.51 ns	0.088 ^{ns}	0.078 ^{ns}

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ^{ns} not significant ($P > 0.05$).

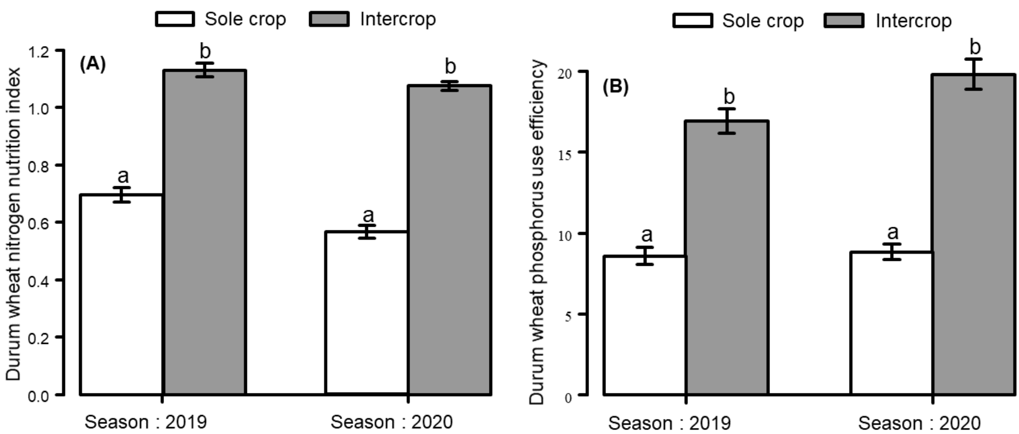

Figure 4. Durum wheat nitrogen nutrition index (A) and P use efficiency (B) for sole crops and intercrops. Means and standard error for sixteen replicates harvested at 130 days after sowing. For each year, mean values labeled with the same letters are not significantly different at $P < 0.05$.

Grain Yield and Land Equivalent Ratio for Yield (LER_Y)

Table 2 shows that cropping systems, not the growing season, had a significant effect on durum wheat grain yield; however, cropping systems and the growing season had no impact on faba bean grain yield. As shown in Fig. 5, A, wheat grain yield was 8 percent higher in intercropping than in sole cropping in 2019 and 7 percent higher in 2020.

Land Equivalent Ratio for yield was higher than 1 ($LER_Y > 1$) as reported in Table 2, indicating that the intercropping was more beneficial than the sole cropping for durum wheat grain yield.

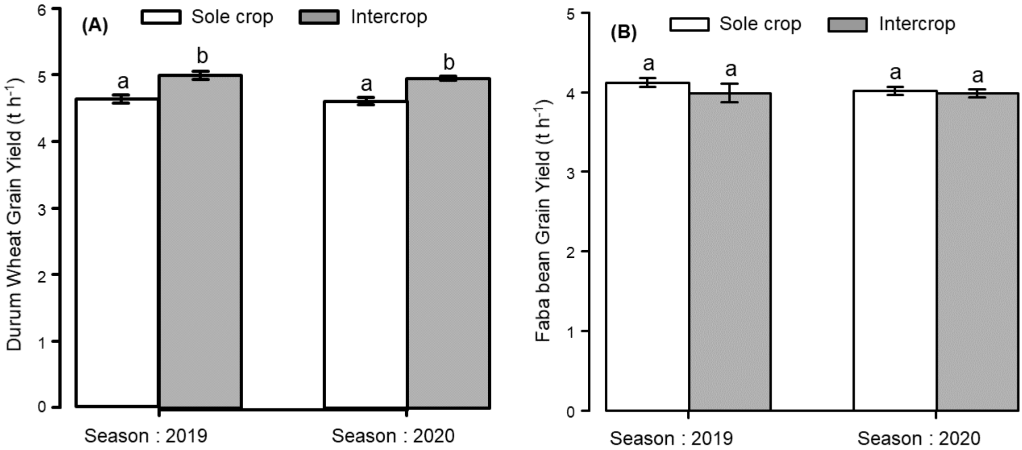

Figure 5. Durum wheat (A) and faba bean (B) grain yield for sole crops and intercrops. Means and standard error for sixteen replicates harvested at total maturity. For each year, mean values labelled with the same letters are not significantly different at $P < 0.05$.

Soil N and P Availability

Fig. 6 shows the average inorganic N (6A) and Olsen P (6B) concentrations in the rhizosphere for intercrops and sole crops, respectively. During both cropping seasons, the inorganic nitrogen concentration in the soil of the intercrops and sole crops was higher than that of the unseeded weeded fallow. The nitrogen concentration in the soil was significantly higher for intercrops than for unseeded weeded fallows by 68% and 72% in 2019 and 2020, respectively. In addition, soil N content was significantly higher under the intercropping system by 21% in 2019 and 2020 than sole cropped faba bean, 51% in 2019 and 46% in 2020 compared to sole cropped wheat (Fig. 6, A).

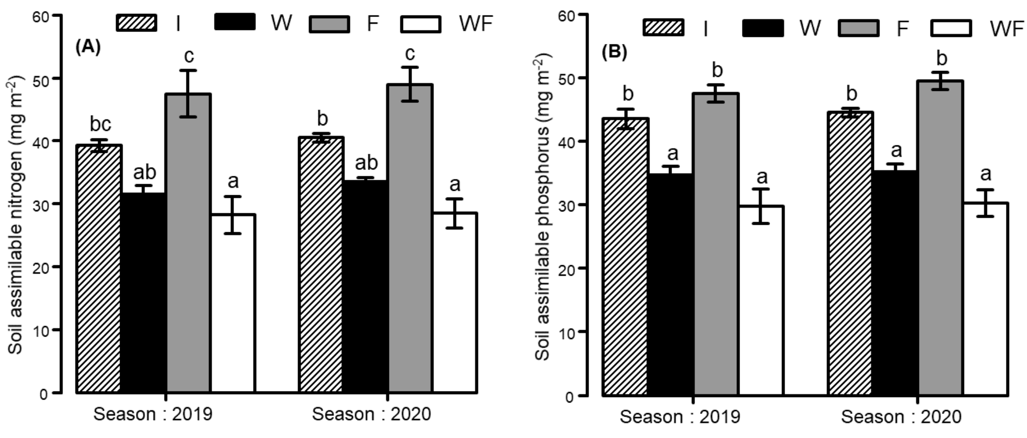

Figure 6. Soil nitrogen (A) and phosphorus (B) contents in sole cropping versus intercropping versus fallow for each year. Means and standard error of four replicates for intercropping and unseeded weeded control and eight replicates for rotation. For each cropping system, mean values labelled with the same letters are not significantly different at $P < 0.05$. Where I – intercrops; W – wheat sole crop; F – faba bean sole crop; WF – weeded fallow.

When faba bean was included in the cropping system, either intercropping or sole cropping, soil Olsen P concentrations were consistently higher compared to sole cropped wheat, and unseeded weeded fallow. Intercropping had the highest Olsen P levels, 37% higher in 2019 and 40% higher in 2020 than sole cropped wheat; it was also 60% higher than unseeded weeded fallow in 2019 and 2020, respectively.

DISCUSSION

To strengthen resilient and sustainable practices in agriculture, crop diversification in the same space (e.g., intercropping cereals-legumes) appears to be one of the agroecological practices that would enhance agroecosystem services (Daryanto et al., 2019; Kherif et al., 2021). Overall, our results indicate a significant increase in dry shoot weight for durum wheat intercropped with faba bean compared to sole cropped durum wheat for an N-deficient and alkaline soil. However, when faba bean was intercropped with durum wheat, the dry weight of shoots and nodules was significantly decreased, but the number of nodules was significantly increased. Previous field studies in the Mediterranean region have investigated the impact of intercropping in various legume-cereal systems on plant growth. It was found that the dry shoot weight of durum wheat was increased when intercropped with faba bean and cowpea (Betencourt et al., 2012; Kaci et al., 2018). Also, these authors confirm that shoot biomass and nodule biomass were significantly lower when cowpea and faba bean were intercropped with durum wheat. However, Maingi et al. (2011) and Banik et al. (2006) reported that nodular biomass of common bean and chickpea was significantly higher under intercropping, which may be due to the beneficial association between cereal and legume. As well, our study shows an increase in durum wheat yield under intercropping in both cropping seasons, confirmed by an LER_Y greater than 1, indicating a benefit to the associated grain. These results are in agreement with those of Kaci et al. (2018), who reported an increase in wheat yield when intercropped with faba bean during three cropping seasons, whereas other studies also report the benefits of cereal/legume association on grain yield (Darch et al., 2018; Mndzebele et al., 2020), such as Kherif et al. (2021) who reported an increase in the yield of wheat associated with chickpea compared to its Sole crop.

Furthermore, intercropping significantly affects the efficiency in the use of rhizobial symbiosis (EURS) in both growing seasons. The faba bean EURS was much higher for intercrops than for sole crops by 12.8 and 8.3 g SDW g^{-1} NDW respectively, in 2019 and 2020. This increase in EURS would result from the strong interspecific competition exerted by the roots of durum wheat on nutrients, especially under these conditions of N deficiency, which would stimulate biological N_2 fixation by legumes. This increase in EURS is also linked to a decrease in nodular biomass and in the number of nodules, which suggests a change in the population of efficient rhizobial strains inducing a high number of small nodules involved in efficient nodulation with intense nitrogenase activity (Alkama et al., 2012; Kaci et al., 2018; Chenene et al., 2021). Kaci et al. (2018) and Latati et al. (2019) also reported an increase in EURS of faba bean and chickpea when intercropped with durum wheat compared to their respective monoculture.

There was also a significant increase in NNI for durum wheat intercropped with faba bean in both growing seasons, with values greater than 1 indicating better nitrogen nutrition for the intercropped wheat compared to the sole cropped. According to

Callaway (1995), the ability of legumes to access the atmospheric N_2 pool via the symbiotic fixation mechanism appears to be an alternative to interspecific competition with cereals for the soil N pool, thus inducing a complementarity of N use between the two species. Furthermore, wheat PUE was significantly higher when intercropped with faba bean in both growing seasons. Our findings agree with those of Tang et al. (2020), who also report a 21% improvement in P use efficiency under the intercropping system. In this study, the increase in PUE is directly linked to the increase in soil Olsen P when legume is included in the cropping system. The increase in Olsen P availability under the association can be explained by several changes induced in the rhizosphere (proton release, organic acids exudation, acid phosphatases, etc.) by the high nitrogen-fixing activity of the legume (Tang et al., 2004; Alkama et al., 2009, 2012; Bargaz et al., 2012).

P availability (Olsen P) was not a limiting factor for crops; however, we found that P availability was higher in intercrops than monocrops and the unseeded weeded fallow. Several pieces of research have shown a significant effect of cereal-legume intercrops on P availability under P-deficiency conditions (Devau et al., 2011; Betencourt et al., 2012; Tang et al., 2020). As suggested by Wang et al. (2015) we presume that low N availability in the rhizosphere can promote P availability through root-induced processes in alkaline soil, such as rhizosphere acidification by legumes (exudation of phosphatases, carboxylates, and/or indirectly through microbial activities). Recent research has demonstrated the benefits of intercropping for cereals through the legume's facilitative mechanisms, which was responsible for increasing the availability of inorganic resources (e.g., inorganic P) through rhizosphere acidification during rhizosphere N_2 fixation, according to the stress gradient theory (Tang et al., 2020; Kherif et al., 2021). Where soil conditions are limited, as in P-deficient alkaline or calcareous soils, these positive interactions are precious (Drevon et al., 2011; Tang et al., 2020).

In this study's N-deficient soil, the availability of N in the rhizosphere of wheat-faba bean intercrops was significantly higher in both cropping seasons compared to sole crops; this increase was also accompanied by improved aboveground biomass and better symbiotic fixation of atmospheric N_2, implying the establishment of an interspecific complementarity that would facilitate nutrient acquisition while reducing competition. An increase in rhizosphere N content in cereal-legume intercrops compared to sole crops was previously reported by several authors like Kaci et al. (2018); Tang et al. (2020) and Rodriguez et al. (2020).

CONCLUSION

During this field trial conducted on a farming site over two cropping seasons, it became evident that wheat/faba bean intercropping is more advantageous than sole cropping. Indeed, we found that intercropped wheat had higher aboveground biomass, grain yield, nitrogen nutrition, and EUP than sole cropped wheat. These significant increases were accompanied by a physiological response of the related faba bean, which increased its capacity to fix atmospheric nitrogen to reduce interspecific competition for soil resources. Furthermore, we found that the availability of N and P in the rhizosphere of intercrops was higher than that of sole crops.

This finding demonstrated intercropping as one of the most effective agroecological systems and an alternative to intensive agriculture for ensuring ecosystem sustainability and reducing greenhouse gas emissions created by contemporary agriculture.

REFERENCES

Alkama, N., Bolou Bi Bolou, E., Vailhe, H., Roger, L., Ounane, S.M. & Drevon, J.J. 2009. Genotypic variability in P use efficiency for symbiotic nitrogen fixation is associated with variation of proton eux in cowpea rhizosphere. *Soil Biology and Biochemestry* **41**, 1814–1823.

Alkama, N., Ounane, G. & Drevon, J.J. 2012. Is genotypic variation of H^+ efflux under P deficiency linked with nodulated-root respiration of N_2-fixing common-bean (*Phaseolus vulgaris* L.)?. *Journal of Plant Physiology* **169**, 1084–1089.

Banik, P., Midya, A., Sarkar, B.K. & Ghose, S.S. 2006. Wheat and chickpea intercropping systems in an additive series experiment: Advantages and weed smothering. *European Journal of Agronomy* **24**, 325–332.

Baraket, F., González-Rosado, M., Brahim, N., Roca, N., Mbarek, H.B., Świtoniak, M., Chaker, R., Bellón, Á.S., Rigane, H., Gargouri, K. & Parras-Alcántara, L. 2021. Short and Long-Term Effect of Land Use and Management on Soil Organic Carbon Stock in Semi-Desert Areas of North Africa-Tunisia. *Agriculture* **11**, 12–67. https://doi.org/10.3390/agriculture11121267

Benlahrech, S., Kaci, G., Teffahi, M. & Ounane, S.M. 2018. Influence of inoculation and phosphorus regimes on symbiotic nitrogen fixation and phosphorus use efficiency of Algerian cowpea (*Vigna unguiculata* L. (Walp.)) landraces. *Agronomy Research* **16**, 337–348.

Betencourt, E., Duputel, M., Colomb, B., Desclaux, D. & Hinsinger, P. 2012. Intercropping promotes the ability of durum wheat and chickpea to increase rhizosphere phosphorus availability in a low P soil. *Soil Biology and Biochemistry* **46**, 181–190.

Cahill, J.F., McNickle, G.G., Haag, J.J., Lamb, E.G., Nyanumba, S.M. & St Clair, C.C. 2010. Plants integrate information about nutrients and neighbors. *Science* **328**, 16–57.

Callaway, R.M. 1995. Positive interactions among plants. *The Botanical Review* **61**, 306–349.

Chen, S., Waghmode, T.R., Sun, R., Kuramae, E., Hu, C. & Liu, B. 2019. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. *Microbiome* **7**(1), 1–13.

Chenene, Y., Blavet, D., Belalmi, M., Kaci, G., Teffahi, M. & Ounane, S.M. 2021. Variation of chickpea nodulation in a Mediterranean agroecosystem: relationship with soil characteristics and thresholds for significant contribution to plant growth. *Agronomy Research* **19**, 42–56.

Cong, W.F., Hoffland, E., Li, L., Six, J., Sun, J.H., Bao, X.G., Zhang, F.S. & Van Der Werf, W. 2015. Intercropping enhances soil carbon and nitrogen. *Global Change Biology* **21**, 1715–1726.

Cordell, D., Drangert, J.O. & White, S. 2009. The story of phosphorus: global food security and food for thought. *Global Environmental Change* **19**, 292–305.

Cu, S.T.T., Hutson, J. & Schuller, K.A. 2005. Mixed culture of wheat (Triticum aestivum L.) with white lupin (Lupinus albus L.) improves the growth and phosphorus nutrition of the wheat. *Plant and Soil* **272**, 143–151.

Darch, T., Giles, C.D., Blackwell, M.S.A., Brown, G.L.K., Menezes-Blackburn, D., Shand, C.A., Stutter, M.I., Lumsdon, D.G., Mezeli, M.M., Wendler, R., Zhanf, H., Wearing, C., Cooper, P. & Haygarth, P.M. 2018. Inter- and intra-species intercropping of barley cultivars and legume species, as affected by soil phosphorus availability. *Plant and Soil* **427**, 125–138.

Daryanto, S., Jacinthe, P.A., Fuab, B., Zhao, W. & Wang, L. 2019. Valuing the ecosystem services of cover crops: Barriers and pathways forward. *Agriculture, Ecosystems & Environment* **270**, 76–78.

Dawson, C.J. & Hilton, J. 2011. Fertiliser availability in a resource-limited world: production and recycling of nitrogen and phosphorus. *Food Policy* **36**, 14–22.

Devau, N., Hinsinger, P., Le Cadre, E. & Gérard, F. 2011. Root-induced processes controlling phosphate availability in soils with contrasted P-fertilized treatments. *Plant and Soil* **348**, 203–218.

Drevon, J.J., Alkama, N., Araujo, A., Beebe, S., Blair, M.W., Hamza, H., Jaillard, B., Lopez, A., Martinez-Romero, E., Rodino, P., Tajini, F. & Zaman-Allah, M. 2011. Nodular diagnosis for ecological engineering of the symbiotic nitrogen fixation with legumes. *Procedia Environmental Sciences* **9**, 40–46.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil* **237**, 173−195.

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., Tang, X. & Zhang, F. 2011. P for two, sharing a scarce resource e soil phosphorus acquisition in the rhizosphere of intercropped species. *Plant Physiology* **156**, 1078–1086.

IUSS Working Group WRB. 2015. World Reference Base for Soil Resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. *World Soil Resources Report* 106, FAO, Rome, Italy, 203 pp.

Kaci, G., Blavet, D., Benlahrech, S., Kouakoua, E., Couderc, P., Deleporte, P., Desclaux, D., Latati, M., Pansu, M., Drevon, J.J. & Ounane, S.M. 2018. The effect of intercropping on the efficiency of faba bean – rhizobial symbiosis and durum wheat soil-nitrogen acquisition in a Mediterranean agroecosystem. *Plant, soil and environment* **64**, 138–146.

Kherif, O., Seghouani, M., Zemmouri, B., Bouhenache, A., Keskes, M.I., Rebouh, Y.N., Ouaret, O. & Latati, M. 2021. Understanding the Response of Wheat-Chickpea Intercropping to Nitrogen Fertilization Using Agro-Ecological Competitive Indices under Contrasting Pedoclimatic Conditions. *Agronomy* **11**, 12–25.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S. & Veneklaas, E. 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of Botany* **98**, 693–713.

Latati, M., Dokukin, P., Aouiche, A., Rebouh, N.Y., Takouachet, R., Hafnaoui, E., Hamdani, F.Z., Bacha, F. & Ounane, S.M. 2019. Species Interactions Improve Above-Ground Biomass and Land Use Efficiency in Intercropped Wheat and Chickpea under Low Soil Inputs. *Agronomy* **9**, 765–780.

Layek, J., Das, A., Mitran, T., Nath, C., Meena, R.S., Yadav, G.S., Shivakumar, G., Kumar, S. & Lal, R. 2018. Cereal+Legume Intercropping: An Option for Improving Productivity and Sustaining Soil Health. In: Meena, R., Das, A., Yadav, G. & Lal, R. (Eds). Legumes for Soil Health and Sustainable Management. *Springer International Publishing*, Singapore, 347–386.

Lemaire, G., Jeuffroy, M.H. & Gastal, F. 2008. Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop N management. *European Journal of Agronomy* **28**, 614–624.

Li, L., Zhang, F., Li, X., Christie, P., Sun, J., Yang, S. & Tang, C. 2003. Interspecific facilitation of nutrient uptake by intercropped maize and faba bean. *Nutrient Cycling in Agroecosystems* **65**, 61–71.

Li, L., Li, S.M., Sun, J.H., Zhou, L.L., Bao, X.G., Zhang, H.G. & Zhang, F.S. 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. The *Proceedings of the National Academy of Sciences* **104**, 11192–11196.

Li, L., Tilman, D., Lambers, H. & Zhang, F.S. 2014. Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. *New Phytologist* **203**, 63–69.

Lynch, J.M. & Barbano, D.M. 1999. Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *Journal of AOAC International* **82**, 1389–1398.

Maingi, M.J., Shisanya, A.C., Gitonga, M.N. & Hornetz, B. 2011. Nitrogen fixation by common bean (*Phaseolus vulgaris* L.) in pure and mixed stands in semi-arid south east Kenya. *European Journal of Agronomy* **14**, 1–12.

Mndzebele, B., Ncube, B., Fessehazion, M., Mabhaudhi, T., Amoo, S., Venter, S. & Modi, A. 2020. Effects of Cowpea-Amaranth Intercropping and Fertiliser Application on Soil Phosphatase Activities, Available Soil Phosphorus, and Crop Growth Response. *Agronomy* **10**, 79–99.

Peoples, M.B., Hauggaard-Nielsen, H., Huguenin-Eli, O., Jensen, E.S., Justes, E. & Williams, M. 2018. The Contributions of Legumes to Reducing the Environmental Risk of Agricultural Production. In Lemaire, G., De Faccio Carvalho, P.C., Kronberg, S. & Recous, S (Eds). *Agroecosystem Diversity Reconciling Contemporary Agriculture and Environmental Quality* **1st edition**. Elsevier, Cambridge, 123–143.

Petibon, P., Roumet, C. & Jouany, C. 2000. Détermination de la teneur en phosphore par la méthode au vert de malachite : adaptation à de petits échantillons végétaux de faible teneur en phosphore. *Cahier Technique INRA* **43**, 3–8.

Raghothama, K.G. 1999. Phosphate acquisition. Annual Review of Plant Physiology and Plant. *Molecular Biology* **50**, 665–693.

Scalise, A., Tortorella, D., Pristeri, A., Petrovičová, B., Gelsomino, A., Lindström, K. & Monti, M. 2015. Legume-barley intercropping stimulates soil N supply and crop yield in the succeeding durum wheat in a rotation under rained conditions. *Soil Biology and Biochemistry* **89**, 150–161.

Tang, C., Drevon, J.J., Jaillard, B., Souche, G., Hinsinger, P. 2004. Proton release of two genotypes of bean (*Phaseolus vulgaris L.*) as affected by N nutrition and P deficiency. *Plant and Soil* **260**, 59–68.

Tang, X., Zhang, C., Yu, Y., Shen, J., Van der werf, W. & Zhang, F. 2020. Intercropping legumes and cereals increases phosphorus use efficiency; a meta-analysis. *Plant and Soil* **460**, 89–104.

Tang, X.Y., Placella, S.A., Daydé, F., Bernard, L., Robin, A., Journet, E.P., Justes, E. & Hinsinger, P. 2016. Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. *Plant and Soil* **407**, 119–134.

Turner, B.L. 2008. Resource partitioning for soil phosphorus: a hypothesis. *Journal of Ecology* **69**, 698–702.

Vadez, V. & Drevon, J.J. 2001. Genotypic variability in P use efficiency for symbiotic N_2 fixation in common bean (*Phaseolus vulgaris* L.). *Agronomie* **21**, 691–699.

Vance, C.P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. *Plant Physiology* **127**, 390–397.

Wang, Z., Bao, X., Li, X., Jin, X., Zhao, J., Sun, J. & Christie, P. 2015. Intercropping maintains soil fertility in terms of chemical properties and enzyme activities on a timescale of one decade. *Plant Soil* **405**, 265–282.

Willey, R.W. 1979. Intercropping-Its mportance and research needs. Part I Competition and yield advantages. *Field Crop Abstract* **32**, 1–10.

Willey, R.W. & Osiru, D.S.O. 1972. Studies on mixtures of maize and beans (*Phaseolus vulgaris*) with special reference to plant population. The *Journal of Agricultural Science* **79**, 519–529.

Zhu, Z.L. & Chen, D.L. 2002. Nitrogen fertilizer use in China - Contributions to food production, impacts on the environment and best management strategies. *Nutrient Cycling in Agroecosystems* **63**, 117–127.

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Technological characteristics of five new apple cultivars of VNIISPK breeding as raw materials for juice industry

N. Levgerova, E. Salina* and I. Sidorova

Russian Research Institute of Fruit Crop Breeding (VNIISPK), RU302530 Orel, Russia
*Correspondence: salina@vniispk.ru

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Abstract. The data of the technological assessment of the suitability of five new winter apple cultivars of VNIISPK breeding (‘Aleksandr Boyko’, ‘Blagodat’, ‘Vavilovskoye, Ivanovskoye’, ‘Patriot’ and ‘Prazdnichnoye’) for the production of raw materials for juice industry are presented. The main technological indicators characterizing the suitability of the cultivar for juice production were studied: the firmness of the pulp, the yield of juice, the content of soluble solids, sugars, titratable acids and catechins in comparison with the standard cultivar ‘Antonovka’. It was found that all of them were distinguished by a higher content of soluble solids and sugars in the juice than in the standard cultivar, and a lower content of titrated acids, as well as higher tasting ratings. ‘Aleksandr Boyko’ surpassed all studied cultivars in terms of such indicators as juice yield, the content of soluble solids, sugars and catechins in it, besides it was distinguished by low acidity. All studied cultivars, especially ‘Alexandr Boyko’, are promising as raw materials for the juice industry.

Key words: juice, unlit, juice yield, quality indicators.

INTRODUCTION

Despite a significant increase in the production of apple fruits, the problem of production of raw materials for processing remains acute in our country (Anonymous, 2021; Maksimova, 2021; Russia's self-sufficiency in apples is below 40%, 2021). The fruit processing industry in Russia is experiencing a negative impact from the lack of a modern raw material base, the growth rate of which is still insufficient for industry and the import level of fruit productions is 19% (Medvedeva, 2018). The issue of production of raw materials for the juice industry is particularly acute. In recent years, there has been a strong increase in interest in local cultivars intended for processing into juice and cider (Zielinski et al., 2014; Rumpunen et al., 2017).

Juices from fruits and vegetables are an integral part of a healthy human diet. They have a high nutritional value, quench thirst well and have a natural taste and aroma (Eremeeva et al., 2016; Makarkina & Pavel, 2017; Tinello, & Lante, 2018; Pepin et al., 2019; Zagoskina & Nazarenko, 2019). Currently, the main volumes of juices produced in Russia account for apple juice - 29.4%. A significant share of raw materials for the production of juice products (up to 70%) is imported to Russia from abroad. Only 25%

of these import fruits are fruits that do not grow in our country (citruses, pineapples, kiwi ets.) (Anonymous, 2020). Difficulties with raw materials for the juice industry are particularly explained by the transition to intensive horticulture. It means that horticulture focused on growing 75~80% of commercial fruits has deprived the manufacturers of 'technical apples', i.e., non-standard apples and windfall apples that has been traditionally used as raw materials. In this regard, it is legitimate to raise the question of the target development of technical intensive gardening, which would satisfy the needs of producers in domestic raw materials (Anonymous, 2017). The urgent need for laying out raw (technical) orchards requires the development of the assortment that meets the requirements of both intensive gardening and the fruit processing industry and contributes to improving the food safety of processed products (Sedov, 2011; Kozlovskaya, 2015; Savelieva, 2016; Mieszczakowska-Frąc et al., 2021). The suitability of apples for processing can be based on various technological indicators: technological (for example, suitability for a particular product), chemical (content of soluble solids, acidity, bioactive compounds, etc.), sensory (balance of aroma, taste, appearance) and economic (for example, juice yield) (Roman et al., 2007; Paganini et al., 2004; Salina et al., 2019). Due to the requirements of the customs regulation TR TS 023/2011, which does not allow the addition of sugar and sweeteners to fruit juices, low variability and the correct ratio of soluble solids and acids in apples can be of great importance for direct juice production (TR TS 023/2011, 2011). Thus, the quality of the final product depends on the quality of raw materials, which, in turn, strongly depends on the genotype (Mieszczakowska-Frąc et al., 2021). Therefore, the technological assessment of new cultivars for juice production becomes important.

The purpose of the research is the technological evaluation of new promising apple cultivars bred by the Russian Research Institute of Fruit Crop Breeding (VNIISPK) for juice production and the selection of the best cultivars according to technological indicators.

MATERIALS AND METHODS

In this study it was investigated five new apple cultivars of winter-ripening suitable for intensive gardening: 'Aleksandr Boyko' - a triploid scab immune cultivar; 'Blagodat' - a triploid; 'Vavilovskoye' - a triploid scab immune cultivar; 'Ivanovskoye' - a scab immune cultivar; 'Patriot' - a triploid and 'Prazdnichnoye' - a triploid scab immune cultivar (Sedov et al., 2018). All cultivars are of VNIISPK breeding, grown on the territory of the Orel district of the Orel region.

The experiment was carried out for three years and included experimental processing for juice, sensory evaluation, and determination of the biochemical composition of juice by the main components. 'Antonovka' served as the standard because this cultivar is one of most popular in Central Russia and its fruits are traditionally used in processing industry.

The fruits were harvested at the stage of the optimal picking time and stored in a refrigerator at a temperature of 2~4 °C until the onset of consumer maturity, after which the juice was made. We determinated the harvest ripeness by the starch iodine test (Smith et al., 1979; Blenpied, & Silsby, 1992). We took 3 kg apples to prepare each sample. Processing apples included washing fruits, inspection, pressing using an electric juicer

'Bork JU 24150 SI', pasteurization at 90 °C with successive cooling and sealing in glass bottles. The sealed bottles were stored for 3 months at room temperature, after which a sensory evaluation and chemical analysis of the samples were carried out.

The following technological and biochemical parameters were studied (Sedov, & Ogoltsova, 1999):

– apple firmness (kg cm^{-2}) – using a digital fruit tester FHT 803

– apple firmness (kg cm^{-2}) – using a digital fruit tester FHT 803

– juice yield (%) - according to the formula:

$$C = \frac{A - B}{A} \cdot 100,$$

where C is juice yield; A is fruit weight before pressing, g; B is the mass of marc, g; 100 is a conversion factor to percent (Daskalov et al., 1969).

– soluble solids content (°Brix) – using a refractometer (Atago refractometer, mod. PAL-1);

– the content of titrated acids (%) – volumetric method by 0.1 M NaOH using a phenolphthalein;

– content of sugars (%) – volumetric method using the Fehling reagent;

– Ratio – sugar/acid ratio

– the content of P-active catechins (mg per 100 g) was determined by the colorimetric method modified by A.I. Vigorov (Vigorov, 1964).

The sensory assessment of the juice appearance and palate was made by the tasting panel at closed tastings on a 5-point scale (5 – excellent quality, 1 – not usable). For a more accurate assessment, tenths of a point were indicated. The overall tasting score was considered as an arithmetic mean between the ratings of appearance and palate (Sedov, & Ogoltsova, 1999).

Obtained date was expressed as an average value ± standard error (SE). Statistical processing of the obtained data was carried out by generally accepted methods using standard Microsoft Excel computer programs. The reliability of the results was evaluated by $LSD_{0.5}$.

RESULTS AND DISCUSSION

The degree of maturity, at which the juice was made, was close to consumer maturity. It was characterized by the firmness of the pulp in three cultivars ('Blagodat', 'Vavilovskoye', 'Ivanovskoye') at the standard cultivar level, but in the fruits of two cultivars ('Alexandr Boyko' and 'Patriot') reliably exceeded it. The varietal variability of the pulp firmness was quite high because both the genotype and external conditions affected it. Firmness of analyzed apple cultivars and physicochemical parameters of produced juice was shown in Table 1.

The juice yield depends on the degree of maturity of apples, the characteristics of the cultivar (genotype) and vegetation conditions. The juice yield during the study period was low - on average 57.6 ± 3.0%, while in the standard it was 62.1 ± 1.8%. According to this indicator, all cultivars were at the standard level with the exception of Vavilovskoye, which had a reliably lower juice yield. Varietal variability was characterized as average (V = 13.26 %). It should be noted that although the juice yield did not exceed 60%, the pretreatment of the pulp with enzymes or microwaves can significantly increase this indicator (Oszmiański et al., 2009; Sharma et al., 2017; Nilova et al., 2020; Mieszczakowska-Frąc et al., 2021). This method is most often used at the present time, since enzymatic treatment before mechanical extraction significantly improves quality and quantity of extracted juice (Sharma et al., 2017).

The content of soluble solids and titratable acidity are considered important quality parameters due to their effect on the sensory perception of apple juices and are of great importance in the production of concentrated juices. According to both domestic and European standards, the minimum content of soluble solids in direct-pressed apple juices should be at least 10% (TR TS 023/2011, 2011; AIJN, 2012). This requirement was met for all cultivars. In general, the studied cultivars were distinguished by high juice extractivity - the content of soluble solids averaged 14.22 ± 0.64°Brix, in standard juice it was 11.17 ± 0.19°Brix. The VNIISPK breeding cultivars reliably exceeded the standard cultivar for this indicator. 'Aleksandr Boyko' and 'Vavilovskoye' were characterized by a higher content of soluble solids in juice than all other studied cultivars (more than 15°Brix) (Table 1).

Table 1. Technological and biochemical indicators in apples and apple juices in comparison with a standart

Cultivar	Firmness (kg cm^{-2})	Juice yield, %	Biochemical composition of juice				
			Soluble solids, % (Brix°)	Sum of sugars, %	Titrated acid, %	Ratio	Catechins, mg (100 g)$^{-1}$
Aleksandr Boyko	9.01 ± 0.45	62.30 ± 3.68	15.53 ± 1.55	14.31 ± 0.31	0.49 ± 0.12	24.34 ± 3.75	122.90 ± 13.40
Blagodat	7.02 ± 0.41	52.03 ± 5.10	14.78 ± 0.22	13.77 ± 0.32	0.77 ± 0.05	17.94 ± 3.43	22.50 ± 18.62
Vavilovskoye	7.24 ± 0.68	43.01 ± 8.40	15.11 ± 0.44	13.16 ± 0.52	0.81 ± 0.12	16.23 ± 2.71	60.39 ± 27.11
Ivanovskoye	7.03 ± 0.27	59.50 ± 8.50	14.65 ± 0.15	13.13 ± 1.23	1.03 ± 0.11	13.03 ± 2.59	87.80 ± 32.25
Patriot	9.51 ± 0.79	58.10 ± 3.47	14.05 ± 0.32	12.25 ± 0.05	0.50 ± 0.04	27.66 ± 1.55	56.95 ± 36.35
Antonovka (st.)	6.36 ± 0.47	62.05 ± 1.76	11.17 ± 0.19	9.73 ± 0.32	1.13 ± 0.05	8.98 ± 0.39	53.95 ± 7.10
$\overline{X}$	7.70 ± 0.51	56.17 ± 3.04	14.22 ± 0.64	13.90 ± 0.59	0.70 ± 0.10	18.03 ± 2.84	67.42 ± 13.97
max	9.51	62.30	15.53	14.31	1.13	27.66	122.90
min	6.36	43.01	11.17	9.73	0.49	8.98	22.50
$LSD_{0.5}$	1.87	11.05	2.33	2.41	0.39	10,34	50.76
V%	16.35	13.26	11.05	12.75	33.47	38.64	50.75

With regard to the acidity of the juice of new cultivars, a significant variation of this indicator was noted from 0.49% for 'Aleksandr Boyko' to 1.13% for the 'Antonovka' standard. A very moderate acidity of the juice was noted in the juice of 'Aleksandr Boyko' and Patriot (0.49 and 0.50%, respectively). In general, the standard significantly showed the highest titratable acidity of the juice of the group of cultivars. Only the 'Ivanovskoye' juice had acidity at the level of 'Antonovka'. The titratable acidity of juices, according to the standard (TR TS 023/2011, 2011), should not exceed

0.8% for babies and 1.3% for preschool and school-age children. All juices are suitable for children food, and juices of the 'Aleksandr Boyko', 'Patriot' and 'Blagodat' are suitable for feeding babies.

Taking into account the differences in the ranges of soluble solids and acidity, it becomes obvious that the ratio of both components (Ratio) primarily depends on the content of organic acids. It is believed that the most balanced taste in apple juices is noted with a Ratio equal to 16 or close to it (Daskalov et al., 1969; Sedov, 2011; Kozlovskaya, 2015). There is statement that the high Ratio values positively influence at the juice taste (Farina et al., 2017), and the optimal ratio of sugars and acids in the juice is in the range of 17~30 (Mieszczakowska-Frąc et al. 2021). Our study showed that all cultivars with a juice taste score of 4.5 points or higher were characterized by a 16.2~27.7 Ratio (Tables 1, 2), which is consistent with the data of Mieszczakowska-Frąc et al. (2021). The lowest ratio of sugars and acids was in the juice of 'Antonovka', and the highest ratio was in the juice of 'Aleksandr Boyko' and 'Patriot', which immediately affected the characteristics of their tastes (Tables 1, 2). The coefficient of correlation between the taste of juice and Ratio was 0.7, which showed a strong negative effect of acidity.

Table 2. Organoleptic indicators of juices of five VNIISPK apple cultivars in comparison with a standart

Cultivar	Taste assessment (points)			Appearance of juice	Taste of juice	Aroma
	Appearance	Taste	Overall			
Aleksandr Boyko	4.5	4.5	4.5	Rich yellow, opaque (muddy), opalescent	Sweet, pleasant, with a slight astringency, typical	Apple, medium
Blagodat	4.7	4.5	4.6	Yellow, transparent	Sweet and sour, pleasant	Apple, weak
Vavilovskoye	4.6	4.5	4.6	Straw- to dark yellow color, transparent, slightly opalescent	Sweet and sour, pleasant, with a slight astringency, typical	Apple, pleasant, medium
Ivanovskoye	4.6	4.4	4.5	Yellow, transparent	Sweet and sour, with a slight astringency, typical	Apple, medium
Patriot	4.4	4.5	4.5	Yellow, opaque (muddy), slightly opalescent	Sweet, pleasant, with a slight astringency, typical	Apple, weak
Antonovka (st.)	4.3	4.1	4.2	Straw-yellow, opaque (muddy), opalescent	Sour, with a slight astringency, typical	Apple, pleasant, medium
$\overline{X}$	4.5	4.5	4.4			
max	4.7	4.5	4.6			
min	4.3	4.1	4.2			
$LSD_{0.5}$	0.2	0.2	0.2			
V%	3.3	3.3	3.6			

The content of polyphenols in juice is most influenced by two factors: the genotype and the method of juice processing. The influence of the second factor in our study is minimized, which brings the role of the genotype to the foreground.

In the studied juices, a high variation in the content of catechins was revealed - from 22.5 mg per 100 g (‘Blagodat’) to 122.9 mg per 100 g (‘Aleksandr Boyko’). This may be due to genotype affecting the retention of catechins in the juice (Van der Sluis et al., 2001; Shafi et al., 2019).

The analysis of the tasting ratings showed that all studied cultivars surpassed the standard cultivar in organoleptic indicators. So, the most attractive was the juice of Blagodat, Vavilovskoye and Ivanovskoye. According to the taste qualities of the juice, Blagodat, Vavilovskoye, Patriot and Aleksandr Boyko especially stood out. According to the general tasting assessment, the juice of all studied cultivars reliably surpassed the standard Antonovka (Table 2).

CONCLUSIONS

The comparative analysis of the technological indicators of new apple cultivars for juice production showed that they all surpassed the standard ‘Antonovka’ in terms of the content of soluble solids in the juice, more moderate acidity and higher organoleptic indicators. The studied cultivars suitable for cultivation by intensive technologies can be recommended for cultivation in technical (raw) orchards. ‘Aleksandr Boyko’ stood out especially, characterized by a juice yield not lower than that of the standard cultivar, a high content of soluble solids in the juice and low acidity. This cultivar also distinguished by a fairly high content of catechins in the juice, which cause antioxidant properties.

REFERENCES

AIJN (European Fruit Juice Association). 2012. AIJN Code of Practice. The Reference Guidelines. Available at: Juice Quality | AIJN – European Fruit Juice Association

Anonymous, 2021. Apple production in Russia increased 1.3 times. Available at: https://mcx.gov.ru/press-service/news/proizvodstvo-yablok-v-rossii-uvelichilos-v-1-3-raza/?ysclid=l346uyp1og Accessed 16.11.2021. (in Russian).

Anonymous, 2020. Juice production in Russia: why is the market going down? Available at: https://delprof.ru/press-center/open-analytics/proizvodstvo-sokov-v-rossii-pochemu-rynok-idet-na-snizhenie-/ Accessed 09.09.2020. (in Russian).

Anonymous, 2017. Evgeny Akhpashev: the development of horticulture will increase the production of raw materials for juices. Available at: https://mcx.gov.ru/press-service/news/evgeniy-akhpashev-razvitie-sadovodstva-pozvolit-uvelichit-obemy-proizvodstva-syrya-dlya-sokov/?ysclid=l34alx1fel Accessed 18.04.2017. (in Russian).

Blenpied, G.D. & Silsby, K.J. 1992. Predicting Harvest Date Windows for Apple. In: *Information Bulletin 221, Cornell Cooperative Extension Publication, Ithaca*, *New-York*, pp. 12.

Daskalov, P., Aslanyan, R., Tenov, R., Zhivkov, M. & Bayadzhiev, R. 1969. *Fruit and vegetable juices* (translated from Bulgarian). Moscow, 424 pp. (in Russian).

Eremeeva, N.B., Makarova, N.V., Demidova, A.V. & Bykova, T.O. 2016. Chemical composition and antioxidant activity of varietal cherries growing in the Samara region. *Storage and processing of agricultural raw materials* **3**, 34–37. 00021 (in Russian, English abstract).

Farina, V., Gianguzzi, G., Mazzaglia, A. & Sortino, G. 2017. Instrumental and sensory evaluation of seven apple (Malus domestica Borkh.) cultivars under organic cultivation in Sicily. *Agronomy Research* **15**(5), 1878–1889. doi: 10.15159/AR.17.048

Kozlovskaya, Z.A. 2015. *Apple breeding in Belarus*. Minsk, 457 pp. (in Russian).

Makarkina, M.A. & Pavel, A.R. 2017. Biologically active substances in strawberry berries grown in Orel region. *Sovremennoe sadovodstvo – Contemporary horticulture* **2**, 10–16. doi: 10.24411/2218-5275-2017-00021 (in Russian, English abstract).

Maksimova, E. 2021. Apple production will continue to grow. Available at: https://www.agroinvestor.ru/markets/news/35620-proizvodstvo-yablok-prodolzhit-rasti/ Accessed 10.04.2021. (in Russian).

Medvedeva, A. World and Russian trends in fruit and vegetable processing. 19.09.2018. Available at: https://www.agroxxi.ru/analiz-rynka-selskohozjaistvennyh-tovarov/mirovye-i-rossiiskie-trendy-v-pererabotke-plodov-i-ovoschei.html (in Russian).

Mieszczakowska-Frąc, M., Płocharski, W., Kruczyńska, D. & Markowski, J. 2021. Scab Resistant Apple Cultivars for Juice Production. *Journal of Horticultural Research* **29**(1), 23–34. doi: 10.2478/johr-2021-0004

Nilova, L., Ikramov, R. & Malyutenkova, S. 2020.The possibility of using microwaves to obtain extracts from berry press residues and jelly products with bioactive characteristics. *Agronomy Research* **18**(S3), 1829 1843. doi: 10.15159/ar.20.044

Oszmiański, J., WojdyŁo, A. & Kolniak, J. 2009. Effect of enzymatic mash treatment and storage on phenolic composition, antioxidant activity, and turbidity of cloudy apple juice. *Journal of Agricultural and Food Chemistry* **57**(15), 7078–7085. doi: 10.1021/jf900806u

Paganini, C., Nogueira, A., Denardi, F. & Wosiacki, G. 2004. Industrial fitness analysis of six apple cultivars, considering their physico-chemical evaluation. *Ciencia e agrotecnologia* **28**(6), 1336–1343.

Pepin, A., Stanhope, K. & Imbeault, P. 2019. Are fruit juices healthier than sugar-sweetened beverages? A review. *Nutrients* **11**, 1006. doi: 10.3390/nu11051006

Roman, I., Ardelean, M., Mitre, V. & Harsan, E. 2007. Phenotypic, genotypic and environmenal correlations between fruit characteristics confirming suitability to juice extraction in winter apple. *Buletin USAMV-CN. Horticultural* **64**(1–2), 58–62. doi: 10.15835/BUASVMCN-HORT:1922

Rumpunen, K., Ekholm, A. & Nybom, H. 2017. Swedish apple cultivars vary in traits for juice and cider making. *Acta Horticulturae* **1172**, 255–258. doi: 10.17660/ActaHortic.2017.1172.48

Russia's self-sufficiency in apples is below 40%. 17.05.2021. Available at: Russia's self-sufficiency in apples is below 40%. Market research, ready-made business plans, business ideas, investments - Business blogs - Sostav.ru (in Russian).

Savelieva, N.N. 2016. *Biological and genetic features of apples and breeding of scab-immune and columnar cultivars*. Michurinsk, 280 pp. (in Russian).

Salina, E, Levgerova, N. & Sedov, E.N. 2019. Apple cultivars and hybrids for juice production. *Fruit growing and viticulture of South Russia* **55**(01), 120–13. doi: 10.30679/2219-5335-2019-1-55-120-130

Sedov, E.N. 2011. *Breeding and new apple cultivars*. Orel, 624 pp. (in Russian).

Sedov, E.N. & Ogoltsova, T.P. (Eds.) 1999. *Program and methodology of fruit, berry and nut cultivar study*. Orel, 608 pp. (in Russian).

Sedov, E.N., Serova, Z.M., Yanchuk, T.V., Makarkina, M.A. & Korneeva, S.A. 2018. *The best apple cultivars of the Russian Research Institute of Fruit Crop Breeding (popularization of breeding achievements)*. Orel, 64 pp. (in Russian).

Shafi, W., Mansoor, S., Jan, S., Singh, D.B., Kazi, M., Raish, M., Alwadei, M., Mir, J.I. & Ahmad, P. 2019. Variability in catechin and rutin contents and their antioxidant potential in diverse apple genotypes. *Molecules* **24**(5), 943. doi: 10.3390/molecules24050943

Sharma, H., Patel, H. & Sugandha. 2017. Enzymatic added extraction and clarification of fruit juices –A review. *Critical Reviews in Food Science and Nutrition* **57**(6), 1215–1227. doi: 10.1080/10408398.2014.977434

Smith, R.B., Lougheed, E.C., Franclin, E.W. & Mcmillan, I. 1979. The starch iodine test for determining stage of maturation in apples. *Canadian Journal of Plant Science* **59**(3), 725–735. doi: 10.4141/cjps79-113

Tinello, F. & Lante, A. 2018. Recent advances in controlling polyphenol oxidase activity of fruit and vegetable products. *Innovative Food Science & Emerging Technologies* **50**, 73–83. doi: 10.1016/j.ifset.2018.10.008

TR TS 023/2011 (Technical regulations of the Customs Union). 2011. *Technical regulations for juice products from fruits and vegetables*. Moscow, 80 pp. (in Russian).

Van der Sluis, A.A., Dekker, M., Jager, A. & Jongen, W.M.F. 2001. Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *Journal of Agricultural and Food Chemistry* **49**(8), 3606-3616. doi: 10.1021/jf001493u

Vigorov, L.I. 1964. Determination of various forms of catechins in fruits and berries. In: *Proceedings of the II All–Union seminar on biologically active (therapeutic) substances of fruits and berries,* Sverdlovsk, pp. 310–322 (in Russian).

Zagoskina, N.V. & Nazarenko, L.V. 2019. Secondary metabolites of plants: distribution, history of study, practical application. *Bulletin of the Moscow City Pedagogical University. Series: Natural Sciences* **2**(34), 8–19. doi: 10.25688/2076-9091.2019.34.2.1 (in Russian, English abstract).

Zielinski, A.A.F., Alberti, A., Braga, C.M. … & Wosiacki; G. 2014. Effect of mash maceration and ripening stage of apples on phenolic compounds and antioxidant power of cloudy juices: A study using chemometrics. *LWT–Food Science and Technology* **57**(1), 223–229. doi: 10.1016/j.lwt.2014.01.029

Agronomy Research **20**(3), 625–636, 2022
https://doi.org/10.15159/AR.22.066

Comparison of the yeast *Saccharomyces cerevisiae var. boulardii* and top-fermenting brewing yeast strains during the fermentation of model nutrient media and beer wort

D. Manshin[1,*], T.V. Meledina[1], T. Britvina[1], S.G. Davydenko[2], N.V. Shelekhova[3], V. Andreev[2] and A. Andreeva[1]

[1]ITMO University, Faculty of Biotechnologies (BioTech), Lomonosov street 9, RU191002 Saint-Petersburg, Russia
[2]Innovation & Research Department, Baltika Breweries, 6-th Verkhnij ln. 3, RU194292 St. Petersburg, Russia
[3]Russian Research Institute of Food Biotechnology, Department of Biotechnology and Food Safety, Samokatnaya street 4b, RU111033 Moscow, Russia
[*]Correspondence: dvmanshin@itmo.ru

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Abstract. Recently, the yeast *Saccharomyces cerevisiae var. boulardii* have attracted the attention of Food Science researchers due to their unique properties, the main among which are probiotics. Thus, research is conducted on the use of this yeast as a starter culture in the technology of yogurt, fermented vegetables, fruit, vegetable juices, as well as beer. This paper is aimed at studying *Saccharomyces cerevisiae var. boulardii 's* fermentation performance compared to top-fermenting brewing yeast strains during fermentation of model nutrient media and beer wort. Fermentation activity of the studied strains was assessed based on the character of fermentation curves, as well as the values of the maximum substrate assimilation rate and apparent degree of fermentation. Moreover, during the study, beer was produced using the yeast *Saccharomyces cerevisiae var. boulardii* as a starter culture. According to the obtained results, it can be concluded that *Saccharomyces cerevisiae var. boulardii* have less fermentation activity compared to brewing strains. In turn, beer produced with *Saccharomyces cerevisiae var. boulardii* significantly differed in physicochemical, microbiological, and organoleptic parameters from the control sample obtained using the *047A* brewing strain. Thus, it contained less ethanol and secondary metabolites; however, the concentration of living cells was significantly higher, which indicates a relatively high viability of the yeast *Saccharomyces cerevisiae var. boulardii*. From an organoleptic point of view, final beer has a positive sensory profile. The aroma of the product had a complex character: it included caramel, spicy, fruity and phenolic notes, as well as smoked and wine elements; while honey was the dominant note of the taste.

Key words: brewing, functional beer, fermented drinks, fermentation activity, probiotics, Saccharomyces cerevisiae var. boulardii, yeast.

INTRODUCTION

The yeast *Saccharomyces cerevisiae var. boulardii* is currently the only registered eukaryotic probiotic microorganism recommended as a drug for both adults and children (Vandenplas et al., 2008; Łukaszewicz, 2012).

Initially, this yeast culture was identified as a separate species of the genus *Saccharomyces*. However, it was further shown that this yeast is a strain of the species *Saccharomyces cerevisiae* (Pais et al., 2020). It was found that *Saccharomyces cerevisiae var. boulardii* and *Saccharomyces cerevisiae* are more than 99% similar in terms of genome sequence considering Average Nucleotide Identity (ANI). At the same time, using the methods of molecular phylogenetics and typing, it was shown that *Saccharomyces cerevisiae var. boulardii* form a separate cluster within the species *Saccharomyces cerevisiae*, which is closer to wine yeast strains. Comparative genomic hybridization experiments also established that *Saccharomyces cerevisiae var. boulardii* and *Saccharomyces cerevisiae* are different strains of the same species but the loss of all intact Ty1/2 elements was reported only in *Saccharomyces cerevisiae var. boulardii*, which is one of the key differences from a genetic view point (Mitterdorfer et al., 2002; Khatri et al., 2017).

Despite the similarity from a genetic point of view, the yeast *Saccharomyces cerevisiae var. boulardii* significantly differ in physiological properties from other industrial and laboratory strains of *Saccharomyces cerevisiae* (see Table 1) (Pais et al., 2020). In particular, they are more resistant to both high temperature and acidity of the environment; unable to use galactose as a carbon source; and always diploid.

Table 1. Physiological features of *Saccharomyces cerevisiae var. boulardii* and *Saccharomyces cerevisiae* (Pais et al., 2020)

Physiological feature	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae var. boulardii*
Optimum growth temperature	30 °C	37 °C
Resistance to high temperature (52 °C)	45% viability	65% viability
Resistance to high acidity (pH = 2 for 1 hour)	30% viability	75% viability
Assimilation of galactose	+	-
Ploidy	diploid and haploid	diploid

The resistance of the probiotic strain to the high acidity of the medium can be explained by the cell wall characteristics. Hudson et al. (2016) showed that *Saccharomyces cerevisiae var. boulardii* have a thickened cell wall compared to laboratory strains of *Saccharomyces cerevisiae* (*BY4741 and W303*). When yeast culture was treated with the antifungal drug caspofungin, which inhibits β-1,3-D-glucan synthesis, and then placed in a nutrient medium with different acidity, a significant decrease in growth was observed at low pH values, indicating that the cell wall is responsible for the resistance of *Saccharomyces cerevisiae var. boulardii* to high acidity.

Another important property of *Saccharomyces cerevisiae var. boulardii* is that they are highly osmotolerant, which consists in maintaining the ability to ferment the substrate and multiply in environments with a high sugar concentration. Thus, Ávila-Reyes et al. (2016) reveled that even at sucrose concentrations more than 60%, this yeast strain showed little biomass growth and formed ethanol (1.5% – v v^{-1}).

The listed properties of *Saccharomyces cerevisiae var. boulardii*, which can be called techno-functional, have attracted the attention of Food Science researchers. We can find some scientific data on the use of this yeast as a starter culture in the technology of yogurt (Lourens-Hattingh & Viljoen, 2001; Karaolis et al., 2013), fermented vegetables (Campbell et al., 2016), fruit and vegetable juices (Fratianni et al., 2014; Değirmencioğlu et al., 2016) as well as beer (Capece et al., 2018; Mulero-Cerezo et al., 2019; Pereira de Paula et al., 2021). In addition to the fact that the use of yeast as a starter culture allows imparting probiotic properties to food products this yeast strain improves its functionality and the functionality of accompanying bacteria in fermented food products. This effect is related to the capacity of *Saccharomyces cerevisiae var. boulardii* strains to stabilize pH and acidity of food matrices for prolonged periods of time. However, in some cases *Saccharomyces cerevisiae var. boulardii* may affect the sensorial characteristics of finished food products. Besides, it has an interesting beneficial effect on the nutritional value of foods since they synthesize folates and eliminate phytates and other antinutrients. In addition, *Saccharomyces cerevisiae var. boulardii* generates several metabolites or nutraceuticals known to exert positive health benefits (Lazo-Vélez et al., 2018).

The use of Saccharomyces cerevisiae var. boulardii in brewing is of a particular interest because strains of the species to which this culture belongs have been used for centuries as one of the indispensable raw materials in beer production. Although the use of *Saccharomyces cerevisiae var. boulardii* in brewing is considered by researchers as very promising; for the producer, in practical implementation, it seems extremely important to know how this yeast culture differs from traditional brewing strains in terms of fermentation performance. Thus, this research is aimed at studying *Saccharomyces cerevisiae var. boulardii 's* fermentation potential compared to top-fermenting brewing yeast strains during fermentation of model nutrient media and beer wort.

MATERIALS AND METHODS

Objects of study

Research objects were top-fermenting brewer's yeast strains (the culture bank of the BSG laboratory, Russia): *002A, 047A, 052A*, and *Saccharomyces cerevisiae var. boulardii* (coded with *S.c.b*) isolated from Enterol® (Biocodex, France). Some characteristics of brewing yeast strains (*002A, 047A, 052A*) are presented in Table 2.

Table 2. The characteristics of the top-fermenting brewer's yeast strains

Characteristics	Yeast strain		
	002A	*047A*	*052A*
Beer style	India pale ale	Wheat beer	Pale ale
Optimal fermentation temperature, °C	18–20	20–22	18–24
Flocculation	Medium	Low	Medium

Cultivation of yeast

Pure yeast cultures were stored on the YPD-agar (1% yeast extract, 2% peptone, 2% glucose, 2% agar) at 4 °C. The inoculum was grown in simple batch culture on YPD medium (1% yeast extract, 2% peptone, 2% glucose) by successive transfers with increasing amounts of nutrient medium. The multiplicity of increasing the volume of medium in relation to the inoculum was 4:1. The flasks were filled with nutrient medium to 1/3 of the volume. The reseeding of the yeast culture was performed after the cells passed from the exponential growth stage to the stationary stage. Cultivation was proceeded at 28 °C.

Study of the kinetics of the fermentation process on model nutrient media

The fermentation kinetics of *Saccharomyces cerevisiae 002A, 047A, 052A* strains, and *Saccharomyces cerevisiae var. boulardii* was studied on the following model media of different compositions:

- Medium 1: malt wort prepared from malt extract (Muntons 'Light', England)
- Medium 2: 1% yeast extract, 2% peptone, 9% glucose (YPD)
- Medium 3: 1% yeast extract, 2% peptone, 9% maltose (YPM).

The initial content of dry matter in nutrient media accounted for 12%, and pH was 5.5. The media were sterilized at 121 °C (1.1 atm) for 20 minutes. The fermentation process was performed for seven days in a simple batch culture in 100 mL Erlenmeyer flasks filled with 30 % sterile nutrient media at 20 °C. The pitching rate was 12×10^6 cells mL^{-1}. During the fermentation process, the concentration of the extract (g $(100\ g)^{-1}$) was determined by refractometer (PTR-46, Index Instruments Ltd, England). The value of pH was determined on titrator (848 Titrino plus, Metrohm AG, Switzerland).

Based on the obtained data, the rate of substrate assimilation (V_{assim}, Eq. 1) and apparent degree of fermentation (ADF, Eq. 2) were calculated.

$$V_{assim.} = \frac{\Delta S}{\Delta \tau} \qquad (1)$$

where ΔS – the change in substrate concentration (g $(100\ g)^{-1}$) over a period of time $\Delta\tau$ (h).

$$ADF = \frac{OE - AE}{OE} \times 100\% \qquad (2)$$

where OE – original extract (% – m m^{-1}); AE – apparent extract (%– m m^{-1}) (Briggs, 2004).

Processing of beer using *Saccharomyces cerevisiae var. boulardii* and top-fermenting brewer's yeast strains *047A*

Industrial beer wort provided by OJSC Baltika (St. Petersburg, Russia) was used to produce beer with *Saccharomyces cerevisiae var. boulardii*. Table 3 shows the main physicochemical parameters of beer wort used in the experiment.

Table 3. Quality indicators of hopped wort

Indicator	Original wort extract (% – m m^{-1})	pH	Bitterness (IBU)	FAN (mg L^{-1})
Value	11.85	5.15	20	250

To determine the completion time of the wort fermentation in connection with the strain characteristics of the starter cultures, the real degree of fermentation (RDF) was calculated for both strains according to the Eq. 3 (Briggs, 2004).

$$RDF = \frac{OE - RE}{OE} \times 100\% \qquad (3)$$

where OE – original extract (% – m m^{-1}); RE – real extract (% – m m^{-1}).

The real degree of fermentation for *S.c.b.* was 62.8%, for *047A* strain - 70.3%.

The fermentation process was performed in five-liter flasks, filled with 70% hopped wort (3.5 L). The concentration of yeast cells at the beginning of fermentation was 12×10^6 cells mL^{-1}. The process of obtaining beer included two stages: a) fermentation of the wort at the temperature of 20 °C until the RDF was reached; b) maturation of beer.

The maturation process included the bottling young beer after fermentation under aseptic conditions in 1.5-liter PET bottles. The degree of filling the bottles was 90%. Then, sterile glucose solution was added to each bottle at the rate of 0.5% carbohydrate content, and the bottles were closed with plastic plugs. For carbonation, young beer was kept in PET bottles for one day at 20 ± 2 °C, cooled to the temperature of 5 °C, and then kept for seven days.

Analysis of young and final beer

In young and final beer, the real extract (RE, % – m m^{-1}), alcohol concentration (% – v v^{-1}), and the real degree of fermentation (RDF, %) were determined using an Alcolyzer Plus Beer analyzer (Anton Paar, Austria). The pH value was determined by an 848 Titrino plus titrator (Metrohm AG, Switzerland). The total concentration of yeast cells was determined using a Goryaev chamber while the concentration of living and dead cells was determined by staining with methylene blue.

The concentrations of secondary metabolites in beer samples were determined by gas chromatography using Hewlett-Packard - HP 6890 GC System with flame ionization detector (Agilent Technologies Inc., USA). Finally, the organoleptic indicators of beer (smell and taste) were assessed by certified tasters of OJSC Baltika (St. Petersburg, Russia).

Statistical analysis

All experiments were performed in triplicate, the data were processed by the method of mathematical statistics with using MS Excel, finding the confidence interval and a probability of 0.95.

RESULTS AND DISCUSSION

Study the kinetics of the fermentation process on model nutrient media

Fermentation activity is a key property of yeast, which partly determines its use in the fermented beverage industry, including brewing. For this reason, the first stage of the study was to compare the fermentation activity of *Saccharomyces cerevisiae var. boulardii* (coded with *S.c.b*) and top-fermenting brewing strains (*002A, 047A, 052A*) during fermentation of unhopped beer wort made from malt extract (medium 1). Fermentation activity of the studied strains was assessed based on the character of fermentation curves (Fig. 1, a), as well as the values of the maximum substrate assimilation rate (Fig. 1, b) and apparent degree of fermentation (Fig. 1, c).

According to the obtained data, it can be concluded that the yeast *Saccharomyces cerevisiae var. boulardii* have less fermentation activity compared to brewing strains. Firstly, this yeast culture can be characterized by a relatively low maximum substrate assimilation rate - 0.090 ± 0.005 g $(100\ g)^{-1}\ h^{-1}$ (Fig. 1, b), which entails a longer fermentation process duration. Thus, there were required five days to complete fermentation for *Saccharomyces cerevisiae var. boulardii*, while for brewing strains this period accounted for three days (Fig. 1, a). Secondly, the apparent degree of fermentation in the case of *Saccharomyces cerevisiae var. boulardii* was approximately 7% lower than the top-fermenting brewing strains (Fig. 1, c), which indicates the inability of this yeast culture to ferment all the beer wort sugars. The latter fact was also noted by other researchers; for example, according to Pereira de Paula et al. (2021) when producing wheat beer using *Saccharomyces cerevisiae var. boulardii*, the degree of fermentation was 6.82% less compared to the control strain *Saccharomyces cerevisiae WB-06*. Capece et al. (2018) noted not only a lower degree of attenuation of the yeast *Saccharomyces cerevisiae var. boulardii* compared to brewing strains (10.29–19.85% less), but also a decrease in this indicator when obtaining beer using the co-fermentation process. Therefore, the inclusion of *Saccharomyces cerevisiae var. boulardii* in the composition of the mixed-starter culture in the ratio with *Saccharomyces cerevisiae* 1:1 led to the decrease in the degree of fermentation by 1.85% in average.

As it is known, the carbohydrate composition of beer wort is mainly represented by sugars fermentable by brewing strains such as glucose (10–15%), maltose (50–60%) and maltotriose (15–20%), as well as non-fermentable dextrins (20–30%) (Stewart, 2016). Apparently, as it was abovementioned, a lower degree of fermentation may be due to the inability of the yeast *Saccharomyces cerevisiae var. boulardii* to assimilate all wort sugars. In its turn, the relatively low rate of substrate assimilation during the fermentationof beer wort may be associated with a low assimilation rate of the quantitatively dominant sugar -

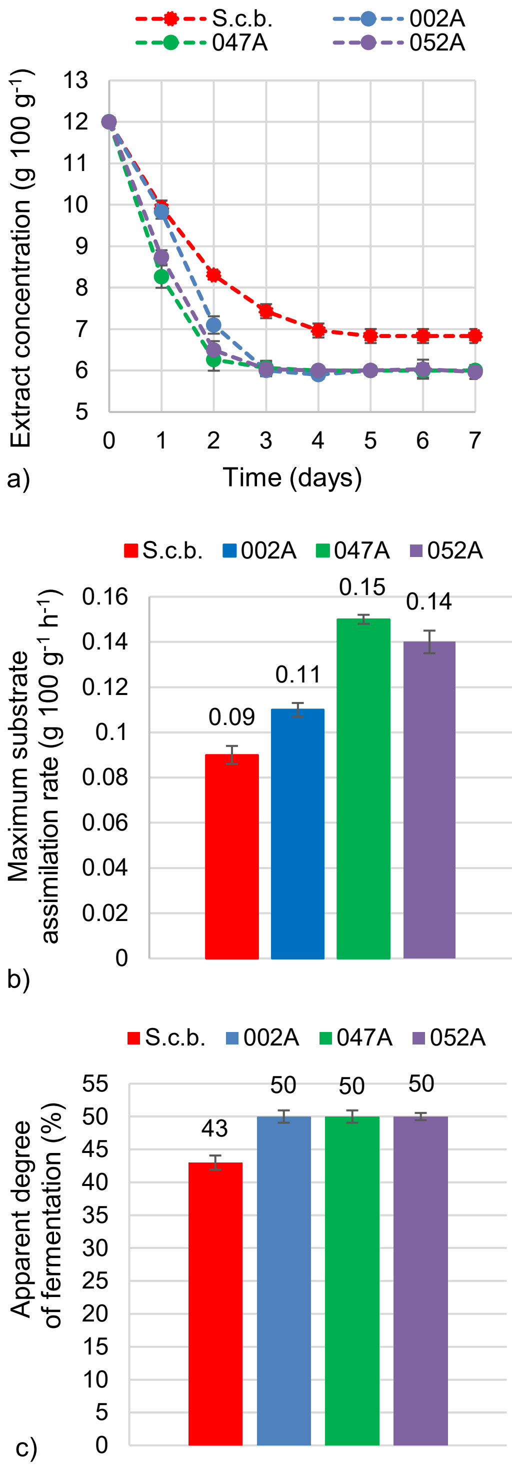

Figure 1. Change in the concentration of the extract (a); maximum substrate assimilation rate (b); apparent degree of fermentation (c).

maltose. To confirm these assumptions, fermentation with *Saccharomyces cerevisiae var. boulardii* was performed in model nutrient media (YPD and YPM), which differed only in the type sugar, and brewing strain *047A* was taken as a control.

Based on the presented data, it can be concluded that the *Saccharomyces cerevisiae var. boulardii* is completely capable of fermenting maltose (Fig. 2, a), although the consumption rate of this disaccharide is significantly inferior to the rate of glucose fermentation (Fig. 2, b). In contrast, in the case of control (strain *047A*), the rates of glucose and maltose consumption were approximately equal and significantly higher than those of *Saccharomyces cerevisiae var. boulardii* (Fig. 2, b), which is typical for brewing strains. In addition, a lag phase was noticeable on the fermentation curve (Fig. 2, a) during the fermentation of the medium with maltose (YPM). All this indicates a relatively low maltase activity of the yeast *Saccharomyces cerevisiae var. boulardii*. It is also worth noting that the values of the maximum rates of substrate assimilation during the fermentation of unhopped beer wort and the model medium with maltose are equal (Fig. 1, b and 2, b). This means that in the kinetics of beer wort fermentation with *Saccharomyces cerevisiae var. boulardii*, the process of maltose assimilation is decisive. Since both glucose and maltose are fermentable sugars by *Saccharomyces cerevisiae var. boulardii*, the reason for the low apparent attenuation in beer wort fermentation seems to be related to the inability of this strain to consume maltotriose or to the extremely low rate of this process.

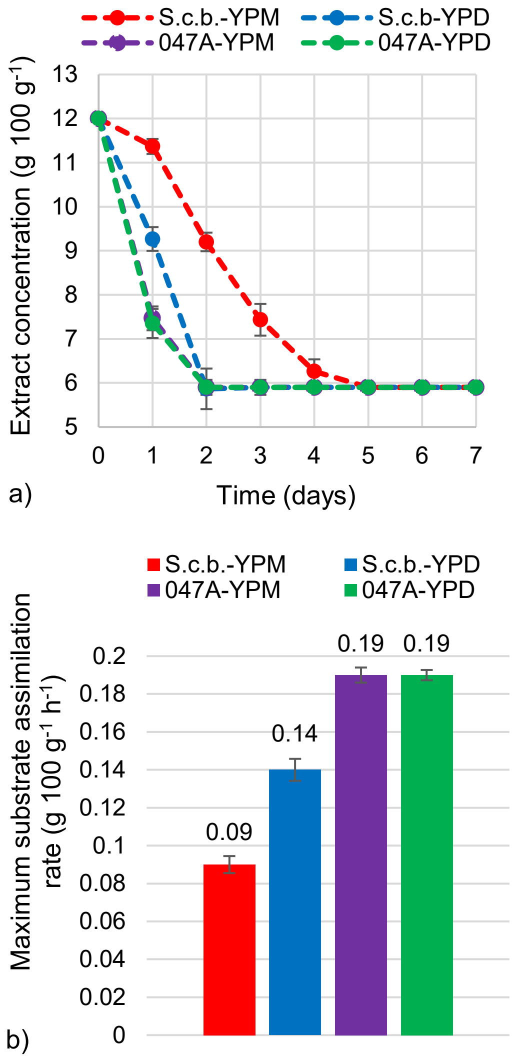

Figure 2. Change in the concentration of the extract (a); maximum substrate assimilation rate (b).

In addition, it is interesting to note that for *Saccharomyces cerevisiae var. boulardii*, the biomass yield on the maltose medium was higher than on the glucose one - 9.15 ± 0.18 and 6.98 ± 0.30%, respectively (data not presented). This observation suggests that the cultivation of this yeast strain on maltose as the main carbon source, despite the significant duration (five days), can be effective. However, we may assume that this process can be intensified by selecting cultivation modes due to varying such factors as temperature, agitation, aeration, and initial sugar concentration. In addition, ultrasound is recognized as a promising tool for intensifying the enzymatic activity of microorganisms, which may be also applied to *Saccharomyces cerevisiae var. boulardii* (Suchkova et al., 2014).

Processing of beers using *Saccharomyces cerevisiae var. boulardii* and top-fermenting brewer's yeast strains 047A, and their analysis

The final stage of the work was to produce beer from industrial hoped beer wort using the yeast *Saccharomyces cerevisiae var. boulardii* as a starter culture. For the purposes of obtaining beer with potential probiotic properties, the product has to be unfiltered and unpasteurized; in fact, wheat beer often possesses these criteria. Thus, strain *047A*, which is intended for the production of wheat beer, was taken as a control. In addition to monitoring the main fermentation process by changes in real extract and ethanol concentrations (Fig. 3), the basic physicochemical and microbiological analysis of young and final beers was performed (Table 4) as well as determination of secondary metabolites concentrations (Table 5). Finally, an organoleptic analysis of the final products was made.

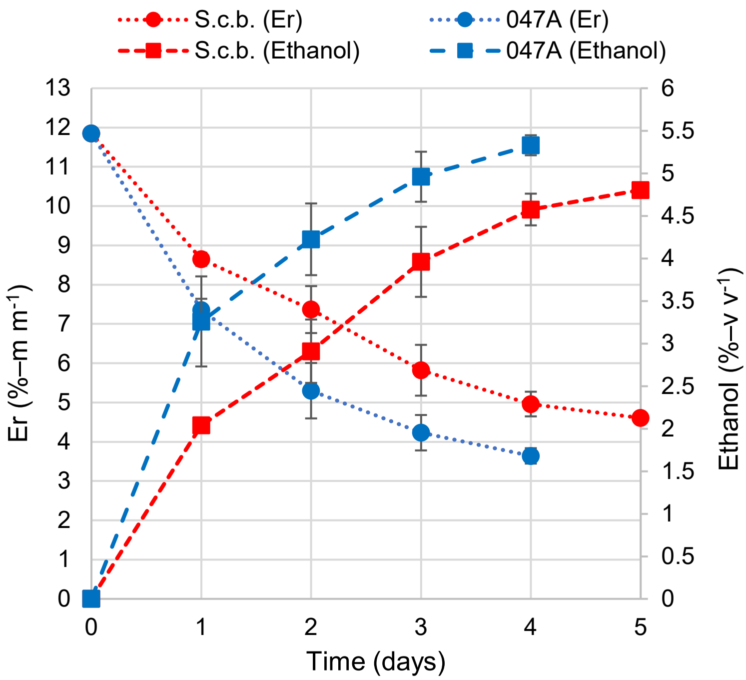

Figure 3. Change in the real extract and alcohol concentration during the main fermentation.

Fig. 3 shows the change in the real extract (Er) and alcohol concentration during the main fermentation process. From the presented data, we can conclude that the real degree of fermentation of the wort using *047A* strain was achieved in four days, while for *Saccharomyces cerevisiae var. boulardii* five days were required. The difference in real degree of fermentation (RDF) between strains was 11.9%, which corresponds to the concentration range of maltotriose in beer wort. As expected, a beer sample made using

Saccharomyces cerevisiae var. boulardii was characterized by a high content of residual extract and, accordingly, a lower ethanol concentration (Table 4).

According to our research, in young beer, obtained using a probiotic strain (*S.c.b.*), the concentration of living cells was quite high and amounted to 8.29 million mL^{-1} compared to the control, which was 2.10 million mL^{-1} (Table 4). Although both the total concentration of cells and living cells decreased during the maturation process, the total concentration of cells and living cells was still quite high in the finished product compared to the control: 2.90 million mL^{-1} and 1.17 million mL^{-1}, respectively. Consequently, the yeast *Saccharomyces cerevisiae var. boulardii* demonstrate not only low flocculation activity, but also high viability. The relatively high viability of the probiotic strain is also noted by Mulero-Cerezo et al. (2019). Thus, a sample of craft beer obtained with *Saccharomyces cerevisiae var. boulardii*, after 45 days of storage, contained $8.3 \pm 1.4\times10^6$ CFU mL^{-1}, while the control sample (beer produced using the *SF- 04* brewing strain) contained only $1.1 \pm 0.2\times10^5$ CFU mL^{-1}.

Table 4. Results of physicochemical and microbiological analysis of young and final beers

Indicator	Young beer		Final beer	
	S.c.b.	*047A*	*S.c.b.*	*047A*
Er (% – m m^{-1})	4.61 ± 0.07	3.64 ± 0.09	4.46 ± 0.12	3.52 ± 0.13
Ethanol (% – v v^{-1})	4.81 ± 0.13	5.33 ± 0.21	5.56 ± 0.04	5.85 ± 0.18
RDF (%)	62.79 ± 0.87	70.38 ± 1.33	66.84 ± 0.94	72.96 ± 1.76
pH	4.21 ± 0.17	4.06 ± 0.08	4.26 ± 0.11	4.10 ± 0.07
Cell concentration (million mL^{-1})	9.67 ± 1.45	2.96 ± 0.44	2.90 ± 0.43	0.90 ± 0.14
Concentration of living cells (million mL^{-1})	8.29 ± 1.24	2.10 ± 0.32	1.17 ± 0.18	0.15 ± 0.02

Table 5. The content of secondary metabolites in young and final beers (mg L^{-1})

Compound	Organoleptic threshold (mg L^{-1})[1]	Young beer		Final beer	
		S.c.b.	*047A*	*S.c.b.*	*047A*
	Aldehydes				
Acetaldehyde	25.00	10.02 ± 1.50	16.61 ± 2.50	29.73 ± 4.46	19.13 ± 2.87
	Vicinal diketones				
Diacetyl	0.15	< 0.01	1.12 ± 0.17	< 0.01	0.56 ± 0.08
2,3-Pentanedione	1.00	< 0.01	0.14 ± 0.02	< 0.01	< 0.01
	Higher alcohols				
n-Propanol	800.00	16.65 ± 2.50	27.73 ± 4.16	20.82 ± 3.12	39.14 ± 5.87
Isobutyl alcohol	200.00	21.16 ± 3.17	85.48 ± 12.82	23.83 ± 3.57	97.56 ± 14.63
Amyl alcohol	65.00	68.13 ± 10.22	100.01 ± 15.00	74.16 ± 11.12	109.60 ± 16.44
	Esters				
Ethyl acetate	21.00	3.53 ± 0.53	16.77 ± 2.52	5.17 ± 0.76	22.06 ± 3.31
Isoamyl acetate	1.40	< 0.01	2.48 ± 0.37	< 0.01	2.90 ± 0.44
Ethyl caproate	0.17	0.13 ± 0.02	< 0.01	0.15± 0.02	< 0.01

[1]Blanco et al. (2016).

Table 5 demonstrates that *Saccharomyces cerevisiae var. boulardii* produce fewer secondary metabolites than control. At the same time, in the final product, the concentration of only such components as acetaldehyde and amyl alcohol exceeded the concentrations corresponding to the perception threshold. The increase in the concentrations of secondary metabolites after fermentation can be explained by secondary fermentation during beer carbonization using a primer. The particular attention should be paid to the concentration of diacetyl, which presence in the final product is considered to be one of the main beer defects. Both in young and final beers, the concentration of vicinal diketones did not exceed the perception threshold. This suggests that, when we use the yeast *Saccharomyces cerevisiae var. boulardii* in brewing, the probability of including a diacetyl rest in the main fermentation mode is insignificant.

When using new non-traditional cultures of yeast in brewing, the organoleptic properties of the final product are of particular interest. According to the data presented in Table 6, the use of the yeast *Saccharomyces cerevisiae var. boulardii* as a starter culture produces a beer with a positive sensory profile. The product aroma had a complex character: it included caramel, spicy, fruity and phenolic notes, as well as smoked and wine elements; while honey was the dominant note of the taste. It is worth mentioning that phenolic notes can be noticed in the composition of the sensory profile. This fact suggests that the yeast *Saccharomyces cerevisiae var. boulardii* is probably a positive for phenolic off-flavor yeast (POF+), as most wheat strains.

Table 6. Organoleptic characteristics of final beer

Indicator	Strain	
	S.c.b.	*047A*
Aroma	Barely perceptible: alcohol, caramel, spicy Slight: wine, fruity, smoked Easily noticeable: phenolic Explicit: hoppy	Barely perceptible: alcohol, wine, fruity, solvent-like Slight: green apple, phenolic, fatty, oily Easily noticeable: hoppy, nutty, herbal Explicit: banana, pear
Flavor	Easily noticeable: sour, hop bitterness with some coarse bitterness (residual), fullness of taste Explicit: honey	Easily noticeable: sour, honeyed, with slight hop bitterness, watery Explicit: coarse bitterness (residual)

According to the data obtained, it can be concluded that the use of the yeast *Saccharomyces cerevisiae var. boulardii* as a starter culture in beer technology is promising. The main advantage of its use is the possibility of obtaining beer with potential probiotic properties. In addition, it should be noted that the use of the yeast *Saccharomyces cerevisiae var. boulardii* in brewing allows us to obtain beer with a low RDF, which has a beneficial effect on such organoleptic properties of beer as body and drinkability, and this fact can also be used in the technology of non-alcoholic and low-alcohol beer. However, the fermentation activity of *Saccharomyces cerevisiae var. boulardii* is lower than brewing strains, which entails a longer fermentation process. To accelerate the fermentation process, further research is needed regarding its optimization, for example, by varying such parameters as pitching rate and temperature.

CONCLUSIONS

In this work, the fermentation performance of the probiotic yeast culture *Saccharomyces cerevisiae var. boulardii* was studied compared to top-fermenting brewing yeast strains during fermentation of model nutrient media and beer wort. According to the obtained results, it can be concluded that *Saccharomyces cerevisiae var. boulardii* have less fermentation activity compared to brewing strains, which consists in a relatively low substrate assimilation rate and the value of the final degree of attenuation. It was determined that both glucose and maltose are fermentable sugars by the yeast *Saccharomyces cerevisiae var. boulardii*. Consequently, the low apparent attenuation in beer wort fermentation seems to be related to the inability of this strain to consume maltotriose or to the extremely low rate of this process. It was also noted that the biomass yield on the maltose medium was higher than on the glucose one.

Beer produced with *Saccharomyces cerevisiae var. boulardii* significantly differed in physicochemical, microbiological, and organoleptic parameters from the control sample obtained using the *047A* brewing strain. It was primarily characterized by a high content of residual extract and, accordingly, a lower concentration of ethanol. This beer also contained significantly more live yeast cells, which indicates the relatively high viability of *Saccharomyces cerevisiae var. boulardii*. In its turn, the content of secondary metabolites was lower than in the control.

REFERENCES

Ávila-Reyes, S.V., Camacho-Diaz, B.H., Acosta-Garcia, M.C., Jimenez-Aparicio, A.R. & Hernandez-Sanchez, H. 2016. Effect of salt and sugar osmotic stress on the viability and morphology of Saccharomyces boulardii. *International Journal of Environment, Agriculture and Biotechnology* **1**, 593–602. doi: 10.22161/ijeab/1.3.43

Blanco, C.A., Andrés-Iglesias, C. & Montero, O. 2016. Lowalcohol Beers: Flavor Compounds, Defects, and Improvement Strategies. *Critical Reviews in Food Science and Nutrition* **56**(8), 1379–1388. doi: 10.1080/10408398.2012.733979

Briggs, D.E., Boulton, C.A., Brookes, P.A. & Stevens, R. 2004. *Brewing Science and practice*, 863 pp.

Campbell, C., Nanjundaswamy, A.K., Njiti, V., Xia, Q. & Chukwuma, F. 2016. Value-added probiotic development by high-solid fermentation of sweet potato with Saccharomyces boulardii. *Food Sci. Nutr* **5**(3), 633–638. doi: 10.1002/fsn3.441

Capece, A., Romaniello, R., Pietrafesa, A., Siesto, G., Pietrafesa, R., Zambuto, M. & Romano, P. 2018. Use of Saccharomyces cerevisiae var. boulardii in co-fermentations with S. cerevisiae for the production of craft beers with potential healthy value added. *International Journal of Food Microbiology* **284**, 22–30. doi: 10.1016/j.ijfoodmicro.2018.06.028

Değirmencioğlu, N., Gurbuz, O. & Şahan, Y. 2016. The Monitoring, Via an In vitro Digestion System, of the Bioactive Content of Vegetable Juice Fermented with Saccharomyces cerevisiae and Saccharomyces boulardii. *J. Food Process Preserv* **40**, 798–811. doi: 10.1111/jfpp.12704

Fratianni, F., Cardinale, F., Russo, I., Iuliano, C., Tremonte, P., Coppola, R. & Nazzaro, F. 2014. Ability of symbiotic encapsulated Saccharomyces cerevisiae boulardii to grow in berry juice and to survive under simulated gastrointestinal conditions. *J. Microencapsul* **31**, 299–305. doi: 10.3109/02652048.2013.871361

Hudson, L.E., McDermott, C.D., Stewart, T.P., Hudson, W.H., Rios, D., Fasken, M.B., Corbett, A.H. & Lamb, T.J. 2016. Characterization of the probiotic yeast Saccharomyces boulardii in the healthy mucosal immune system. *PLoS ONE* **11**, 1–21. doi: 10.1371/journal.pone.0153351

Karaolis, C., Botsaris, G., Pantelides, I. & Tsaltas, D. 2013. Potential application of Saccharomyces boulardii as a probiotic in goat's yogurt: survival and organoleptic effects. *International Journal of Food Science & Technology* **48**(7), 1445–1452. doi: 10.1111/ijfs.12111

Khatri, I., Tomar, R., Ganesan, K., Prasad, G.S. & Subramanian, S. 2017. Complete genome sequence and comparative genomics of the probiotic yeast Saccharomyces boulardii. *Sci. Rep*. **7**, 1–13. doi: 10.1111/ijfs.12111

Lazo-Vélez, M.A., Serna-Saldívar, S.O., Rosales-Medina, M.F., Tinoco-Alvear, M. & Briones-García, M. 2018. Application of Saccharomyces cerevisiae var. boulardii in food processing: a review. *J Appl Microbiol* **125**(4), 943–951. doi: 10.1111/jam.14037

Lourens-Hattingh, A. & Viljoen B.C. 2001. Growth and survival of a probiotic yeast in dairy products. *Food Research International* **34**(9), 791–796.

Łukaszewicz, M. 2012. Saccharomyces cerevisiae var. boulardii – Probiotic Yeast. In Everlon Rigobelo (eds) *Probiotics*. Books on Demand, Rijeka, Croatia, 385–388 pp.

Mitterdorfer, G., Mayer, H.K., Kneife, W. & Viernstein, H. 2002. Clustering of Saccharomyces boulardii strains within the species S. cerevisiae using molecular typing techniques. *Journal of Applied Microbiology* **93**, 521–530. doi: 10.1046/j.1365-2672.2002.01710.x

Mulero-Cerezo, J., Briz-Redón, A. & Serrano-Aroca, A. 2019. Saccharomyces Cerevisiae Var. Boulardii: Valuable Probiotic Starter for Craft Beer Production. *Applied Sciences* **9**(16), 1–16. doi: 10.3390/app9163250

Pais, P., Almeida, V., Yılmaz, M. & Teixeira, M.C. 2020. Saccharomyces boulardii: What Makes It Tick as Successful Probiotic? *Journal of Fungi* **6**(2), 78. doi: 10.3390/jof6020078

Pereira de Paula, B., de Souza Lago, H., Firmino, L., Fernandes Lemos Júnior, W.J., Ferreira Dutra Corrêa, M., Fioravante Guerra, A., Signori Pereira, K. & Zarur Coelho, M.A. 2021. Technological features of Saccharomyces cerevisiae var. boulardii for potential probiotic wheat beer development. *LWT - Food Science and Technology* **135**, 1–8. doi: 10.1016/j.lwt.2020.110233

Stewart, G.G. 2016. Saccharomyces species in the Production of Beer. *Beverages* **2(**34), 1–18. doi:10.3390/beverages2040034

Suchkova, E., Shershenkov, B. & Baranenko, D. 2014. Effect of ultrasonic treatment on metabolic activity of Propionibacterium shermanii, cultivated in nutrient medium based on milk whey. *Agronomy Research* **12**(3), 813–820.

Vandenplas, Y., Brunser, O. & Szajewska, H. 2008. Saccharomyces boulardii in childhood. *European Journal of Pediatrics* **168**(3), 253–265. doi: 10.1007/s00431-008-0879-7

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Role of planting density on the growth efficiency of *Juniperus virginiana* L. under open-air hydroponic conditions of the Ararat Valley

Kh.S. Mayrapetyan, A.S. Eghiazaryan, S.A. Eloyan and A.S. Karapetyan*

G.S. Davtyan Institute of Hydroponics Problems, NAS RA, 108 Noragyugh, AM0082 Yerevan, Armenia
*Correspondence: astghik84k@rambler.ru

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Abstract. *Juniperus virginiana* (*J. virginiana*) is an evergreen coniferous tree, which has wide usage not only in green construction, but also in folk medicine as a source of valuable bioactive substances. The high demand for the tree forces the development of new methods for plant cultivation. Hydroponics is considered to be one of the most popular methods. As the soilless growing perspectiveness of *J. virginiana* in Armenia has previously been confirmed by our experiments, the optimization of growing conditions, which bests suits for the enhancement of the growth efficiency and accelerated production of viable trees remains an actual issue. Optimization of the planting density (PD) is one of them. Taking into account the above-mentioned in the frame of this study the role of PD on the growth efficiency of *J. virginiana* has been studied in open-air hydroponic conditions of the Ararat Valley for the first time. The saplings of the tree were planted in volcanic red slag with three different PDs: 10, 12 and 14 plants per square meter. According to the biometrical measurements, no significant differences between the variants were observed at the end of the experimental period. In October an average height and stem diameter of the plants grown in various PDs were fluctuated between 71.7–76.5 cm and 13.9–14.5 mm, accordingly. Positive relationship between the plant height and stem diameter of *J. virginiana* during the whole vegetation period has been observed. Our preliminary studies showed, that all the applied PDs are preferable for early years of hydroponic growing of *J. virginiana* in open-air hydroponic conditions of the Ararat Valley.

Key words: *J. virginiana*, hydroponics, planting density (PD), plant height, stem diameter.

INTRODUCTION

Junipers are coniferous trees and shrubs in the genus *Juniperus* of *Cupressaceae* family. The genus consists of approximately 76 species and 27 varieties. The representatives of the genus are geographically wide distributed. Depending on taxonomic viewpoint, they are widely distributed throughout Eastern and Western Hemisphere, Europe, Central Asia, China, Far East, Continental North America, United States and Canada, Mexico and Guatemala, Caribbean, Africa (Adams, 2014; Adams, 2019).

Junipers are widespread in Armenia as well. In addition to use in landscaping *Juniperus virginiana* (*J. virginiana*) is also widely used in folk medicine as a source of valuable biologically active substances. In the paper of Zhang & Yao (2018) anxiolytic effect of essential oil from *J. virginiana* has been established. Semerdjieva et al. (2019) approved antioxidative capacity of the essential oil from *J. virginiana*. It should be noted, that the composition of essential oil from *J. virginiana* is differed depending on the sampling place. Cantrell et al. (2013) studying the chemical composition of the essential oil of the trees, which were sampled from 49 locations of Mississippi, Alabama, Tennessee and North Dakota, divide the accessions of the plant into different groups. Based on essential oil composition the *J. virginiana* accessions were divided into various chemotypes: safrole-limonene-linalool, safrole-β-pinene-limonene-linalool, β-pinene-limonene, limonene, limonene-linalool, limonene-safrole, limonene-safrole-β-pinene, β-pinene-limonene-bornyl acetate, β-pinene-limonene-linalool-bornyl acetate, and myrcene-limonene. Moreover, various chemotypes showed different antioxidative activity. Leaves (needles) of *J. virginiana* also contains a natural product - podophyllotoxin, which is used to manufacture drugs for treatment of cancer, rheumatoid arthritis, psoriasis, genital warts, and multiple sclerosis. It's important to note that *J. virginiana* is distinguished with highest concentration of podophyllotoxin compared to the other representatives of the genus (Cushman et al., 2003; Gawde et al., 2009; Cantrell et al., 2013). Eller et al. (2014) approved that essential oil from *J. virginiana* may also be used for pest control, as it showed high efficiency against synthetic acaricides for *I. scapularis*. In another study Eller et al. (2010) approved that *J. virginiana* wood extract conferred resistance against subterranean termites and wood-rot fungi.

The high value and demand of *J. virginiana* requires new methods of production. Soilless culture represents a suitable method for cultivation of plants (Ebrahimi et al., 2012; Dehnavard et al., 2017) that can also be applied for tree species like *J. virginiana*.

In our previous studies the growth efficiency and perspectiveness of *J. virginiana* in open-air hydroponic conditions of the Ararat Valley has been confirmed (Mayrapetyan et al., 2021).

Among the many advantages, hydroponics allows to increase planting density (PD) per unit surface compared to the traditional method of cultivation, which is one of the most critically important factors in agriculture.

Many studies were done around the influence of PDs on biomass efficiency of various crops (Maboko & Du Plooy, 2012; Katrevičs et al., 2018; Marziliano et al., 2018; Berbeć & Matyka, 2020; Postma et al., 2021; Zhang et al., 2021). Berbeć & Matyka (2020) showed that yield parameters of plants cultivated as short rotation coppice are strongly depending on PDs and came to the conclusion that the optimal PD should be chosen to best suit the needs of the plants and industry as well. Zhang et al. (2021) showed that various cultivars response differently to the PD and that the optimization of PD is a promising approach for sustainable development of agriculture in semiarid climate. In the paper Postma et al. (2021) several comparisons between the influence of PD were presented for different decades. And as it shown in modern agriculture crops are planted with higher PDs than in traditional agriculture which is due to the improved management, increased fertilization, etc.

Taking into account the above-mentioned, the aim of this work was to study the influence of different PDs on the growth peculiarities and efficiency of *J. virginiana* in open-air hydroponic conditions of the Ararat Valley for the first time. This will allow to develop the optimal PD, which best suits for the accelerated production of high-quality and maximum number of trees per unit surface.

MATERIALS AND METHODS

The experiments were carried out at G.S. Davtyan Institute of Hydroponics Problems of National Academy of Sciences of the Republic of Armenia. Two-year-old planting materials were introduced from nursery into open-air hydroponic conditions of the Ararat Valley. The planting was done in the vegetational experimental station of the Institute. As a growing substrate volcanic red slag with 3–15 mm diameter of particles was used. During the vegetation period the plants were nourished with nutrient solution elaborated by academician Davtyan (Davtyan & Mairapetyan, 1976). The following PDs were applied in the experiments:

A) 10 plants per square meter,
B) 12 plants per square meter,
C) 14 plants per square meter.

During the vegetation biometrical measurements were done. The plant height was measured with ruler, as the length from the collar to the apex. The diameter of the stem was measured near the base with caliper. At the moment of the measurements the age of the trees were two years old.

The obtained data were subjected to the statistical elaboration with GraphPad Prism 8 Software Package. Standard deviations (SD) were calculated for each variant.

RESULTS AND DISCUSSION

No significant differences were observed between the variants regarding *J. virginiana* (Fig. 1) height and stem diameter at the end of the experimental period.

The heights of the plants grown with different PDs were fluctuated in May between: 36.5–38.9 cm, June: 45.4–49.2 cm, July: 53.4–56.3 cm, August: 61.5–65.9 cm, September: 68.2–72.6 cm, October: 71.7–76.5 cm. The diameters of the stems were fluctuated in May between: 7.3–7.5 mm, June: 8.0–8.4 mm, July: 9.7–10.1 mm, August: 11.3–11.9 mm, September: 12.4–13.6 mm, October: 13.9–14.5 mm. From May to October an average heights and diameters of the stems of the plants grown in 10, 12, 14 plants sqm^{-1} PDs increased 37.6, 37.6 and 34.1 cm and 7.2, 6.9,

Figure 1. *J. virginiana* in hydroponic conditions.

6.6 mm, correspondingly (Figs 2, 3). Survival rate of the plants was 100%.

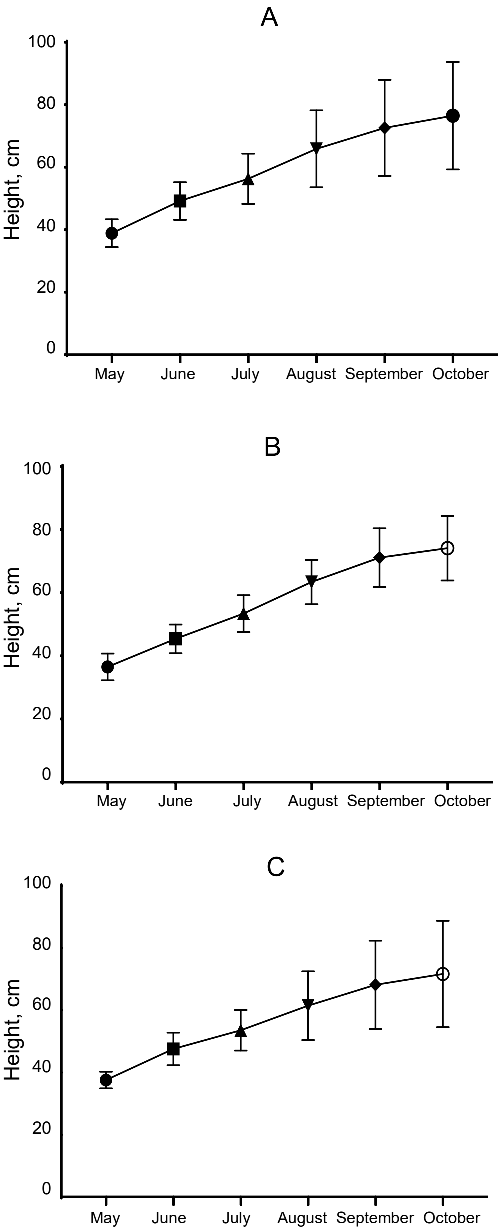

Figure 2. Height of *J. virginiana* during vegetation, grown in various PDs: A – 10 plants sqm^{-1}; B – 12 plants sqm^{-1}; C – 14 plants sqm^{-1}.

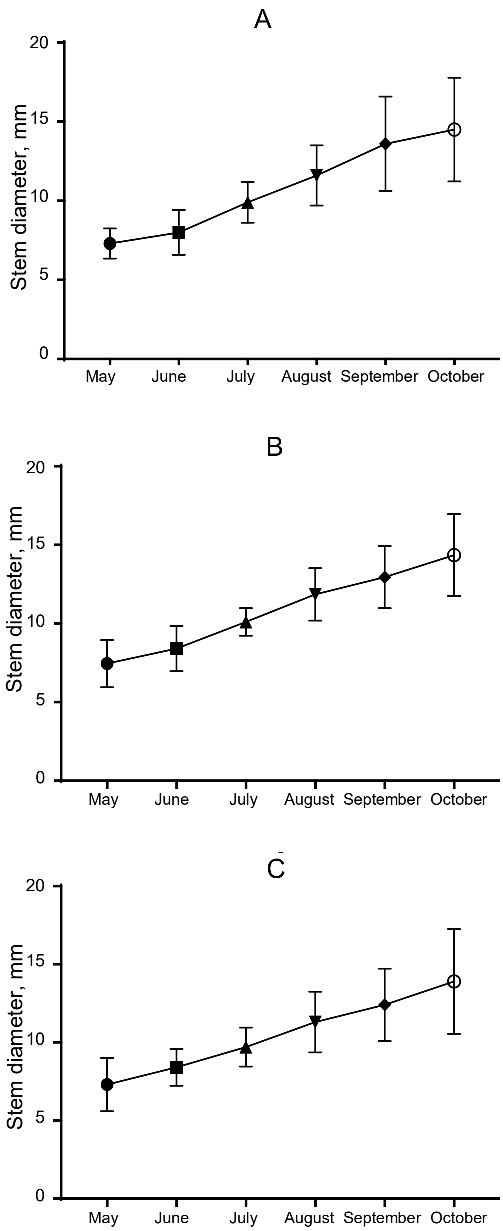

Figure 3. Stem diameter of *J. virginiana* during vegetation, grown in various PDs: A – 10 plants sqm^{-1}; B – 12 plants sqm^{-1}; C – 14 plants sqm^{-1}.

The linear regressions between the plant height and stem diameter for each variant during the vegetation were calculated and the graphs built (Fig. 4). For all the variants positive relationships between them are exist. $R^2 = 0.9692$, $R^2 = 0.9835$ and $R^2 = 0.9755$ for 10, 12, 14 plants sqm^{-1} variants, accordingly.

Zhao et al. (2011) showed that during the first two years after planting no significant differences were observed between PDs of loblolly pine. In later years PDs affected significantly on average diameter at breast height.

Gadow & Kotze (2014) find out that maximum densities of *Pinus patula* and *Pinus elliottii* may differ depending on the growing site. At the same time maximum densities of the two species, which were grown in different sites were also greatly differ.

Dong et al. (2016) demonstrated the responses of *Cunninghamia lanceolata* to a high PD. As a result, *Cunninghamia lanceolata* showed less growth, biomass accumulation and lower photosynthetic rate.

Although the number of the trees between the selected variants does not differ strongly calculated per one square meter surface, and no significant differences were observed regarding plant height and stem diameter at end of the experimental period, meanwhile the difference may be visible in case of large-scale production. For example: in case of 100% survival rate, by applying 10, 12, 14 plants sqm^{-1} PDs, per 1 ha surface it will be possible to obtain 100,000, 120,000 and 140,000 trees, accordingly.

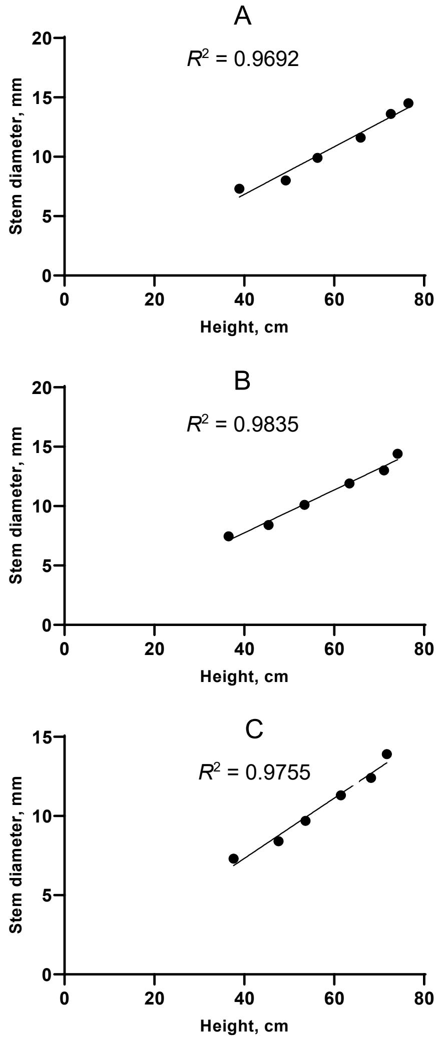

Figure 4. Linear relationship between the plant height and stem diameter of *J. virginiana* during the vegetation, grown in various PDs: A – 10 plants sqm^{-1}; B – 12 plants sqm^{-1}; C – 14 plants sqm^{-1}.

CONCLUSIONS

The high value and demand of *J. virginiana* is forcing to the development of new biotechnological ways for accelerated production of the saplings. Plant soilless culture or hydroponics is considered to be one of the most effective methods for obtaining tree-shrubs planting material, and enhancement plant productivity. Among the many advantages, hydroponics allows to increase PD per unit surface compared to the traditional method of cultivation, which is one of the most critically important factors in agriculture.

From May to October, during the one vegetation period the trees with 71.7–76.5 cm height, 13.9–14.5 mm stem diameter and well developed root system are possible to obtain. For the early years of cultivation all the applied PDs are preferable for hydroponic growing of *J. virginiana* in open-air hydroponic conditions of the Ararat Valley.

Though no significant differences were observed between PDs for our experimental period, the results may differ for big trees. Moreover, for large-scale production, by increasing the PD the biomass yield from the unit surface could be visible. Positive relationship between the plant height and stem diameter of *J. virginiana* during the whole vegetation period has been observed.

As the conducted experiments are preliminary, PDs were choosed randomly to understand the dependence of the plant growing pecularities on the PD. For further studies the fluctuations between the number of the trees per unit surface should be changed more strictly in order to determine the upper and the lower borders, where the plant growth could reach critical state.

REFERENCES

Adams, R.P. 2014. *Junipers of the World: The Genus Juniperus*, 4th Edition, 422 pp.

Adams, R.P. 2019. *Juniperus* of Canada and the United States: TaxonomyKey and Distribution. *Lundellia* **21**(1), 1–34. https://doi.org/10.25224/1097-993X-21.1

Berbeć, A.K. & Matyka, M. 2020. Planting density effects on grow rate, biometric parameters, and biomass calorific value of selected trees cultivated as SRC. *Agriculture* **10**(12), 583. https://doi.org/10.3390/agriculture10120583

Cantrell, C.L., Zheljazkov, V.D., Osbrink, W.L.A., Castro, A., Maddox, V., Craker, L.E. & Astatkie, T. 2013. Podophyllotoxin and essential oil profile of Juniperus and related species. *Industrial Crops and Products* **43**, 668–676. https://doi.org/10.1016/j.indcrop.2012.07.075

Cushman, K.E., Maqbool, M., Gerard, P.D., Bedir, E., Lata, H. & Moraes, R.M. 2003. Variation of podophyllotoxin in leaves of Eastern red cedar (*Juniperus virginiana*). *Planta Med.* **69**(5), 477–478. https://doi.org/10.1055/s-2003-39707

Davtyan, G.S. & Mairapetyan, S.Kh. 1976. *Soilless production of Rose Geranium*, AS of ASSR Publ., Yerevan, 134 pp. (in Armenian).

Dehnavard, S., Souri, M.K. & Mardanlu, S. 2017. Tomato growth responses to foliar application of ammonium sulfate in hydroponic culture. *Journal of Plant Nutrition* **40**(3), 315–323. https://doi.org/10.1080/01904167.2016.1240191

Dong, T., Zhang, Y., Zhang, Y. & Zhang, S. 2016. Continuous planting under a high density enhances the competition for nutrients among young *Cunninghamia lanceolata* saplings. *Annals of Forest Science* **73**, 331–339. https://doi.org/10.1007/s13595-015-0518-1

Ebrahimi, R., Souri, M.K., Ebrahimi, F. & Ahmadizadeh, M. 2012. Growth and yield of strawberries under different potassium concentrations of hydroponic system in three substrates. *World Appl. Sci. J.* **16**(10), 1380–1386.

Eller, F.J., Clausen, C.A., Green, F. & Taylor, S.L. 2010. Critical fluid extraction of Juniperus virginiana L. and bioactivity of extracts against subterranean termites and wood-rot fungi. *Industrial Crops and Products* **32**(3), 481–485. https://doi.org/10.1016/j.indcrop.2010.06.018

Eller, F.J., Vander Meer, R.K., Behle, R.W., Flor-Weiler, L.B. & Palmquist, D.E. 2014. Bioactivity of cedarwood oil and cedrol against arthropod pests. *Environmental Entomology* **43**(3), 762–766, https://doi.org/10.1603/EN13270

Gadow, K.V. & Kotze, H. 2014. Tree survival and maximum density of planted forests - Observations from South African spacing studies. *For. Ecosyst.* **1**, 21, https://doi.org/10.1186/s40663-014-0021-4

Gawde, A.J., Cantrell, C.L., Valtcho, D. & Zheljazkov, V.D. 2009. Dual extraction of essential oil and podophyllotoxin from Juniperus virginiana. *Industrial Crops and Products* **30**(2), 276–280. https://doi:10.1016/j.indcrop.2009.05.005

Katrevičs, J., Džeriņa, B., Neimane, U., Desaine, I., Bigača, Z. & Jansons, Ā. 2018. Production and profitability of low density Norway spruce (*Picea abies* (L.) Karst.) plantation at 50 years of age: case study from eastern Latvia. *Agronomy Research* **16**(1), 113–121, https://doi.org/10.15159/AR.18.014

Maboko, M.M. & Du Plooy, C.P. 2012. Effect of plant density and harvesting method on yield components of hydroponically grown amaranth. *Acta Hortic.* **947**, 415–421. https://doi.org/10.17660/ActaHortic.2012.947.54

Marziliano, P.A., Russo, D., Altieri, V., Macrì, G. & Lombardi, F. 2018. Optimizing the sample size to estimate growth in I-214 poplar plantations at definitive tree density for bioenergetic production. *Agronomy Research* **16**(3), 821–837, https://doi.org/10.15159/AR.18.092

Mayrapetyan, Kh.S., Hovsepyan, A.H., Eghiazaryan, A.S., Hakobjanyan, A.A., Eloyan, S.A. & Karapetyan, A.S. 2021. The production of *virginian juniper* (*Juniperus virginiana* L.) in conditions of Ararat Valley and Dilijan forest zone. *Biological Journal of Armenia* **72**(1–2), 132–136.

Postma, J.A., Hecht, V.L., Hikosaka, K., Nord, E.A., Pons, T.L. & Poorter, H. 2021. Dividing the pie: A quantitative review on plant density responses. *Plant, Cell & Environment* **44**, 1072–1094. https://doi.org/10.1111/pce.13968

Semerdjieva, I.B., Shiwakoti, S., Cantrell, C.L., Zheljazkov, V.D., Astatkie, T., Schlegel, V. & Radoukova, T. 2019. Hydrodistillation extraction kinetics regression models for essential oil yield and composition in *Juniperus virginiana*, *J. excelsa*, and *J. sabina*. *Molecules* **24**(5), 986. https://doi.org/10.3390/molecules24050986

Zhang, Y., Xu, Z., Li, J. & Wang, R. 2021. Optimum planting density improves resource use efficiency and yield stability of rainfed maize in semiarid climate, *Front. Plant Sci.* https://doi.org/10.3389/fpls.2021.752606

Zhang, K. & Yao, L. 2018. The anxiolytic effect of Juniperus virginiana L. essential oil and determination of its active constituents. *Physiology & Behavior* **189**, 50–58. https://doi.org/10.1016/j.physbeh.2018.01.004

Zhao, D., Kane, M. & Borders, B. 2011. Growth responses to planting density and management intensity in loblolly pine plantations in the southeastern USA Lower Coastal Plain, *Annals of Forest Science* **68**, 625–635. https://doi.org/10.1007/s13595-011-0045-7

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Tartaric acid synthetic derivatives effect on phytopathogenic bacteria

A.R. Mikaelyan[1], B.G. Babayan[2], A.A. Vartanyan[3,*] and H.V. Tokmajyan[4]

[1]National Polytechnic University of Armenia, Teryan Srt., 105, AM0009 Yerevan, Armenia
[2]Armbiotechnology Scientific and Production Center, Gyurjyan Str., 14, AM0056 Yerevan, Armenia
[3]Moscow Aviation Institute (National Research University), Volokolamskoe highway 4, RU125993 Moscow, Russia
[4]Shushi University of Technology, Armenakyan Str., 125, AM0047 Yerevan, Armenia
[*]Correspondence: arevshadvartanyan@mail.ru

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Abstract. The scientific goals of current research were devoted to targeted derivatization of natural tartaric acid (TA) for the enhancement of antimicrobial properties of it such as like the effects of them on multi-drug resistant phytopathogenic bacteria, depends to their structure features and the genetic parameters of studied microorganisms. The main utilitarian goal is to develop new class of antimicrobial biodegradable compounds with possible prospective application as moresafe alternative to traditional antibiotics applicable for: agriculture, horticulture, food industry as well as in medicine. These compounds were developed in basic research laboratory: 'Agrarian Pesticide Creation and The Quality Control' at National Polytechnic University of Armenia (NPUA). TA and tartrates are safe antimicrobial food additives. According to the results of *in vitro* studies, the synthesized cyclohexyl-, benzyl- and phenyl- derivatives of it in the form of amine complex salts (correspondently CAS, BAS and PhAS) and cyclic imides (correspondently CI, BI and PhI) are effective against the model multi-drug resistant strains of Gram-negative microorganisms. Bactericidal effects of TA derivatives were demonstrated on 19 model native soil strains of phytopathogenic *Xanthomonas beticola* (*6 strains*), *X. vesicatoria and Pseudomonas syringae* (*13 strains*) representatives, which are differing in antibiotic resistance. Regarding the transformation results, the absence of transfer of resistance to TA imides and amine complex salts by plasmids, makes them promising objects for further research. Primary studies have not shown any antibacterial effect on various non-pathogenic soil bacteria (*Pseudomonas chlororaphis, P. taetrolens*, etc.). The described compounds are recommended for further more detailed toxicological studies.

Key words: antimicrobial agents, complex salts of tartaric acid, imides of tartaric acid, multi-drug resistance, phytopathogen, *Pseudomonas, Xanthomonas.*

INTRODUCTION

Phytopathogenic bacteria from *Pseudomonadaceae* and *Xanthomonadaceae* families, which are the causative agents of plant bacteriosis (fruits, leaves, etc.) are very significant. These microorganisms are ubiquitous on the planet due to their high adaptability and plasticity of metabolism (Yoshimoto et al., 2010).

High adaptability and antibiotic resistance, especially pan-drug resistance and multi-drug resistance of *Pseudomonas* and *Xanthomonas* representatives are well known (*Pseudomonas syringae, pv. syringae, P. syringae, pv. tabaci, P. syringae, pv. lachrymans, Xanthomonas vesicatoria, X. beticola*) (Santi et al., 2021). Their representatives are common inhabitant of almost any wet surface, being harmful for crops, agricultural animals (Morris et al., 2007). In this regard, these bacteria are a convenient object for studying the mechanisms of resistance and ways to overcome it.

The problem of antimicrobial drugs resistance has become particularly acute in recent decades. According to WHO reports for 2019, antimicrobial drugs resistance is one of the top five research priorities for the XXI century (World Health Organization, 2019). Due to the global coronavirus pandemic that has swept the planet, the use of antibiotics and other antimicrobial drugs has increased significantly. It has led to an increase in the number of resistant bacteria (Livermore, 2021). Therefore, the development of new classes of antimicrobial drugs is especially acute (Rahman & Das, 2021). However, promising synthetic antibiotics, such as azalide macrolides or fluoroquinolones, to which no natural specific resistance has been found, are often quite non-selective in their effect. Also, they have a number of undesirable side effects for patients. For example, vancomycin and the analogous antibiotics, used against super bacteria are often cardiotoxic and neurotoxic (Martinez et al., 2018).

Various types of cross-resistance, such as pan-drug resistance, as well as multi-drug resistance in case of phytopathogenic representatives of *Pseudomonadaceae* and *Xanthomonadaceae*, lead to a low efficacy of agricultural crop protection agents containing antibiotics. This problem is well-known also in scope of medicine and veterinary. It's notable by the decrease of antibiotic therapy efficiency of *Pseudomonas, Stenotrophomonas* and *Xanthomonas* infections treatment of animals and plants. That appropriately is caused by the abilities of mentioned bacteria to transmit the resistance by the intraspecific gene horizontal transfer. In contribution to it the enhancement of properties resistance take place, with the uncontrolled spread of antibiotics resistance genes by mobile genetic elements (transposons and plasmids) of phytopathogenic strains and agricultural animal opportunistic pathogens, as well as human pathogens.

As a result, it led to decrease of efficiency of therapy for plant, human and animal *Pseudomonas, Stenotrophomonas* and *Xanthomonas* infections treatment (Monteil et al., 2016). The negative impact of this problem on health care, gardening, horticulture, agriculture and animal husbandry is quite significant (Vidaver, 2002).

Being involved in a huge number of food chains of various biogeocenoses and other ecosystems, as decomposers, *Pseudomonadaceae* and *Xanthomonadaceae* representatives participate in interspecific gene horizontal transfer. And it led to spread of antibiotics resistance genes by mobile genetic elements (transposons and plasmids). Thus, the number of resistant pathogens of agricultural crops is increasing. In response to this, the volumes of the used antimicrobial plant protection agents are being increased, what adversely affects the quality of agricultural production. It transfers the problem of multi-drug resistance of microorganisms to the plane of agriculture, ecology, and health care (Dolejska & Literak, 2019). The changes of resistance profiles of microorganisms can potentially lead to dramatic changes in animals and plants biodiversity in biogeocenoses and other ecosystems (Aslam et al., 2018). Thus, the main research problem is the elaboration of compounds which can be more effective and less harmful for human organism and for environment.

That is why, the search for new alternatives to classical, complex and combined agents of the protection of agricultural plants and animals, containing antibiotics of various classes is a very urgent theoretical and practical problem (Granados-Chinchilla & Rodríguez, 2017).

For this reason, one of the most promising directions is the targeted derivatization of natural antimicrobial compounds with the potential enhancement of antimicrobial properties. One of the alternatives for solving of this problem is the use of natural organic acids and their derivatives (Melaku et al., 2021). One of the common groups of antimicrobial compounds are natural organic acids, especially aldaric acids (Liang et al., 2021). These compounds are widespread in plants and constitute one of the most important components of the antimicrobial barrier of human skin (Cartron et al., 2014). As it is known from literature data TA, lactic and citric acids, as well as their derivatives, have proven themselves as effective antimicrobials. Dicarboxylic and tricarboxylic acids and their salts, have shown in many experiments an unexpected ability to enhance the antimicrobial activity of a number of disinfectants. One per cent of citrate content can significantly increase the bactericidal and bacteriostatic activity of antibiotics. It also decreases the viability of strains, which are resistant to them. Citrates have intrinsic antimicrobial properties and they are synergistic effective against bacterial colonization of tissue samples by pathogens. They also enhance the antimicrobial properties of phytogenic polyphenols (activators of the innate immune systems of plants), and disinfecting organic dyes (crystal violet, methylene blue) (Andrews et al., 2011).

Another direction of search for new antimicrobial drugs is the development of polymeric, polyamide and nanopolymeric derivatives of carboxylic acids. Natural aldaric acids derivatives creation by the targeted derivatization and functionalization are the most important directions for development of new classes of antimicrobial agents. The advantage of this method consists in obtaining compounds with alternative mechanisms of activity, which are sharply different from the ways of e of known antibiotics (D'Mello, 2003; Kumari & Jaisankar, 2017).

The principal scientific goal of current research was natural TA targeted derivatization for the increase in antimicrobial potential of it. The main practical goal of a particular study was an application of the elaborated compounds, as new potentially ecologically safe alternative of generally used antibiotics, which are not always effective and moreover often have harmful side effects. Due to well-known safety and antimicrobial properties of TA and its salts, amine complex salts and cyclic imides with improved antimicrobial properties against multi-drug resistant pathogens were synthesized. The effect of cyclohexyl-, phenyl-, benzyl-substituted imides and complex salts of natural TA on strains of native phytopathogenic *Xanthomonas* 13 strains of different species, isolated form soil, was considered (Fig. 1) (Bagdasaryan et al., 2020).

Due to well-known antimicrobial properties of TA and its salts, this paper considers the possibility of the successful use of TA synthetic derivatives: amine complex salts and cyclic imides with improved antimicrobial properties against multi-drug resistant pathogens. The effect of cyclohexyl-, phenyl-, benzyl-substituted imides and complex salts of natural TA on strains of phytopathogenic bacteria various types was considered (Fig. 1) (Bagdasaryan et al., 2020). According to the earlier studies, cyclohexyl- and benzyl-substituted imides and amine complex salts demonstrated activity against

K. pneumonia, St. aureus, Salmonella enteritis, P. aeruginosa, S. maltophilia and other opportunistic pathogens of humans and animals. Field tests have shown that the developed TA derivatives are not toxic for fish (Ageyets et al., 2019).

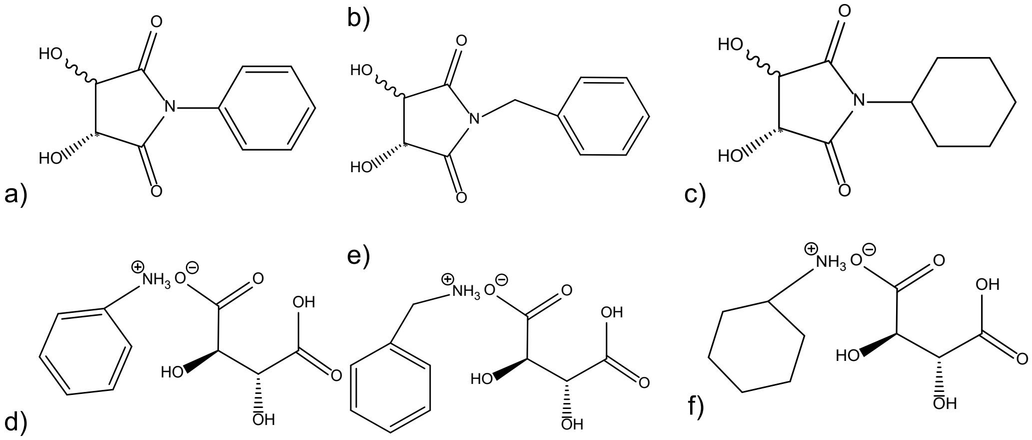

Figure 1. Synthetic derivatives of TA; a – phenylimide (PhI); b – benzylimide (BI); c – cyclohexylimide (CI); d – phenylamine complex salt (PhAS); e – benzylamine complex salt (BAS); f – cyclohexylamine complex salt (CAS).

The obtained results are of particular interest, taking into account the potential ability of *P. taetrolens* and other representatives of the *P. chlororaphis* group to utilize complex salts of TA as a carbon source (Baghdasaryan et al., 2019). This suggests the potential environmental safety of using these compounds as antiphytopathogenic agents.

MATERIALS AND METHODS

Tartaric acid derivatives obtaining

TA derivatives were generated according to synthetic methodology (Fricke et al., 2010), which was improved in 'Laboratory of Agrarian Pesticide Creation and The Quality Control' at NPUA (Fig. 2) (Dashchyan et al., 2015).

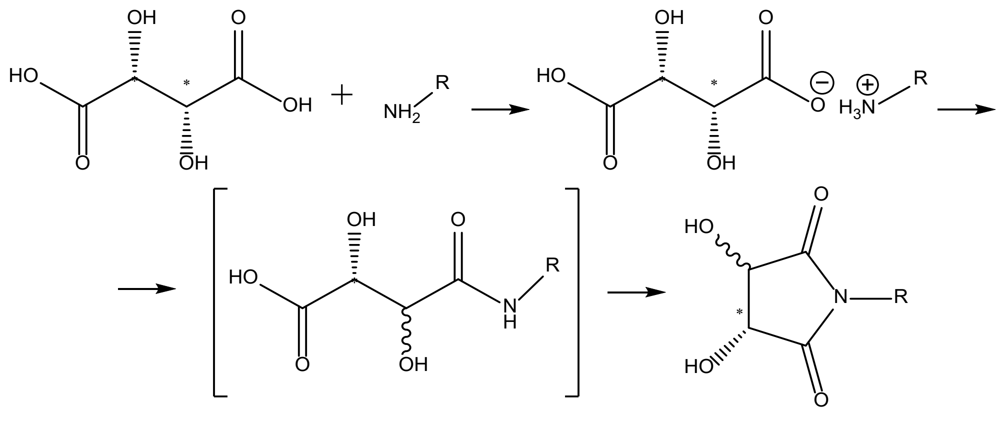

Figure 2. TA derivatization principal scheme. R = benzyl-, phenyl-, cyclohexyl-.

Antibiotic resistance study

In current research for the microbiological experiments, strains of the National Collection of Microorganisms of Microbial Depository Center (MDC), 'Armbiotechnology' Scientific and Production Center (SPC) of the National Academy of Sciences of the Republic of Armenia (NAS RA) were used. For the research of antibiotic-resistance there were studied 18 strains of *Xanthomonas* following species: *Xanthomonas vesicatoria, X. beticola and Pseudomonas the following strains: Pseudomonas syringae, pv. lachrymans, P. syringae, pv. tabaci*. As the control there were used *E. coli* the following strains: *E. coli DH5α* (non-plasmid sensitive to the used 13 antibiotics), *E. coli DH5α/VOG16 (VOG16* plasmid-containing resistant to kanamycin), *E. coli pUC18 (pIUC18 plasmid containing resistant to ampicillin), E. coli DH5α/PEC7 (PEC7* plasmid-containing resistant to chloramphenicol).

The resistance of the selected strains was defined using solid agarised selective-differential media. Sterilized by autoclaving beef-extract broth and beef-extract agar were used. For qualitative evaluation of antibiotic-resistance of bacteria, the cultivation was carried out by streaking, using 30mm diameter Petri dishes, at 37 °C, under aerobic conditions, on selective-differential media with 50 μg mL^{-1} content of the following antibiotics: Pcn/Penicillin, Amp/ampicillin, Amx/amoxicillin, Amc/augmentin, Cfx/cefixime, Cro/ceftriaxone (aminopenicillins and cephalosporins of β-lactam antibiotics group), Kan/kanamycin, Stp/streptomycin, Gnc/gentamicin (aminoglycosides), Cip/ciprofloxacin (of fluoroquinolones), Tcn/tetracycline (of tetracyclines), Cam chloramphenicol, also Azm/azithromycin (of azalide macrolides), produced by 'Astoria' (Mikaelyan et al., 2019). The choice of antibiotics was due to their wide applicability in medicine, as well as in agriculture and food industry (Landers et al., 2012).

TA new derivatives antimicrobial properties monitoring

Microbiological tests to determine the activity of new TA derivatives were carried out on selective cultivation media. For effect detection on solid cultural media, it was used bilayer media method with some modifications, elaborated in our laboratory (Totten et al., 1982, ThermoFisher, 2008): instead of blood agar it was used meat-peptone agar media. 1.8%-agar containing MPA (meat peptone agar) was poured into Petri dishes (30 mm diameter). Then separately for each tested strain of bacteria there were prepared mixtures of appropriate microbial culture suspension and molten 0.7% agar containing MPA at 37 °C, in sterile test tubes. On surface of solid 1.8%-MPA cultivation media in Petri dishes, the mentioned mixture of suspension of the object microbial strain (~10^8 CFU) with 0.7% - agar containing MPA was added as the second layer, for the uniform distribution of microbial culture. After the solidifying of second layer, 3 mcL of each of TA synthetic derivatives (benzylimide, cyclohexylimide, phenylimide, cyclohexyl amine complex salt, benzyl amine complex salt, and phenyl amine complex salt) in concentrations: 0.001M, 0.01M, 0.025M, 0.05M, 0.1M, 0.5M were sterilely added. Solutions were prepared in water for complex amine salts, and in DMSO (dimethyl sulfoxide) for imides, due to their solubility features. The appropriate controls were considered as micro-drops of sterile water and DMSO. The growth of microorganisms under aerobic conditions at temperature optimum of a given species (37 °C) on appropriate cultivation media containing test compounds was visually registered within a period: 12 h, 24 h, 48 h, 76 h. The sterile zones of bacterial growth inhibition in area of tested compound effect were calculated visually in mm. As a positive control it was considered the growth of the studied bacteria on same cultural media upon the same conditions with sterile pure solvents (water for amine complex salts and dimethyl sulfoxide for imides), without an addition of testing compound. (Eswaranandam et al., 2004).

Determination of minimum inhibitory concentrations were defined according to the generally accepted methods by multiple dilution procedures for each substance beginning from 0.5M to 0.0001M (Andrews, 2001).

Genetic study of observed *Pseudomonas* and *Xanthomonas* resistance mechanisms

For the definition of differences in resistance diapason of the studied strains to antibiotics and TA new derivatives, the series of genetic analysis were carried out. Genetic research of the studied bacteria was carried out by isolation of total and plasmid DNA from cells of various strains of phytopathogens, and 0.8–2.5% agar gel-electrophoresis. Plasmid analysis of sensitive and resistant strains of phytopathogens was carried out by electrophoresis and transformation (Thean et al., 2021). As positive control there were used: *E. coli DH5α/VOG16, E. coli pUC18, E. coli DH5α/PEC7; as negative control: E. coli DH5α.*

Mechanism of resistance was studied by the detection of resistance dependence to antibiotic concentration, as well as by Polymerase Chain Reaction (PCR) analysis of genes of antibiotic modifying enzymes (Table 1) (Dumas et al., 2006).

Table 1. Conditions of PCR analysis of the studied bacteria resistance

Gene primer	Subsequence	Temperature regime (T_{lid} 96 °C),
blaOXA-10	F:5'TATCGCGTGTCTTTCGAGTA3'; R:5'TTAGCCACCAATGATGCCC3'	94 °C-150s, 14 cycles: 94 °C-45s, 63 °C-45s, 72 °C-90s, 22 cycles: 94 °C-45s, 59 °C-45s, 72 °C-90s.
aph(3')VI	F:5'AGGTGACACTATAGAATACGGAAACAGCGTTTTAGAGC3'; R:5'GTACGACTCACTATAGGGAGGTTTTGCATTGATCGCTTT3'	94 °C-150s, 14 cycles: 94 °C-45s, 66 °C-45s, 72 °C-90s, 23 cycles: 94 °C-45s, 63 °C-45s, 72 °C-90s.
aac(6')II	F:5'AGGTGACACTATAGAATATTCATGTCCGCGAGCACCCC3'; R:5'GTACGACTCACTATAGGGAGACTCTTCCGCCATCGCTCT3'	94 °C - 150s, 14 cycles: 94 °C-45s, 64 °C-45s, 72 °C-90s, 23 cycles: 94 °C-45s, 61 °C-45s, 72 °C-90s.
pCAT639	F:5'AGGGACGACGGTCATATGGGCAAC3'; R:5'CCTTCGTCCAAGCTTCAGGCCGT3'	94 °C-150s, 14 cycles: 94 °C-45s, 66 °C-45s, 72 °C-90s, 23cycles: 94 °C-45s, 63 °C-45s, 72 °C-90s.

The presence of aminoglycoside, amphenicole and β-lactam modification enzymes genes in phytopathogens genome was studied via PCR analysis. The genes of the following enzymes (which are widespread in representatives of the same genera, isolated form clinical samples) were identified: *aph(3')IV, aac(6')II, catB7, blaOXA-10.* The gene *blaOXA-10* is responsible for synthesis of to β-lactamase OXA-10, the gene *catB7* is responsible for chloramphenicol acetyltransferase CATB7. The genes *aph(3')IV* and *aac(6')II* are responsible for synthesis of enzymes of aminoglycoside antibiotics: modification: aminoglycoside N-acetyltransferase AAC and O-phosphotransferase APH, respectively.

The resistance transmission study

The transformation was carried out by the method of obtaining of chemically competent cells, according to Mandel, using low-temperature centrifugation in presence of calcium chloride, with a modification developed in the laboratory of ecological safety of the SPC 'Armbiotechnology', NAS RA (Fig. 3) (Babayan et al., 2020a, 2020b).

Refreshing of 3 mL overnight culture of sensitive strain refreshing
(Titer of overnight culture $1*10^8$ cells)
(1:50 dilution, 37 °C)
↓
Centrifugation of 1.5 mL refreshed overnight culture (4,500 rpm, 5 min, -7 ÷ -4 °C)
↓
Resuspension of sediment in (5 mL sterile 0.1M - $CaCl_2$, 5 min, 0 °C)
↓
Centrifugation (4,500 rpm, 5 min, -7 ÷ -4 °C)
↓
Resuspension of sediment in (5 mL sterile 0.1 M - $CaCl_2$)
↓
Direct use of obtained competent cells or cells conservation
(in 40% Vol. sterile absolute glycerin, at -70 ÷ -40 °C)
↓
50 µl of competent cells of a sensitive recipient strain add to 5 µl
of plasmid DNA (DNA_p), isolated form the resistant donor strain
↓
Incubation (20–40 min, 0 °C)
↓
Heating (up to 42 °C within 5 min)
↓
Incubation (1 min, 42 °C)
↓
Cell spatuling on selective medium (50 µg mL^{-1} antibiotic, 24 h, 37 °C)
↓
Cultivations series of transformants on selective media, which contains antibiotics to which the donor strain is resistant, with alternating cultivation on non-selective media
↓
Analysis of plasmid replication stability in transformants, after the multiple cultivations on non-selective media, through the replica

Figure 3. Scheme of transformation of strains, sensitive to TA derivatives by plasmids, isolated from strains, resistant to these compounds.

Statistical assessment

All the experiments were carries out in 5 series of 3 repeats for each probe. MS Excel was used for data analysis of TA synthetic derivatives antimicrobial effect evaluation. The data of growth inhibition zones are given in mm (Fig. 4–6), SEM (Standard Error of the Mean) were ± 0.23–0.37. Significance was tested by applying Student *t*-test and estimated as *p*-value less than 0.05.

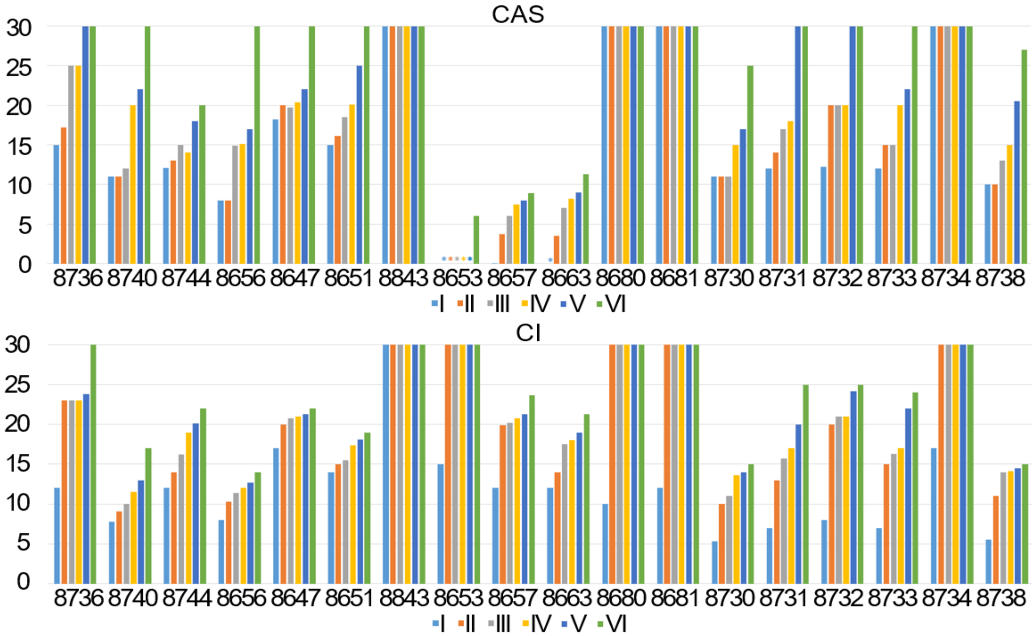

Figure 4. Antimicrobial effect of Cyclohexylimide (CI) and Cyclohexyl amine complex salt (CAS) of TA against *Pseudomonas* and *Xanthomonas* different strains. Concentrations: I – 0.001M; II – 0.01M; III – 0.025M; IV – 0.05M; V – 0.1M; VI – 0.5M; ‘*’ – absence of effect. The values of inhibition zones are presented in mm and are average means of 15 independent experiments.

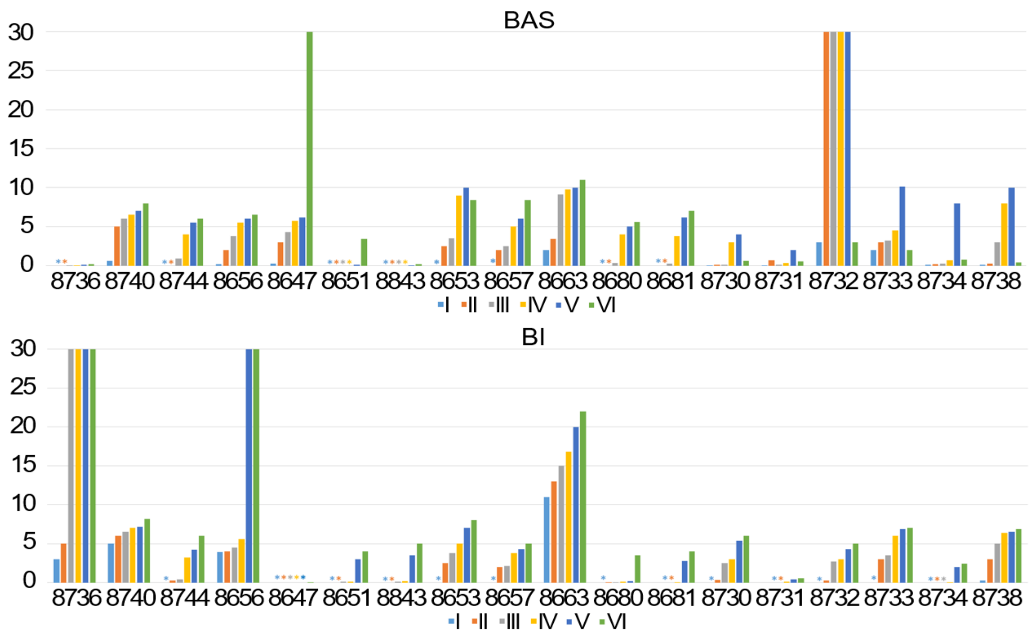

Figure 5. Antimicrobial effect of Benzylimide (BI) and Benzyl amine salt (BAS) of TA against *Pseudomonas* and *Xanthomonas* different strains. Concentrations: I – 0.001M; II – 0.01M; III – 0.025M; IV – 0.05M; V – 0.1M; VI – 0.5M; ‘*’ – absence of effect. The values of inhibition zones are presented in mm and are average means of 15 independent experiments.

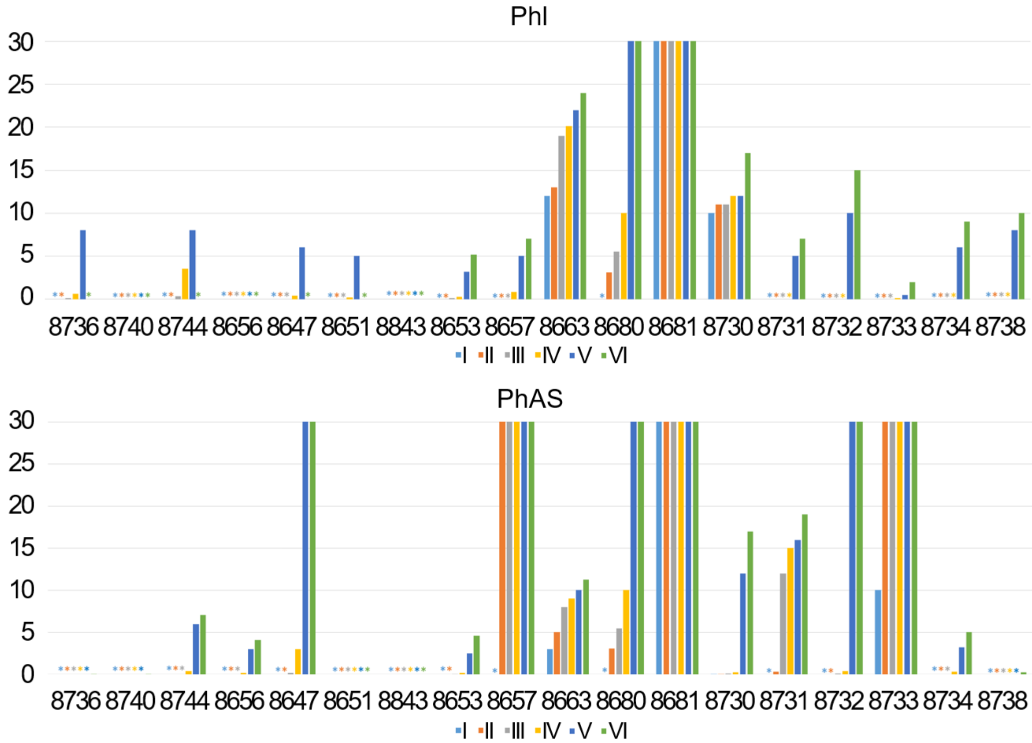

Figure 6. Antimicrobial effect of Phenylimide (PhI) and Phenyl amine complex salt (PhAS) of TA against *Pseudomonas* and *Xanthomonas* different strains. Concentrations: I – 0.001M; II – 0.01M; III – 0.025M; IV – 0.05M; V – 0.1M; VI – 0.5M; '*' – absence of effect. The values of inhibition zones are presented in mm and are average means of 15 independent experiments.

RESULTS AND DISCUSSION

The data obtained during the study of antibiotic resistance of group of phytopathogenic bacteria of various *Pseudomonas* and *Xanthomonas* species are summarized in Table 2. The resistance study of *X. vesicatoria* and *P. syringae* 19 native soil strains to 13 antibiotics of different classes and generations (including antibiotics of the third generation, such as ceftriaxone) revealed a wide diversity of resistance diapason in most strains. The presence of pan-drug resistant and multi-drug resistant representatives was noted.

Table 2 shows that majority of the studied strains are resistant to various antibiotics, including tetracycline and streptomycin, which are widely used in agriculture (Adam et al., 2008). In subsequent experiments, the studied bacterial strains were tested for the effect of various derivatives of TA on solid cultural media, what is presented at figures 4–6 (the detailed method is given in materials and methods).

Table 2. Resistance of phytopathogenic *Pseudomonas* and *Xanthomonas* from the National Collection of Microorganisms of MDC, SPC 'Armbiotechnology' NAS RA. Species name (A – *P. syringae, pv. lachrymans*, B – *P. syringae, pv. syringae*, C – *X. vesicatoria, D – P. syringae, pv. tabaci, E – X. beticola*), 50 μg mL^{-1} antibiotic content: 1 – Kan, 2 – Stp, 3 – Gnc, 4 – Cam, 5 – Amc, 6 – Amx, 7 – Amp, 8 – Pcn, 9 – Cfx, 10 – Cro, 11 – Tcn, 12 – Azm, 13 – Cip; control – positive control on a full-fledged cultivation environment; '+' – growth, '-' – inhibition of growth, '- *'- single colonies after the third day of cultivation

Strain		Antibiotic resistance													Control
Phytopathogen		1	2	3	4	5	6	7	8	9	10	11	12	13	
8730	A	-	-	-	-	+	+	+	+	+	+	-	+	+	+
8731		-	-	-	+	+	+	+	-	-*	+	-	-	+	+
8732		-	-	-*	-	-	+	-	-	+	+	-	-	-	+
8733		+	+	-	+	+	+	+	+	+	+	-	-	-	+
8734		-*	-	-	-	-	+	-	+	+	+	-	-	-	+
8742		+	+	+	-	-	+	-	+	+	+	+	+	+	+
8738		+	+	+	+	+	+	-	+	+	+	+	+	+	+
8736	B	-	-	+	-	+	+	+	+	+	+	+	-	+	+
8740		+	+	+	+	-	+	+	+	+	+	+	+	+	+
8744		-	-	-	-	+	+	+	+	+	+	+	+	-	+
8647		+	-	+	-	+	+	+	+	+	+	+	+	-	+
8843		-	+	-	-	-	-	+	+	-	-	+	-	-	+
8651	C	-	-	-	-	-	+	-	-	-*	-*	+	-	-*	+
8653		-	-	-	-	+	-*	-	-	-*	-*	-*	+	-*	+
8657	D	+	-	+	-	+	+	+	+	+	+	+	-	-	+
8656		+	+	-*	+	+	+	-	+	+	+	+	+	+	+
8663	E	+	+	+	+	-	+	-	+	+	+	+	+	+	+
8680		-	-	-	-	-*	-*	-	-*	-*	-	-	-	-	+
8681		-	-	-	-	-	-*	-	-	-*	-	-	-	-	+

Antibiotic resistance tests were carried out in 5 series of 3 repeats of each particular strain of bacteria. Antimicrobial activity was detected by the evaluation of growth inhibition on media. For all the tested compounds, the results of measurements of inhibition zones were considered as average of at least fifteen independent experiments for each bacterial strain. Significance was considered at $p < 0.05$.

For strains *P. syringae, pv. tabaci* 8657, *X. beticola 8680, 8681, P. syringae 8733* effects of phenyl-substituted complex amine salts were higher than in case of phenylimide ($p < 0.01$). In case of benzyl- functional group containing complex amine salts, the effect was higher then in case of imide form for strains *P. syringae pv. lachrymans 8736, P. syringae, pv. tabaci 8656* ($p < 0.01$), while for strains *P. syringae pv. lachrymans 8736, 8740, 8744*, *P. syringae, pv. tabaci 8656* the imide was more effective growth inhibitor, then the appropriate complex amine salt. For both cyclohexyl- functional group substituted derivatives there were noted the comparable high effects of bacterial growth inhibition for the majority of *Pseudomonas* and *Xanthomonas* studied strains of different species ($p < 0.01$), while for the strains *X. beticola 8653, 8657, 8663 the effect of imide was higher than in case of complex amine salt* ($p < 0.05$). *Due to the results, the antimicrobial effect of TA synthetic derivatives is a function from both substituent chemical nature and individual genetical features of each particular strain. That is why the genetical analyses of antimicrobial resistance mechanisms were carried out.*

According to the observations data, the effective concentrations of cyclohexyl derivatives are lower than the concentrations of benzyl- and phenyl- derivatives both for imides and amine complex salts. According to the results, the minimum inhibitory concentrations (MIC) of tested compounds are differing depends to strain of bacteria (Table 3).

Probably, this is due to the differences in bioavailability of salt and imide forms of these compounds and due to the species-specific features of the permeability systems of bacterial cells. This also might be explained by the affinity of cyclohexyl- (CAS and CI) derivatives to certain species-specific membrane proteins of *Pseudomonas* representatives, what was observed via docking analyses of analogues compounds, had been shown in earlier research (Chang et al., 2015).

Table 3. Minimum Inhibitory Concentrations (MIC) of TA new derivatives estimated for *P. syringae, pv. tabaci 8736* and *X. beticola 8681*

TA derivative	Minimum Inhibitory Concentrations (MIC)	
	P. syringae, pv. syringae 8736	*X. beticola 8681*
BI	(0.001M) 6.6 mcg mL^{-1}	(0.0001M) 66 ng mL^{-1}
BAS	(0.5M) 3.84 mg mL^{-1}	(0.0001M) 76 ng mL^{-1}
CAS	(0.0001M) 74.7 ng mL^{-1}	(0.001M) 7.47 mcg mL^{-1}
CI	(0.0001M) 31.8 ng mL^{-1}	(0.0001M) 31.8 ng mL^{-1}
PHI	(0.022M) 50 mcg mL^{-1}	(0.001M) 6.18 mcg mL^{-1}
PhAS	(0.01M) 618 mcg mL^{-1}	(0.001M) 7.29 mcg mL^{-1}

During the genetic analyses the presence of plasmids and genes of antibiotic modification enzymes was studied, as well as their localization and grade of transmission ability by the interspecies gene horizontal transfer under selective and non-selective conditions. Studies have shown that some of strains contain plasmids that differ in molecular weight and are capable to transmit antibiotic resistance. The other part of strains has plasmids that are not responsible for transferring of aminoglycosides and β-lactam resistance genes. There are also strains in which no plasmids have been found. For all the studied strains, plasmids with an ability of transmission of resistance to any TA synthetic derivatives were not detected (Fig. 7).

Figure 7. Electrophoretic study of DNA of various phytopathogenic *Pseudomonas* and *Xanthomonas* from the National Collection of Microorganisms of MDC, SPC ‘Armbiotechnology’ NAS RA on 0.8% – 2.5% of agarose gel. 1 – *P. syringae path. lachrymans 8732*; 2 – *X. vesicatoria 8647*; 3 – *P. syringae, path. syringae 8736*; 4 – *X. beticola 8680*; 5 – *P. syringae, path. tabaci 8663*; 6 – *P. syringae, path. tabaci 8665*; 7 – *S. maltophilia 9286*; 8 – *X. beticola 8681*.

The results of the analysis comparing the different genetic properties of *Pseudomonas* and *Xanthomonas* with their resistance to antibiotics of various classes and generations are listed in Table 4. The presence of plasmids, their ability of antibiotic resistance transmission and its stability in the case of various recipients were studied using appropriate selective media.

Microbiological experiments have shown that in studied group of *Pseudomonas* and *Xanthomonas*, the resistant representatives prevail, most of which are multi-drug resistant or pan-drug resistant. Antibiotic sensitive strains are in the minority, or show lagging growth on an appropriate selective media. It should be noted the high stability of resistance in this group of strains, which were deposited and cultivated on nutrient media without contact with any antimicrobial compound. That resistance maintains as a result of both nucleoid and plasmid genes. Possibly this is caused by the presence of genes of primary metabolism enzymes on their plasmids, what determines the stability of the plasmids under non-selective conditions of cultivation. In that aspect, the strains *X. vesicatoria 8656* and *P. syringae 8740*, which demonstrate the resistance to almost all the used antibiotics, are of particular interest.

Table 4. Genetic properties of plasmids of various phytopathogenic *Pseudomonas* and *Xanthomonas*. I – *X. beticola*, II – *P. syringae, pathovar tabaci*, III – *P. syringae, pv. lachrymans*, IV – P. *syringae, pv. syringae*, V – *X. vesicatoria*, '+' – the presence of a feature, '-' – the absence of a feature

Strain of Bacteria		Plasmid content	Ability to transfer the genes of resistance to 13 studied antibiotics	Ability to transfer the genes of resistance to TA derivatives
I	*8680*	-	-	-
	8681	-	-	-
	8663	+	+	-
II	*8656*	+	+	-
	8657	+	+	-
III	*8730*	+	+	-
	8731	+	+	-
	8732	-	-	-
	8733	+	+	-
	8734	-	-	-
	8738	+	+	-
	8742	+	+	-
IV	*8740*	+	+	-
	8744	+	+	-
	8736	+	+	-
V	*8843*	+	-	-
	8651	+	-	-
	8653	+	-	-
	8647	+	-	-

The results of PCR analysis had shown the *blaOXA-10* gene was found in *P. syringae 8740*. According to the negative results of transformation of sensitive strains *P. aeruginosa 9056*, *E. coli DH5α* (not containing plasmids), *X. vesicatoria 8647* (according to this study, the plasmids of this phytopathogenic microorganism do not transfer resistance to the given 13 antibiotics) by plasmids of the given strain, the *blaOXA-10* gene is located on the bacterial chromosome of the particular microorganism. Plasmids were also found in *P. syringae 8736, 8656, 8740* and in *X. vesicatoria 8647* by gel electrophoresis and transformation. Some of them are not related to resistance (*P. syringae 8740*) to the studied antibiotics, while the rest of them carry genes of resistance to β-lactam antibiotics. According to the transformation experiments, resistance to aminoglycoside and amphenicole antibiotics in all studied strains of *P. syringae* and *X. vesicatoria* is represented by nucleoid genes (Niu et al., 2015). All the studied strains, which contains the appropriate plasmids are able to transmit antibiotic resistance and are demonstrating stable replication of the particular inserted plasmids, in case of *Pseudomonas* recipients, as well as *Stenotrophomonas* *maltophilia* (belonging to the same *Xanthomonadaceae* family) recipient. However, in case of *Xanthomonas* and *E. coli* recipients, stability was not observed.

In subsequent experiments it was observed that the effect of TA derivatives does not depend to content of plasmids. Both investigated phenyl- derivatives of TA (PhAS and PhI) don't have an effect on strains containing the *blaOXA-10* gene, while benzyl- and cyclohexyl- derivatives (BAS, CAS, BI, CI) are active against all studied bacteria. However, in contrast to the Tcn-resistant *P. chlororaphis* strains, the growth of similar *P. syringae* and *X. beticola* representatives is suppressed by benzyl- and cyclohexyl- complex salts and TA imides.

Perhaps this is related to differences in the substrate specificity of polyphenol oxidases and other enzymes of Tcn-resistance, which are encoded by the nucleoid, as the characteristics of a particular bacterial species (Sultan et al., 2018). Our studies have shown that the most of *P. syringae* representatives have plasmids, which are responsible for β-lactams resistance transmission. No plasmids were found in *Xanthomonas* representatives. Genes *aph(3')IV, blaOXA-10, aac(6')II,* and *catB7* were also not found. The resistance of these strains is caused by other plasmid and nucleoid encoded genes. Literature data indicate a wide variety of similar genes and their localization in *Xanthomonas* genome (Tamir-Ariel et al., 2007). During the cultivation of transformants constructed on the basis of sensitive strains and plasmids isolated from resistant strains, the stable replication of plasmids on non-selective media was revealed for *X. beticola*, *P. syringe* plasmids in *P. aeruginosa 9056* recipients. In contrast to it, the replication of plasmids in *E. coli DH5α* is stabile only on selective media containing the corresponding antibiotic, the modification gene of which is located on the plasmid.

CONCLUSIONS

Antibacterial effect of TA new derivatives was studied on 19 model strains of phytopathogens from *Xanthomonas beticola (6 strains), X. vesicatoria and Pseudomonas syringae (13 strains)*, isolated form soil and different in their sensitivity to antibiotics. New synthetic derivatives of TA have demonstrated effect against the range of phytopathogenic bacteria of *Pseudomonas* and *Xanthomonas* genera. Cyclohexyl- and benzyl- imides and complex salts of TA are more effective than phenyl-derivatives. The effect of the discussed TA derivatives on resistant strains *P. syringae*, *X. beticola* and *X. vesicatoria* species does not correlate with the presence of plasmids and genes of β-lactam, amphenicol and aminoglycoside modification enzymes in genome. Thus, the mechanisms of effect of the discussed compounds are related with another molecular targets in cells. And the impossibility of transfer of resistance to TA all the discussed derivatives by plasmids was demonstrated. It makes these substances recommended for other further research for the elaboration of new classes of potentially ecologically safe anti-phytopathogenic drugs for crop and garden trees protection. For that aim, more detailed *in silico, in vitro*, *in vivo* eco-toxicological research are under evaluation. And further more detailed research of the discussed substances effects on bacteria, as well as antimicrobial resistance mechanisms features influence studies needed as a next step of research.

REFERENCES

Adam, J., Kriz, Z., Prokop, M., Wimmerova, M. & Koca, J. 2008. In Silico Mutagenesis and Docking Studies of Pseudomonas aeruginosa PA-IIL Lectin-Predicting Binding Modes and Energies. *Journal of chemical information and modeling* **48**(11), 2234–2242.

Ageyets, V.Y., Mikaelyan, A.R., Koshak, Z.V. & Babayan, B.G. 2019. Ways to improve efficiency of compound feed for fish. *Proceedings of the National Academy of Sciences of Belarus, Agrarian Series* **57**(3), 323–333.

Andrews, M.A., Figuly, G.D., Chapman, J.S., Hunt, T.W., Glunt, C.D., Rivenbark, J.A. & Chenault, H.K. 2011. Antimicrobial hydrogels formed by crosslinking polyallylamine with aldaric acid derivatives. *Journal of Applied Polymer Science* **119**(6), 3244–3252.

Andrews, J.M. 2001. Determination of minimum inhibitory concentrations. *Journal of antimicrobial Chemotherapy* **48**(1), 5–16.

Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., Nisar, M.A., Alvi, R.F., Aslam, M.A., Qamar, M.U., Salamat, M.K.F. & Baloch, Z. 2018. Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance* **11**, 1645–1658.

Babayan, B.G., Mikaelyan, A.R., Asatryan, N.L., Bagdasaryan, S.A. & Melkumyan, M.A. 2020a. The effect of tartaric acid new derivatives against the multidrug resistant opportunistic pathogenic soil strains of pseudomonas fluorescens. *Test Engineering and Management* **83**(5–6), 8516–8521.

Babayan, B., Mikaelyan, A., Asatryan, N., Soghomonyan, T., Baghdasaryan, A., Melkumyan, M., Bagdasaryan, S. & Grigoryan, A. 2020b. Tartaric Acid Synthetic Derivatives for Multi–Drug Resistant Phytopathogen Pseudomonas and Xanthomonas Combating. *International Journal of Sciences: Basic and Applied Research (IJSBAR)* **52**(1), 21–30.

Bagdasaryan, S., Babayan, B., Melkumyan, M. & Kinosyan, M. 2020. Polysorbates Biodegradation Potential and Plasmid Stability of Soil Pseudomonas. *Austrian Journal of Technical and Natural Sciences* **5–6**, 3–7.

Baghdasaryan, A.S., Soghomonyan, T.M., Mikaelyan, A.R., Asatryan, N.L., Babayan, B.G., Bagdasaryan, S.A. & Melkumyan, M.A. 2019. Tartaric Acid New Derivatives Antibacterial Activity and Biodegradation by Non-Pathogenic Soil Strains Of P. chlororaphis. In *Book of Abstracts 'Microbes: Biology and Application'*, pp 20.

Cartron, M.L., England, S.R., Chiriac, A.I., Josten, M., Turner, R., Rauter, Y., Hurd, A., Sahl, H.-G., Jones, S. & Foster, S.J. 2014. Bactericidal Activity of the Human Skin Fatty Acid cis-6-Hexadecanoic *Acid on Staphylococcus aureus. Antimicrobial agents and chemotherapy* **58**(7), 3599–3609.

Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M. & Hanage, W.P. 2015. Antibiotics in agriculture and the risk to human health: how worried should we be? *Evolutionary applications* **8**(3), 240–247.

Dashchyan, N.A., Asatryan, N.L., Galstyan, G.F. & Mikaelyan, A.R. 2015. Obtaining Bioactive Additives of Cyclic Structure on the Basis of Optically Active Tartaric Acid. *Bulletin of NPUA, Collection of scientific papers, part II*, 682–688.

D'Mello, J.P.F. 2003. *Food Safety: Contaminants and Toxins*. CABI publishing, Wallingford. doi: 10.1079/9780851996073.0000

Dolejska, M. & Literak, I. 2019. Wildlife is overlooked in the epidemiology of medically important antibiotic-resistant bacteria. *Antimicrobial agents and chemotherapy* **63**(8), e01167–19.

Dumas, J.L., Van Delden, C., Perron, K. & Köhler, T. 2006. Analysis of antibiotic resistance gene expression in Pseudomonas aeruginosa by quantitative real-time-PCR. *FEMS microbiology letters* **254**(2), 217–225.

Eswaranandam, S., Hettiarachchy, N.S. & Johnson, M.G. 2004. Antimicrobial activity of citric, lactic, malic, or tartaric acids and nisin-incorporated soy protein film against Listeria monocytogenes, Escherichia coli O157: H7, and Salmonella gaminara. *Journal of Food Science* **69**(3), 79–84.

Fricke, Y., Kopp, N. & Wünsch, B. 2010. Synthesis of Iminodiacetaldehyde Derivatives as Building Blocks for Pharmacologically Active Agents. *Synthesis* **2010**(5), 791–796.

Granados-Chinchilla, F. & Rodríguez, C. 2017. Tetracyclines in food and feedingstuffs: from regulation to analytical methods, bacterial resistance, and environmental and health implications. *Journal of Analytical Methods in Chemistry* **2017**, 1315–1317.

Kumari, C.M. & Jaisankar, V. 2017. A comparative study on antimicrobial properties of certain polymeric nanocomposites. *International Journal of Current Research* **9**(8), 56325–56329.

Landers, T.F., Cohen, B., Wittum, T.E. & Larson, E.L. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. *Public health reports* **127**(1), 4–22.

Liang, C., Gao, W., Ge, T., Tan, X., Wang, J., Liu, H., Wang, Y., Han, C., Xu, Q. & Wang, Q. 2021. Lauric Acid Is a Potent Biological Control Agent That Damages the Cell Membrane of Phytophthora sojae. *Frontiers in Microbiology* **12**, 666–761.

Livermore, D.M. 2021. Antibiotic resistance during and beyond COVID-19. *JAC-Antimicrobial Resistance* **3**(1), 5–16.

Martinez, E., Sahni, S., Cheema, M.A. & Iftikhar, A. 2018. Vancomycin-induced coronary artery spasm: a case of Kounis syndrome. *Case Reports* 2018, bcr-2017.

Melaku, M., Zhong, R., Han, H., Wan, F., Yi, B. & Zhang, H. 2021. Butyric and Citric Acids and Their Salts in Poultry Nutrition: Effects on Gut Health and Intestinal Microbiota. *International Journal of Molecular Sciences* **22**(19), 10392.

Mikaelyan, A.R., Asatryan, L.N., Bagdasaryan, S.A. & Babayan, B.G. 2019. *Antimicrobial Activity of Newly Synthesized Derivatives of TA Against the Multidrug Resistant Soil Strains of Pseudomonas and Stenotrophomonas*. Abstract Book of ARICON at Cambridge, Cambridge, UK, pp 37.

Morris, C.E., Kinkel, L.L., Xiao, K., Prior, P. & Sands, D.C. 2007. Surprising niche for the plant pathogen Pseudomonas syringae. *Infection, Genetics and Evolution* **7**(1), 84–92.

Monteil, C.L., Yahara, K., Studholme, D.J., Mageiros, L., Méric, G., Swingle, B., Morris, C.E., Vinatzer, B.A. & Sheppard, S.K. 2016. Population-genomic insights into emergence, crop adaptation and dissemination of Pseudomonas syringae pathogens. *Microbial genomics* **2**(10), e000089.

Niu, Xi.N., Wei, Zh. Q., Zou, H.F., Xie, G.G., Wu, F., Li, K.-J., Jiang, W., Ji-Liang, T. & He, Y.Q. 2015. Complete sequence and detailed analysis of the first indigenous plasmid from Xanthomonas oryzae pv. oryzicola. *BMC microbiology* **15**(1), 1–15.

Rahman, S. & Das, A.K. 2021. Integrated Multi-omics, Virtual Screening and Molecular Docking Analysis of Methicillin-Resistant Staphylococcus aureus USA300 for the Identification of Potential Therapeutic Targets: An In-Silico Approach. *International journal of peptide research and therapeutics* **17**, 1–21.

Santi, I., Manfredi, P., Maffei, E., Egli, A. & Jenal, U. 2021. Evolution of Antibiotic Tolerance Shapes Resistance Development in Chronic Pseudomonas aeruginosa Infections. *Mbio* **12**(1), e03482–20.

Sultan, I., Rahman, S., Jan, A.T., Siddiqui, M.T., Mondal, A.H. & Haq, Q.M.R. 2018. Antibiotics, resistome and resistance mechanisms: a bacterial perspective. *Frontiers in microbiology* **9**, 2066.

Tamir-Ariel, D., Navon, N. & Burdman, S. 2007. Identification of genes in Xanthomonas campestris pv. vesicatoria induced during its interaction with tomato. *Journal of bacteriology* **189**(17), 6359–6371.

Thean, R.K.R., Ong, D.X.Y., Heng, Z.S.L., Gan, S.K.E. & Yeo, J.Y. 2021. To Plate or to Simply Unfreeze, That Is the Question for Optimal Plasmid Extraction. *Journal of Biomolecular Techniques* **32**(2), 57–62.

Totten, P.A., Amsel, R., Hale, J., Piot, P. & Holmes, K.K. 1982. Selective differential human blood bilayer media for isolation of Gardnerella (Haemophilus) vaginalis. *Journal of Clinical Microbiology* **15**(1), 141–147.

ThermoFisher 2008. *Thermo Fisher manuals/IFU1478*. Available at https://tools.thermofisher.com/content/sfs/manuals/IFU1478.pdf

Vidaver, A.K. 2002. Uses of antimicrobials in plant agriculture. *Clinical infectious diseases* **34**(3), 107–110.

World Health Organization 2019. *Follow-up to the high-level meetings of the United Nations General Assembly on health-related issues*. Available at https://apps.who.int/gb/ebwha/pdf_files/WHA72/A72_18-en.pdf

Yoshimoto, K., Takano, Y. & Sakai, Y. 2010. Autophagy in plants and phytopathogens. *FEBS letters* **584**(7), 1350–1358.

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Effect of foliar products on the inflorescence yield of lavender and essential oil

N. Minev[1,*], A. Matev[1], N. Yordanova[1], I. Milanov[1], M. Sabeva[2] and M. Almaliev[1]

[1]Agricultural University - Plovdiv, 12 Mendeleev street, BL4000 Plovdiv, Bulgaria
[2]Agricultural Academy - Sofia, Institute of Plant Genetic Resources 'K. Malkov', 2 Drujba street, BL4122 Sadovo, Bulgaria
[*]Correspondence: nikiminev@abv.bg

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Abstract. The topic of the effect of foliar fertilization on the productivity and oil content of lavender is relevant, but not sufficiently studied. The present study aims to establish the effect of foliar products on the growth, development and productivity of lavender. The field experiment was carried out at the Agricultural University - Plovdiv with lavender of 'Jubileina' variety during 2019–2020. The following variants were included in the study: 1. Untreated control; 2. Treatment with Fertileader Gold (FG) - 3 L ha^{-1}; 3. Treatment with Fertiactyl Trium + Fertileader Vital (FT + FVital) - 1.5 + 1.5 L ha^{-1}; 4. Treatment with Fertileader Viti (FViti) - 3 L ha^{-1}; 5. Treatment with Fertileader Vital (FV) - 3 L ha^{-1}; 6. Treatment with Fertileader Alpha (FA) - 3 L ha^{-1}. Those preparations are bio stimulants for foliar application. The treatments were made in two consecutive lavender vegetation seasons. The first application was carried out in the second growing season (2019) and the second in the next, third growing season (2020). The foliar application of all tested products increased the photosynthetic activity, but it was better expressed when using the plant nutrition products FV, FViti and FT + FVital. A positive effect was also observed in the height and diameter of the bush, but during the third vegetation period. The number of flowering stems increased by 62.9%; 59.4%; 53.3% and 8.4%, respectively, when applying the fertilizers FG, FT + FVital, FViti and FV. The application of FG and FT + FVital increased the yield of fresh inflorescences by 6.1% and 3.7%. The application of the different products affected the oil yield in different ways; the application of FG, FT + FVital and FViti increased it, while FV and FA decreased it by 27 kg ha^{-1} and 16 kg ha^{-1}, respectively, for the first vegetation and by 43.4 kg $ha^{-1}$and 33.1 kg ha^{-1} for the second vegetation. The boron containing products FG, FT + FVital and FViti led to a significant increase in the essential oil yield, while the application of the foliar fertilizers FV and FA reduced it. Based on those results, the first three products are recommended.

Key words: medicinal crops, *Lavandula angustifolia*, foliar fertilization, flower yield, oil yield, oil content.

INTRODUCTION

The quantitative and qualitative parameters of lavender production (number of flowers and essential oil yield) are influenced by a complex set of factors, such as soil, climate, varietal composition, degree of flowering, duration of the distillation process, fertilizer application, etc. So far little is known about the factors that cause variations in lavender essential oil or control the monoterpene profile and abundance of monoterpenes.

Secondary metabolites, such as monoterpenes, perform various environmental functions and therefore they are regulated by the environmental factors. It was suggested that the released volatile substances can serve or support pollinators (Raguso & Pichersky, 1999). It was also found that monoterpenes acted as a barrier against heat stress (Velikova et al., 2006) and their radiation was regulated by the level of irradiation in some plant species (Staudt & Seufert, 1995).

Flowering is a factor influencing the quantitative and qualitative indicators of lavender essential oil (Konakchiev, 2015). The author established the crucial importance of the flowering stage on the quantitative and qualitative parameters of the extracted lavender oil. The duration of distillation process (DT) had also a significant effect on the quantity and quality of lavender oil.

The application of fertilizers is one of the most important factors determining plant productivity (Bardule et al., 2013; Almeida et al., 2015). Among the agronomic practices, nutrient management in oil-bearing crops affected most significantly the yield and quality of essential oils (Pal et al., 2016). The synthesis of essential oil in aromatic plants can be positively or negatively affected by the form, type and amount of fertilizers (Yadegari, 2015; Lamptey et al., 2017; Lukošiūtė et al., 2020). Nutrients such as nitrogen (N), phosphorus (P) and potassium (K) could affect the growth and synthesis of essential oil in aromatic plants and are used to build many organic compounds, such as amino acids, proteins, enzymes and nucleic acids. Those nutrients affect the function and levels of enzymes involved in terpenoid biosynthesis (Hafsi et al., 2014).

The application of N fertilizer reduced the essential oil content of creeping juniper (*Juniperus horizontalis* L.), (Robert & Francis, 1986), although an increase in the yield of Thyme essential oil (*Thymus vulgaris* L.) was established by Baranauskienne et al. (2003) and in cumin (*Cuminum cyminum* L.) by Azizi & Kahrizi (2008). Phosphorus plays an important role in various metabolic processes, such as a component of the nucleic acids, phospholipids, and coenzymes activating the production of amino acids used in the synthesis of proteins, DNA, RNA and ATP (Rouached et al., 2010). High levels of P lowered the essential oil yield of chamomile (*Matricaria chamomilla* L.), (Emonfor et al., 1990), but increased it in feverfew (*Tanacetum parthenium* L.), (Saharkhiz & Omidbaigi, 2008) and common sage (*Salvia officinalis* L.), (Nell et al., 2009).

Nurzynska-Wierdak (2013) found that the deficit of potassium can disrupt the metabolism of nitrogen, provoke changes in the N:K ratio and/or the proportions of nitrogen fractions, as well as the accumulation of harmful amino substances and ammonium ions in the plant. Chrysargyris et al. (2016) reported that the efficiency of N and P levels may be linked to the appropriate levels of K in plants, underscoring the need for further study. The application of K affected plant growth and essential oil yield of lemongrass (*Cymbopogon flexuosus*), ditania (*Origanum dictamnu*s), basil (*Ocimum basilicum*) and rosemary (*Rosmarinus officinalis*), (Economakis, 1993; Puttanna et al., 2010).

Currently, the use of chemical fertilizers in lavender is mainly limited to the application of N and P in soil, while the effect of other macro-and micronutrients has not been studied. There is also a lack of information regarding the influence of organic and organic-mineral fertilizer on lavender and there is no such information about the effect of foliar fertilizer on the quality and productivity of this important for Bulgaria essential oil crop.

The present study aims to establish the effect of foliar products on the growth, development and productivity of lavender.

MATERIALS AND METHODS

In the period 2019–2020, a field trial was carried out at the Agricultural University - Plovdiv to achieve the aim set. The experiment was conducted with lavender of 'Jubileyna' variety. The trial was carried out by the long plot method, in three replications, with the size of the experimental plot 140 m^2 and the harvest plot - 63 m^2. The field trial variants were: 1. Untreated control; 2. Treatment with Fertileader Gold (FG) - 3 L ha^{-1}; 3. Treatment with Fertiactyl Trium + Fertileader Vital (FT + FVital) - 1.5 + 1.5 L ha^{-1}; 4. Treatment with Fertileader Viti (FViti) - 3 L ha^{-1}; 5. Treatment with Fertileader Vital (FV) - 3 L ha^{-1}; 6. Treatment with Fertileader Alpha (FA) - 3 L ha^{-1}. The treatments were made in two consecutive lavender vegetation seasons, the first being carried out in the second growing year (2019) and the second - in the next third growing season (2020).

The following commercial products were used to test the effect of foliar fertilizers on lavender: 1) Fertileader Gold (FG), (5.7% B; 0.35% Mo); 2) Fertiactyl Trium (FT), (5% N; 5% P_2O_5; 7% K_2O; 1.5% Zn); 3) Fertileader Vital (FVital), (9% N; 5% P_2O_5; 4% K_2O; 0.02% Fe; 0.01% Mo; 0.05% Zn; 0.1% Mn; 0,05% B); 4) Fertileader Viti (FViti), (6% P_2O_5; 12% K_2O; 1% B); 5) Fertileader Alpha (FA), (5% N;13% P_2O_5; 2% B).

Soil characteristics

The soil in the experimental field is alluvial-meadow. Geographically the site is located in the Thracian-Strandja region. The alluvial-meadow soils were formed on sandy-loam and sandy-gravel quaternary deposits. According to the International Classification of FAO, they refer to Mollic Fluvisol. They were formed on alluvial deposits, they have a well-formed humus-accumulative horizon, which gradually passes into the C horizon and a gleization process is observed deeply down (below 100 cm) in the soil-forming material - the A-C-G profile. The humus content is usually no more than 1–2%.

The soil sample analysis made before setting the experiment for the c - 8.30 mg kg^{-1}; NO_3^- - 33.20 mg kg^{-1}; Total mineral nitrogen - 41.50 mg kg^{-1}; P_2O_5 - 282, mg kg^{-1}; K_2O - 560 mg kg^{-1}; CaO - 210.3 mg kg^{-1}; MgO - 53.0 mg kg^{-1}; MnO_4 - 210.0 mg kg^{-1}; pH (H_2O) - 7.6.

These data show that the soil has a slightly alkaline reaction, a low content of N and optimal levels of P_2O_5 and K_2O. Exchangeable calcium (CaO) and magnesium (MgO) were in amounts typical for the soil type. The total quantity of MnO_4 was also determined, which defines the soil as well-stocked concerning this element. However, because of the alkaline nature of the soil, mobility and accessibility for the plants are low. The analysis of the specific soil conditions showed that the soil type is particularly suitable for growing lavender (Stoyanova et al., 2009; Minev, 2020).

Plant material

Lavender cultivation began in November 2017 with certified seedlings of the Bulgarian 'Jubileyna' variety, following the conventional technology. The planting density was 20,000 per ha, with a row distance of 35 cm and an inter-row distance of 1.4 m. The variety used in the experiment was created by hybridization. It has rounded tufts, erect, up to 56 cm high, with about 460 flowering stems, dark purple in colour. The average yield of fresh flowers is 5,540 kg ha^{-1}, the essential oil content in the flowers averages 2% and the rate of yield is 52.8.

In both experimental years (in November) the granulated product TOP 34 (5% N; 19% P_2O_5; 10% K_2O; 19% SO_3; 0.1% Zn; 0.1% B) was applied to the entire experimental area in a dosage of 200 kg ha^{-1}, by incorporation into the soil. Spring fertilization with nitrogen was carried out with ammonium nitrate (NH_4NO_3) in a dose of 90 kg ha^{-1} N in the second growing season and 120 kg ha^{-1} N in the third. The foliar products were used in the respective doses in the lavender bud formation stage.

Plant samples

Plant samples were taken in the stage of the full flowering (95–100%). The fresh flowers were harvested by hand, weighed and steam distilled, for each experimental treatment separately. For this purpose, a specially adapted device with a capacity of 100 L and a Florentine vessel for separating the essential oil from the water was used. The distillation time (DT) was 1.5 hours for all variants of the experiment. In the phenological stage of full flowering, the following biometric indicators were determined: bush height (cm), bush diameter (cm), number of flowering stems, length of the inflorescence (cm) and number of flowering vertebrae.

Photosynthetic activity and Chlorophyll content

Photosynthetic activity (µmol per m^2 s^{-1}) for all variants of the experiment was determined a week after foliar treatment using a portable measuring system LCA-4, England. The chlorophyll content in lavender leaves (mg m^{-2}) was determined using a portable measuring device Chlorophyll meter MC-100, seven days after the application of the foliar products.

The software product ANOVA was used to process the data statistically.

RESULTS AND DISCUSSION

The quantity of precipitation during the winter months (November - February) of the first experimental year (Fig. 1) was close to the norm for the region, except for December, which was dry. During the months of the active growing season, the situation was different. The period March - May was relatively dry, while in June, when the lavender plants form the essential oil yield, precipitation significantly exceeded the norm determined based on data for a period of 30 years.

The winter months of the second experimental year (2019/2020) were characterized by a small amount of precipitation, which is a prerequisite for the emergence of a deficit of soil moisture. That had a negative effect on the process of fertilizer absorption, even though November was humid and largely neutralized the negative impact of the subsequent drought. The precipitations in the period March - June restored moisture in the one-meter soil layer, which had a positive effect on the growth and development of the crop. Precipitation above the norm was measured in March and April, while in February, March, May and June the quantity of precipitation was about the norm.

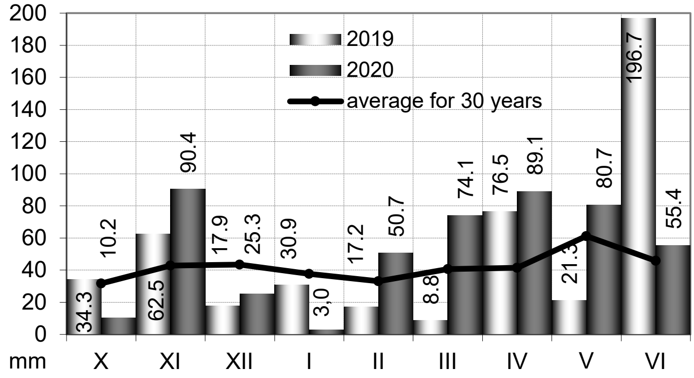

Figure 1. Amount of monthly precipitation (mm) during the study period.

In terms of air temperature, the period November - February in the first experimental year (2018–2019) was characterized by values close to the average for a long period (Fig. 2). February, which is significantly warmer month, was an exception. That was the reason for the earlier resumption of the vegetation of the plantation. The period of active vegetation was characterized by relatively warmer March and June, and, April and May were average in terms of temperature. In general, however, the temperature accumulated during the growing season did not significantly exceed that for the 30 year-period.

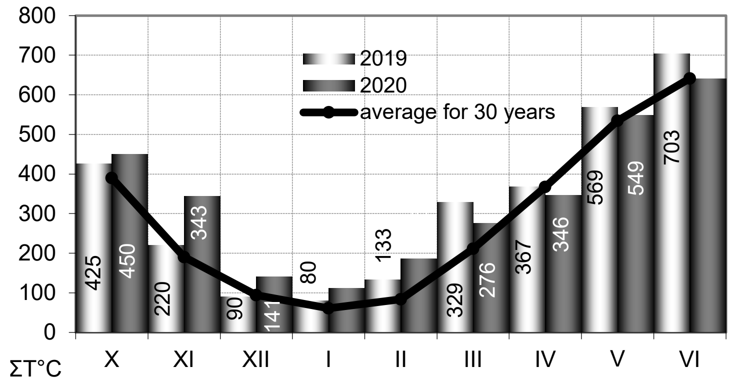

Figure 2. Average monthly temperatures(°C) during the study period.

The temperature values during most of the vegetation of 2019/2020 exceeded that of the long-term period. Exceptions were April and June, which were cooler. As a result, the beginning and the course of the budding and flowering stages were delayed.

Photosynthetic activity and Chlorophyll content

Foliar fertilization is an important agricultural practice worldwide due to the rapid and efficient absorption of the applied plant products (Lasa et al., 2012). It reduced costs and contributed to the sustainable development of organic farming (Fernández & Brown, 2013; Garde-Cerdán et al., 2015). The foliar application of the tested products led to an increase in the photosynthetic activity in all the variants of the experiment (Fig. 3). In the first experimental year (2019), the highest values compared to the control (values above 8 µmol per m^2 s^{-1}) were those treated with FV, FViti, FA and FT + FVital. During the second growing season (2020) the most stimulating activity in terms of this indicator was established in the variants treated with FT + FVital, FV and FViti. That could be due to the neutralizing effect of K_2O on abiotic stress caused by significant amounts of precipitation during flowering (Fig. 1). The application of foliar preparations in the budding stage had some effect on the chlorophyll content of lavender (Fig. 4). For the same reason, the increase in the FT + FVital and FViti variants was more significant in the second experimental year (1,603 and 1,316 mg m^{-2}, respectively) compared to the first.

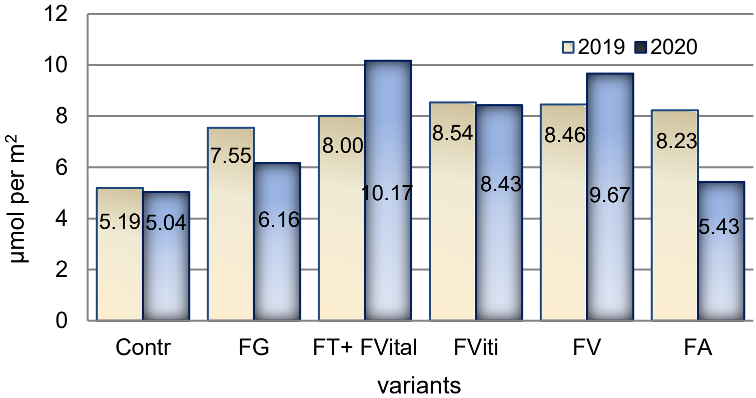

Figure 3. Photosynthetic activity (µmol/m^2s) of lavender 'Jubileyna' variety.

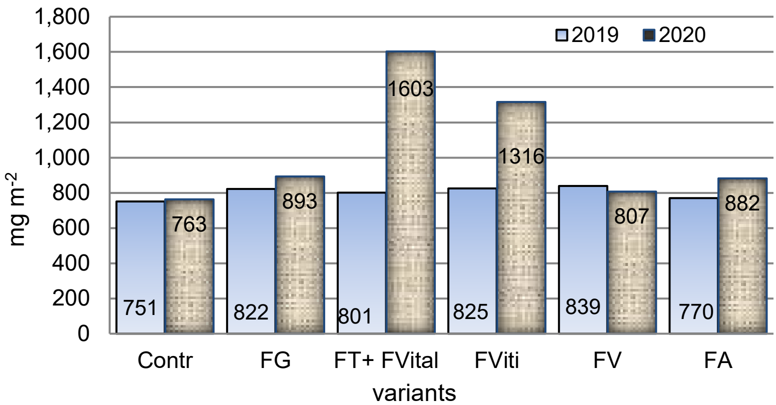

Figure 4. Chlorophyll content (mg m^{-2}) of lavender 'Jubileyna' variety.

Yield and yield components

Fig. 5 shows the data on the yield components of lavender. The bush height in the first year of the experiment ranged from 39.1 cm when applying FViti to 47.0 cm when applying FG. During the second experimental year, the values of the indicator increased significantly compared to the first, ranging from 69.2 cm in the 5[th] variant of foliar fertilization (FV) to 75.0 cm in the 3[rd] variant. The higher values during this vegetation were due to the larger size of the plants, which is an age specific feature. Foliar fertilization, with few exceptions, had a positive effect on the height of the bushes, compared to the control, which was statistically significant. Concerning bush diameter, the results were similar to the previous ones. Higher values of the indicator were reported in 2020 compared to 2019.

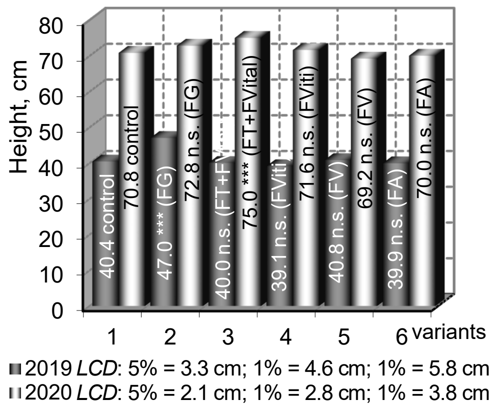

Figure 5, a. Yield components (bush height) of lavender 'Jubileyna' variety.

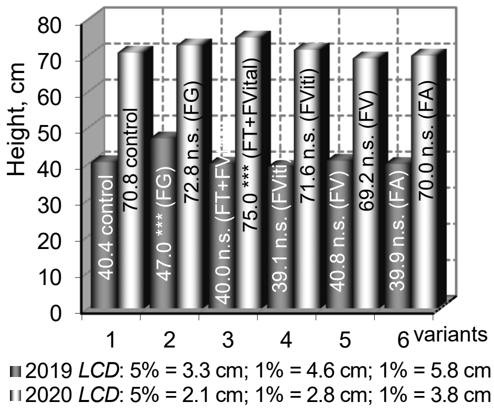

Figure 5, b. Yield components (bush diameter) of lavender 'Jubileyna' variety.

In the first year of the experiment, the largest bush diameter was found in the fertilization variant with FT + FVital, but in the second year - in the fertilization variant with FG, 74 cm and 124 cm, respectively.

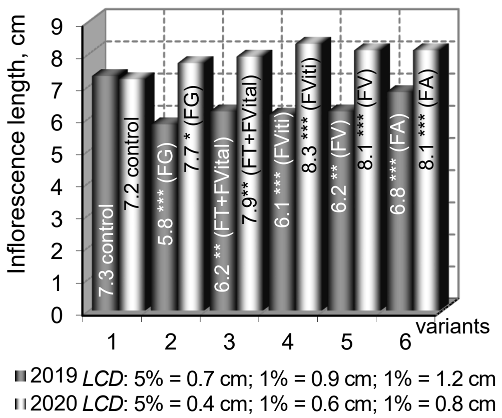

Figure 5, c. Yield components (inflorescence length) of lavender 'Jubileyna' variety.

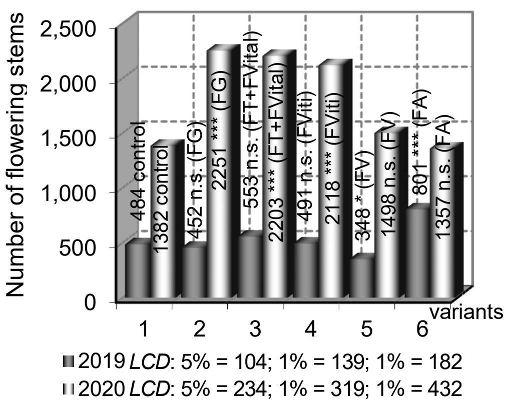

Figure 5, d. Yield components (number of flowering stems) of lavender 'Jubileyna' variety.

The foliar application of all tested preparations in 2020 significantly increased the bush diameter, compared to the untreated variant. The differences between the control and the variants treated with FG, FViti and FV were insignificant during 2019. This is most likely due to the after-effects of the foliar-applied products during the next lavender vegetation.The inflorescence length in the first experimental year was affected negatively by the applied foliar fertilization, and in all studied variants the results were lower compared to the control. A different trend was found in the second year of the study. The length of the inflorescence increased within 0.5–1.1 cm, compared to the unfertilized variant, which was statistically significant. The variants with the application of FV and FA had an influence on the values of this indicator. The application of FViti had the most significant effect on it (Fig. 5, c).

The application of foliar feeding in the second year of the experiment led to an increase in the inflorescence length in lavender by 7% to 15% in the different variants of foliar fertilization. The increase was statistically significant.

The number of flowering stems was significantly affected by the foliar feeding, with more significant differences in the second experimental year, when the values of the indicator ranged from 1,357 in the 6th fertilization variant (FA) to 2,251 in the 2nd variant (FG). Compared to the control, the number of flowering stems increased by 62.9%; 59.4% and 53.3%, respectively, when fertilizing with FG, FT + FVital and FViti. The differences between the control and treated with FV and FA variants were insignificant. This is most likely due to the nitrogen content in these products. Regarding the number of flowering vertebrae, similar trends were found. Higher values of the indicator were found in the second experimental year compared to the first and the positive influence of the applied leaf fertilizers was established, the differences being statistically significant.

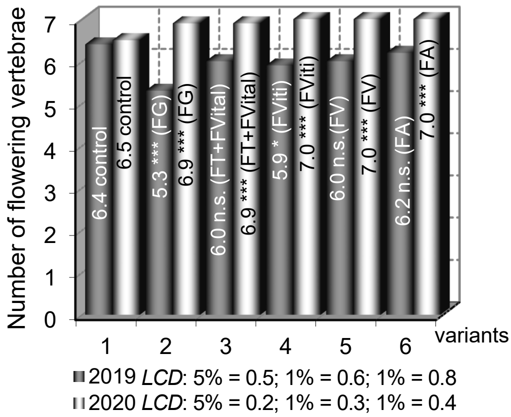

Figure 5, e. Yield components (number of flowering vertebrae) of lavender 'Jubileyna' variety.

The yield of fresh inflorescences (Table 1) in the first year of the study varied from 5,892 kg ha^{-1} for the 4th fertilization variant (FViti) to 6,659 kg ha^{-1} for the 6th variant (FA). Statistically significant differences in lavender productivity were not found only between the control and FV variant.

A significant difference in the values of the indicator was found in all the other products for foliar feeding, compared to the untreated variant. The yield of fresh inflorescences increased significantly compared to the control - by 450 kg ha^{-1} when fertilizing with FA, which is 7.2% more, and, decreased when fertilizing with FViti and FV by 317 kg ha^{-1} and 55 kg ha^{-1}, respectively. In 2020, the difference in the yield of fresh inflorescences between the control and FViti, FV and FA variants was significant. It was negative. The application of FG and FT + FVital foliar fertilizers increased the productivity of the crop by 6.1% and 3.7%, compared to the control, which was statistically significant.

Table 1. The yield of fresh lavender inflorescences

Variants	2019				2020			
	kg ha^{-1}	± kg ha^{-1}	% to the control	Significance	kg ha^{-1}	± kg ha^{-1}	% to the control	Significance
control	6,209	0.0	100.0	control	9944	0.0	100.0	control
FG	6,330	121.0	101.9	*	10547	603.0	106.1	**
FT + FVital	6,556	347.0	105.6	***	10313	369.0	103.7	*
FViti	5,892	-317.0	94.9	***	8337	-1,607.0	83.8	***
FV	6,154	-55.0	99.1	n.s	6013	-3,931.0	60.5	***
FA	6,659	450.0	107.2	***	6134	-3,810.0	61.7	***
LCD	5% = 109; 1% = 151; 0.1% = 209 kg ha^{-1}				5% = 345; 1% = 477; 0.1% = 659 kg ha^{-1}			

Table 2. Yield of lavender essential oil

Variants	2019				2020			
	kg ha^{-1}	± kg ha^{-1}	% to the control	Significance	kg ha^{-1}	± kg ha^{-1}	% to the control	Significance
control	86.0	0.0	100.0	control	159.0	0.0	100.0	control
FG	99.0	13.0	115.1	***	184.1	25.1	115.8	***
FT + FVital	110.0	24.0	127.9	***	183.3	24.3	115.3	***
FViti	115.0	29.0	133.7	***	168.3	9.3	105.8	**
FV	59.0	-27.0	68.6	***	115.6	-43.4	72.7	***
FA	70.0	-16.0	81.4	***	125.9	-33.1	79.2	***
LCD	5% = 0.2; 1% = 4.8; 0.1% = 6.7 kg ha^{-1}				5% = 6.0; 1% = 8.3; 0.1% = 11.5 kg ha^{-1}			

Several studies show that the application of organic fertilizers in different crops increased the yield and quality of essential oils. For example, that was observed in balm (Sodré et al., 2011), mint (Marotti et al., 1994), thyme (Juárez-Rosete et al., 2014) and basil (Bufalo et al., 2015; El-Naggar et al., 2015). The essential oil yield for the entire experimental period of the present study varied from 59 kg ha^{-1} to 183.3 kg ha^{-1} (Table 2). In 2019, the oil yield in the untreated variant was 86 kg ha^{-1}, while in 2020 it was significantly higher - 159 kg ha^{-1}, which is an age specific characteristic. It was found that the treatment with FG, FT + FVital and FViti led to a significant increase in the essential oil yield compared to the untreated variant, while the application of the foliar fertilizers FV and FA led to a decrease in the amount of oil, regardless of the year of cultivation. The decrease was by 27 kg ha^{-1} and 16 kg ha^{-1} in 2019 and by 43.4 kg ha^{-1} and 33.1 kg ha^{-1} in 2020, respectively. Due to the favourable combination between the microelements B and Mo, the highest values of the indicator were reported in the variant treated with FG - 184.1 kg ha^{-1}, exceeding the control by 15.8%. The amount of essential oil (Fig. 6) increased with the increase of the flower

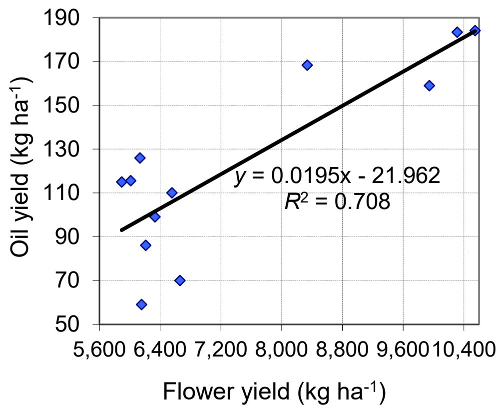

Figure 6. Relationship between flower yield and oil yield of lavender 'Jubileyna' variety.

yield in lavender, and the relationship between the two indicators was linear at $R^2 = 0.708$.

Based on the data for flower and oil yields, as well as for the structural elements of the yield in all the variants for both experimental years, the correlations between them were established. The results are presented in Table 3. According to the values of the correlation coefficients, the yield was strongly influenced by bush height and diameter. Oil yield was strongly correlated with the height and diameter of the bush, as well as with the number of flowering stems and the number of flowering vertebrae. The inflorescence length also affected flower and oil yields, although to a lesser degree.

Table 3. Correlation matrix (Pearson)

Yield components	Bush height, cm	Bush diameter, cm	Number of flowering stems	Inflorescence length, cm	Number of flowering vertebrae	Yield of fresh flowers, kg ha^{-1}	Yield of essential oil, kg ha^{-1}
Bush height	1,000	**0.934**	**0.898**	0.633	0.593	**0.688**	0.842
Bush diameter		1,000	**0.923**	0.638	0.633	**0.656**	**0.851**
Number of flowering stems			1,000	0.650	0.649	0.533	**0.887**
Inflorescence length				1,000	**0.770**	0.425	0.608
Number of flowering vertebrae					1,000	0.461	0.618
Yield of fresh flowers						1,000	**0.841**
Yield of essential oil							1,000

Significant correlation at $p < 0.05$ (bold)

CONCLUSIONS

The foliar application of all the tested products increased the photosynthetic activity of lavender. The positive effect was better expressed when using the preparations FV, FViti and FT + FVital. The application of foliar fertilizers in the budding stage had a certain positive effect on the chlorophyll content, more significant increase being reported in the variants treated with FT + FVital and FViti in the second growing year. That was probably due to the K_2O content in those preparations, which can mitigate the adverse effects of abiotic stress (low air temperature and precipitations during the flowering period).

Foliar fertilization had a positive effect on the height and diameter of the lavender bushes, better expressed during the second growing season. During the first experimental year (second vegetation), the effect of FG, FViti and FV was weak. Inflorescence length was also not affected by the applied foliar fertilization during the first experimental year. During the second experimental year it increased. The most significant changes were reported in the plants treated with FViti, FV and FA. As a result of the foliar fertilization, the number of flowering stems and vertebrae increased more significantly during the third vegetation. FV and FA preparations had practically no effect on the number of flowering stems.

The yield of fresh inflorescences, was positively influenced by the application with FG and FT + FVital. Treatment with the boron containing products FG, FT + FVital and FViti led to a significant increase in the essential oil yield, while the application of the foliar fertilizers FV and FA reduced it. Based on those results, the first three products are recommended.

REFERENCES

Almeida, H.J., Pancelli, M.A., Prado, R.M., Cavalcante, V.S. & Cruz, F.J.R. 2015. Effect of potassium on nutritional status and productivity of peanuts in succession with sugarcane. *J. Soil Sci. Plant Nutr.* **15**(1), 1–10.

Azizi, K. & Kahrizi, D. 2008. Effect of nitrogen levels, plant density and climate on yield and quality in cumin (*Cuminum cyminum*). *Asian J. Plant Sci*. **7**, 710–716.

Bardule, A., Rancane, S., Gutmane, I., Berzins, P., Stesele, V., Lazdina, D. & Bardulis, A. 2013. The effect of fertiliser type on hybrid aspen increment and seed yield of perennial grass cultivated in the agroforestry system. *Agronomy Research* **11**(1), 13–24.

Baranauskienne, R., Venskutonis, P.R., Viskelis, P. & Dambrausience, E. 2003. Influence of nitrogen fertilizer on the yield and composition of thyme (*Thymus vulgaris*). *J. Agr. Food Chem.* **51**, 7751–7758.

Bufalo, J., Cantrell, C.L., Astatkie, T., Zheljazkov, V.D., Gawde, A. & Boaro, C.S.F. 2015. Organic versus conventional fertilization effects on sweet basil (*Ocimum basilicum* L.) growth in a greenhouse system. *Ind. Crops Prod.* **74**, 249–254.

Chrysargyris, A., Panayiotou, Ch. & Tzortzakis, N. 2016. Nitrogen and phosphorus levels affected plant growth, essential oil composition and antioxidant status of lavender plant (*Lavandula angustifolia* Mill.), Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol 3603, Cyprus. *Industrial Crops and Products* **83**, 577–586.

Economakis, C.D. 1993. Effect of potassium on growth and yield of *Origanum dictamnu*s in growth culture. *Acta Hort*. **331**, 339–344.

El-Naggar, A.H., Hassan, M.R.A., Shaban, E.H. & Mohamed, M.E. 2015. Effect of organic and biofertilizers on growth, oil yield and chemical composition of the essential oil of *Ocimum basillicum* L. Plants Alex. *J. Agric. Res.* **60**, 1–16.

Emonfor, V.E., Chweya, J.A., Keya, S.O. & Munavu, R.M. 1990. The effect of nitrogen and phosphorus on the essential oil yield. *East Afr. Agric. For. J*. **55**, 191–194.

Fernández, V. & Brown, P.H. 2013. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Front. Plant Sci.* **4**, 289.

Garde-Cerdán, T., Santamaria, P., Rubio-Bretón, P., González-Arenzana, L., López-Alfaro, I. & López, R. 2015. Foliar application of proline, phenylalanine, and urea to Tempranillo vines: effect on grape volatile composition and comparison with the use of commercial nitrogen fertilizers. *LWT-Food Sci. Technol.* **60**, 684–689.

Hafsi, C., Debez, A. & Abdelly, C. 2014. Potassium deficiency in plants: effects and signaling cascades. *Acta Physiol. Plant*. **36**, 1055–1070.

Juárez-Rosete, C.R., Aguilar-Castillo, J.A. & Rodríguez-Mendoza, M.N. 2014. Fertilizer source in biomass production and quality of essential oils of thyme (*Thymus vulgaris* L.). *Eur. J. Med. Plants* **4**(7), 865–871.

Konakchiev, A. 2015. Essential oils of *Lavandula angustifolia* Mill varieties and species of the genus *Achilea* L., Institute of Organic Chemistry with Center for Physical Chemistry (BAS), *Dissertation*, pp. 64–75.

Lasa, B., Menendez, S., Sagastizabal, K., Cervantes, M.E.C., Irigoyen, I., Muro, J., Aparicio-Tejo, P.M. & Ariz, I. 2012. Foliar application of urea to 'Sauvignon Blanc' and 'Merlot' vines: doses and time of application. *Plant Growth Regul.* **67**, 73–81.

Lamptey, S.S., Yeboah, L.Li & Zhang, R. 2017. Dry matter accumulation and nitrogen concentration in forage and grain maize in dryland areas under different soil amendments. *Agronomy Research* **15**(4), 1646–1658. https://doi.org/10.15159/AR.17.01

Lukošiūtė, S., Šernaitė, L., Morkeliūnė, A., Rasiukevičiūtė, N. & Valiuškaitė, A. 2020. The effect of *Lamiaceae* plants essential oils on fungal plant pathogens in vitro. *Agronomy Research* **18**(S4), 2761–2769. https://doi.org/10.15159/AR.20.225

Marotti, M., Piccaglia, R., Giovanelli, E., Deans, S.G. & Eaglesham, E. 1994. Effects of planting time and mineral fertilization on peppermint (*Mentha x piperita* L.) essential oil composition and its biological activity. *Flavour Fragr. J.* **9**, 125–129.

Minev, N. 2020. Lavander (*Lavandula angustifolia* Mill), *Monography*, Publisher UCHY, 21–23 (in Bulgaian).

Nell, M., Votsch, M., Vierheilig, H., Steinkellner, S., Zitterl-Eglseer, K., Franz, C. & Novak, J. 2009. Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.). *J. Sci. Food Agric*. **89**, 1090–1096.

Nurzynska-Wierdak, R., Borowski, B., Dzida, K., Zawislak, G. & Kowalski, R. 2013. Essential oil composition of sweet basil cultivars as affected by nitrogenand potassium fertilization. *Turk J. Agric. For*. **37**, 427–436. doi:10.3906/tar-1203-43

Pal, P.K., Mahajan, M. & Agnihotri, V.K. 2016. Foliar application of plant nutrients and kinetin modifies growth and essential oil profile in *Rosa damascena* under acidic conditions. *Acta Physiol. Plant.* **38**, 176.

Puttanna, K., Rao, E.V.S.P., Singh, R. & Ramesh, S. 2010. Influence of nitrogen and potassium fertilization on yield and quality of rosemary in relation to harvest number. *Commun. Soil Sci. Plant Anal*. **41**, 190–198.

Raguso, R.A. & Pichersky, E. 1999. New perspectives in pollination biology: Floral fragrances. A day in the life of a linalool molecule: Chemical communication in a plant- pollinator system, Part 1: Linalool biosynthesis in flowering plants. *Plant Species Biology* **14**, 95–120.

Robert, M.D. & Francis, N.A. 1986. *Plant Physiology*, Robert Johnson, Publisher (PWS), a division of Wadsworth Inc., Boston, USA, pp. 786.

Rouached, H., Arpat, A.B. & Poirier, Y. 2010. Regulation of phosphate starvation responses in plants: signaling players and cross-talks. *Mol. Plant*. **3**, 288–299.

Saharkhiz, M.J. & Omidbaigi, R. 2008. The effect of phosphorus on the productivity of feverfew (*Tanacetum parthenium* L.) Schultz Bip). *Nat. Appl. Sci*. 2, 63–67.

Sodré, A.C.B., Luz, J.M.Q., Haber, L.L., Marques, M.O.M., Rodrigues, C.R. & Blank, A.F. 2011. Organic and mineral fertilization and chemical composition of lemon balm (*Melissa officinalis*) essential oil. *Rev. Bras. Farmacogn* **22**, 40–44.

Stoyanova, A., Balinova-Tzvetkova, A. & Georgiev, E. 2009. Lavander. Study of essential oil products in Bulgaria, Publisher Agency 7 D, Plovdiv, 46–48 (in Bulgaian).

Staudt, M. & Seufert, G. 1995. Light-dependent emission of monoterpenes by holm oak (*Quercus ilex* L.). *Naturwissenschaften* **82**, 89–92.

Velikova, V., Loreto, F., Tsonev, T., Brilli, F. & Edreva, A. 2006. Isoprene prevents the negative consequences of high temperature stress in *Platanus orientalis* leaves. *Functional Plant Biology* **33**, 1–10.

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The effect of feed supplementation with inulin on boar taint levels and meat quality of entire male pigs

R. Pereira Pinto[1,2,3,*], M. Vaz Velho[1,2], M. Barros[1,2], N. Reis[1,3] and P. Pires[1,2]

[1]CISAS - Center for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo, Rua Escola Industrial e Comercial de Nun'Álvares, PT4900-347 Viana do Castelo, Portugal
[2]Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Rua Escola Industrial e Comercial de Nun'Álvares, PT4900-347 Viana do Castelo, Portugal
[3]EDIUS - International PhD School of the USC, University of Santiago de Compostela, ES15705 Santiago de Compostela, Spain
[*]Correspondence: rpinto@ipvc.pt

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Abstract. Skatole and androstenone are the two main compounds responsible for the foul odour in entire male pigs' meat, known as boar taint. This study evaluated the effect of feed supplementation with inulin on the boar taint levels of 30 entire male pigs. Two months before slaughter, the animals were allocated into three groups ($n = 10$). The control group received a standard commercial diet. The other groups were fed the same commercial diet with 3% and 6% added inulin, respectively. Results showed that inulin addition to the feed significantly reduced skatole levels in the pigs' adipose tissue compared with the control group. The levels of androstenone were not affected by the dietary approach. Although there were differences in some parameters, the supplementation with inulin did not promote extensive changes in the meat quality parameters between the tested groups. When raising entire males, supplementation with inulin in finishing diets could be considered to reduce the boar taint perception by the consumer.

Key words: androstenone, entire male pigs, inulin, meat quality, skatole.

INTRODUCTION

The surgical castration of male piglets, a common procedure in most European countries, has become an animal welfare concern due to the pain and stress associated with the procedure (EFSA, 2004). Using behavioural and physiological parameters, it has been scientifically proven that castration is a painful surgical intervention even when performed on very young animals, and it is a costly operation for producers (Fredriksen et al., 2011).

The main reason to castrate male piglets is to prevent the development of boar taint, an unpleasant odour found in the carcasses of entire males (EFSA, 2004; Fredriksen et al., 2011; Wauters et al., 2017). This odour becomes especially intense when cooked (Mathur et al., 2012) and is mainly associated with the presence of skatole (3-methylindole) and androstenone (5α-androst-16-en-3-one), which, due to their lipophilic characteristics, tends to accumulate in adipose tissue. Skatole is a metabolite derived from the amino acid tryptophan produced in the lower part of the intestine by the intestinal microbiota and is associated with a faecal and naphthalene smell (Furnols & Oliver, 1999). Androstenone is a steroid produced in the testes with a smell often associated with urine and sweat (Furnols & Oliver, 1999). Skatole levels are usually low in castrated and female animals; also, androstenone is known to interfere with the hepatic elimination of skatole (Doran et al., 2002; Whittington et al., 2004). In contrast to androstenone, skatole is perceived by 99% of consumers (Weiler et al., 2000). However, the average consumer does not know why sometimes pork has a foul smell. A study in four European countries (France, Germany, the Netherlands, and Belgium) reported that 88.7% of consumers were unaware of boar taint or did not know what it was (Vanhonacker & Verbeke, 2011). Also, in a recent survey of Portuguese consumers, nearly half of the respondents did not know what boar taint was or had even never heard about it (Pereira Pinto & Vaz-Velho, 2021).

There are different realities in Europe. In major pig producing countries such as Germany, France, and Denmark, around 75% to 93% of commercial male piglets are surgically castrated (Van Ferneij, 2022), while in countries such as Spain, Portugal, Ireland, and the United Kingdom, male pigs are slaughtered before they reach puberty (approximately 5 months of age), weighing less than 100 kg, being raised without castration (Weiler & Bonneau, 2019). However, producers lose efficiency due to reduced carcass size.

Stakeholders in the production of pigs have been defending the ban on surgical castration in the European Union, which represents a challenge for the pork production chain (Morlein et al., 2015). All over the world, several alternatives to surgical castration are applied, such as raising uncastrated males (entire males) or immunocastration. The application of immunocastration inhibits testosterone production, reducing the boar odour without surgical intervention and the risk of infection. It requires at least two injections into the animal at least 4 weeks apart, with the second dose at least 4 weeks before slaughter (Heyrman et al., 2019). A major disadvantage of this method is that the EU catalogues this chemical in the therapeutic area of sex hormones and modulators of the genital system (EMA, 2020), which is currently unfeasible in organic production modes.

Breeding entire males have some advantages, such as lower production costs, a more natural approach, and improved animal welfare. When compared with uncastrated male pigs, it was found that in castrated males, feed efficiency decreased by 10%, muscle content decreased by 5%, while carcass fat increased by 26% (Bonneau et al., 1994). The disadvantages of breeding entire males are mainly related to the possible formation of boar and meat odour with less advantageous characteristics for the production of meat products. Aluwe et al. (2013) compared meat from entire and castrated males and found differences in pH, colour, drip loss, and cooking loss; these differences did not significantly influence the quality or palatability of the meat.

Several studies (Byrne et al., 2008; Hansen et al., 2008; Kjos et al., 2010; Aluwe et al., 2013; Bilić-Šobot et al., 2014; Backus et al., 2016) have shown that feed supplemented with fermentable carbohydrates, such as inulin obtained from chicory, were effective in reducing the concentration of skatole in the swine intestine. Inulin is a non-digestible fructose polymer found mainly in chicory roots (*Cichorium intybus*) or Jerusalem artichoke tubers (*Helianthus tuberosus*). It contains oligosaccharides and polysaccharides, regarded as a source of prebiotics (Grela et al., 2021). Prebiotics are commonly used to enhance pigs fattening, changing pork physicochemical properties such as increasing the total fat content (Degola & Jonkus, 2018). Dietary inulin is not hydrolysed by mammalian enzymes but is easily fermented by the bacterial community and favours the growth of intestinal bifidobacteria (Grela et al., 2021). Fermentation of inulin by intestinal bacteria produces a large number of short-chain fatty acids, which can stimulate the production and secretion of the mucous layer covering the mucosal surface of the gastrointestinal tract (Wang et al., 2019). Dietary inulin may also alter intestinal bacterial populations due to its iron bioavailability-promoting effect (Patterson et al., 2010), and inulin-type fructans may reduce the production of potentially toxic metabolites (Meyer & Stasse-Wolthuis, 2009).

Thus, this research aimed to evaluate the effect of feed supplementation with inulin in the finishing diets of entire males, assessing meat quality parameters and, mainly, the influence of inulin on the reduction of the boar taint in carcasses.

MATERIALS AND METHODS

Study design and feeding conditions

Thirty entire male pigs males, offspring from a Pietrain terminal sire, crossed with a Large White, Landrace crossed sow were used for the study. The animals were fed the same commercial diet, with *ad libitum* access, up to the age of 5.5 months. At this stage, the pigs were allocated into three distinct groups ($n = 10$), where one group received a standard commercial diet (control group). In the other groups, the same commercial diet included 3% and 6% of inulin (Fibrofos, a by-product of *Cichorium intybus* L.), respectively. Feed was constituted of wheat, corn, soybean bagasse, barley, fibrofos 60, rapeseed bagasse, wheat bran, cane molasses, dicalcium phosphate, L- lysine, salt, L-threonine, DL-methionine, and calcium carbonate. Some slight corrections were made in the relative composition of the diets containing Fibrofos in order to balance the nutritional level. Diets have similar nutritional profiles, as shown in Table 1. All groups received the same feed, 2.8 kg per day per pig, for 2 months until slaughter.

Table 1. Nutrient levels (%) of the standard diet (control) and the diets with added inulin

Nutrients	Control	3% of added inulin	6% of added inulin
Moisture	12.37	12.26	12.24
Crude Protein	15.55	15.56	15.55
Raw fibre	4.53	3.84	3.73
NDF	13.71	12.63	11.64
ADF	5.76	5.01	4.76
Raw ash	4.32	4.30	4.10
Fat	1.99	1.89	1.82
Starch	45.10	42.65	40.45
Calcium	0.72	0.74	0.71
Phosphor	0.48	0.48	0.49
Lysine	0.99	0.99	0.99
Lysine SID	0.90	0.91	0.91
Met + Cis	0.61	0.62	0.62
Met + Cis SID	0.55	0.56	0.57
Threonine	0.69	0.71	0.70
Threonine SID	0.62	0.64	0.64
Tryptophan	0.18	0.18	0.18
Tryptophan SID	0.16	0.16	0.16
Sodium	0.17	0.20	0.20
Linoleic acid	0.97	0.88	0.85
Energy Kcal kg^{-1}	2309	2309	2309
Inulin	0.00	3.00	6.00

Analysis of moisture, pH, loss on thawing, colour, hardness, and intramuscular fat

Meat samples were collected from the ham's muscle tissue (*Biceps femoris*), frozen and kept at -18 °C until analysis. Before further analysis, meat samples were thawed to determine the thawing loss: samples were weighed, thawed at 4 °C for 24 hours, dried with a paper towel and reweighed. To determine the moisture content, samples were minced and dried in an oven at 103 ± 2 °C until constant weight, as described in A.O.A.C (2016), method 950.46. The pH was measured by potentiometry using the equipment Crison pH25+ (Crison Instruments, Barcelona, Spain), inserting the probe electrode into the meat samples. Colour measurements were performed according to the CIELAB colour system described by Honikel (1998), using a Minolta CR-300 colourimeter (Konica Minolta Tokyo, Japan). To determine the hardness, samples of meat (4 cm) were cut to analyse the texture profile (using the TA.XT2 Plus equipment from Stable Micro Systems TPA). Intramuscular fat (IMF) percentage was determined by a Soxhlet extraction procedure using petroleum ether as an extraction agent after sample hydrolysis, as described in the A.O.A.C. (2016) method 960.39. To analyse moisture, pH, thawing loss, and intramuscular fat, measurements were performed in triplicate. For colour and hardness, samples were measured 10 times each.

Boar taint quantification

The quantification of skatole and androstenone in pork fat was done using a high-performance liquid chromatography (HPLC) method adapted from Hansen-Moller (1994), as described in the following sections.

Fat extraction. Sample preparation consisted of cutting the adipose tissue from the subcutaneous layer of the belly and extracting the liquid fat after microwave heating (800 W, 2 min). A sample of water-free liquid fat (1 g) was placed in Falcon tubes, and 1 mLof methanol was added. After vortexing for 30 sec, tubes were incubated for 10 min at 40 °C in an ultrasonic bath (Sonica® Ultrasonic Cleaner). Samples were centrifuged (JP Selecta Mixtasel) for 15 min at 1,100 g and placed in an ice-water bath for 20 min. The liquid fraction was filtered through a 0.2 μm filter before sample derivatisation.

Sample derivatisation. Manual derivatisation of 500 μL of the sample was performed at room temperature by sequentially adding 75 μL of 0.1% dansylhydrazine, 50 μL of deionised water in methanol, and 40 μL of 14% BF_3 solution in methanol. After stirring for 5 min, 20 μl of the mixture was injected into the HPLC.

HPLC operating conditions. A ThermoFisher UltiMate 3000 HPLC system with a Hypersil ODS C18 column (5 μm particle diameter and 250×4.6 mm, Thermo Scientific Portugal), operated at 40 °C, was used. The eluent was: (A) 0.1% v/v acetic acid; (B) acetonitrile; (C) tetrahydrofuran; (D) pH 6.0 buffer solution (25 mM potassium phosphate). The following gradient profile was used: 0–5 min, 50%–60% A, 35%–45% B, 5% C; 5–6 min, 50% A, 45% B, 5% C; 6–6.1 min, 20%–50% A, 30%–45% B, 5%–30% C, 0–20% D; 6.1–12 min, 0–20% A, 30%–40% B, 30%–40% C, 20% D; 12–12.5 min, 0–60% A, 40%–35% B, 5%–40% C, 0–20% D; 12.5–13 min, 60% A, 35% B and 5% C, with a flow rate of 2.0 mL min^{-1}. Fluorescence detection was performed with excitation at 285 nm and emission at 340 nm (0–6.0 min) for detection of skatole; excitation at 346 nm and emission at 521 nm (6.1–13 min) for the detection of androstenone.

Quantification of skatole and androstenone. For quantification, a calibration method using the external method was performed. The range of standards was between 1.60 and 63.0 ng g^{-1} for skatole and 37.6 and 1503.8 ng g^{-1} for androstenone, using 10 standards for each. Calibration curves' linearity coefficient (R^2) was 0.9928 for skatole and 0.9969 for androstenone. Detection and quantification limits (LoD and LoQ) were calculated: LoD of 12.6 ng g^{-1} and 63.2 ng g^{-1} for skatole and androstenone, respectively, and LoQ of 38.2 ng g^{-1} and 191.5 ng g^{-1} for skatole and androstenone, respectively.

Data analysis

Statistical analysis was performed using Statistica for Windows software package, version 14.0.0.15 (TIBCO Software, Palo Alto, California, USA). Data were subjected to Kolmogorov-Smirnov test to assess normality and, depending on the result, followed by an analysis of variance (ANOVA) or the Kruskal-Wallis test. Differences between groups were determined using the Fischer's Least Significant Difference (*LSD*) *post-hoc* test. Significant differences were established in $P < 0.05$.

RESULTS AND DISCUSSION

The values of skatole and androstenone concentrations in the belly fat, determined by HPLC, are shown in Table 2. In the control group, where no inulin was added, the average content of skatole was about 2.7 times higher than in the groups supplemented with inulin (44.3 ng g^{-1} *vs*. 16.4 µg g^{-1}), meaning that inulin addition was effective in skatole reduction independently of the tested amount.

Regarding androstenone, there were no significant differences ($P = 0.36$). Although a slight reduction in mean values can be noticed when inulin is increased, it is not possible to establish a link between the diet and the androstenone production. Androstenone values in fat mainly depend on genetic factors linked to sexual maturity (Bonneau, 2006).

Table 2. Skatole and androstenone concentrations (mean ± standard deviation) in pig belly fat (ng g^{-1} fat)

Group (added inulin)	Skatole (ng g^{-1})	Androstenone (ng g^{-1})
0%	44.3 ± 41.1[a]	459.2 ± 235.4
3%	16.4 ± 10.0[b]	358.3 ± 244.5
6%	15.9 ± 15.9[b]	275.9 ± 191.8
P-value	0.03	0.36

[a,b] Different superscripts within columns indicate significant differences ($p < 0.05$).

Similar results in feeding trials with inulin were obtained (Kjos et al., 2010; Overland et al., 2011; Zammerini et al., 2012; Aluwe et al., 2017), where skatole was reduced, and there were no differences in androstenone values. Nonetheless, in a study conducted by Heyrman et al. (2018), chicory root supplementation for two to three weeks led to a reduction in both skatole and androstenone. However, the author of this study states that the significant differences in androstenone values may be due to the differences in carcass weight since androstenone concentrations were positively linked with carcass weight.

Fig. 1 represents the skatole *vs.* androstenone scatterplot, where are the values obtained for skatole and androstenone for each animal ($n = 30$) and identified according to the provided diet. Some patterns can be identified: lower skatole values in the 3% and 6% groups and higher values in the control group. A wide dispersion of results is reflected in the standard deviation values shown in Table 2. However, this dispersion is typical when skatole and androstenone are measured in the pigs' population, as reported in similar studies (Hansen-Moller, 1994; Aluwe et al., 2013; Aaslyng et al., 2015; Morlein et al., 2016; Liu et al., 2017; Verplanken et al., 2017).

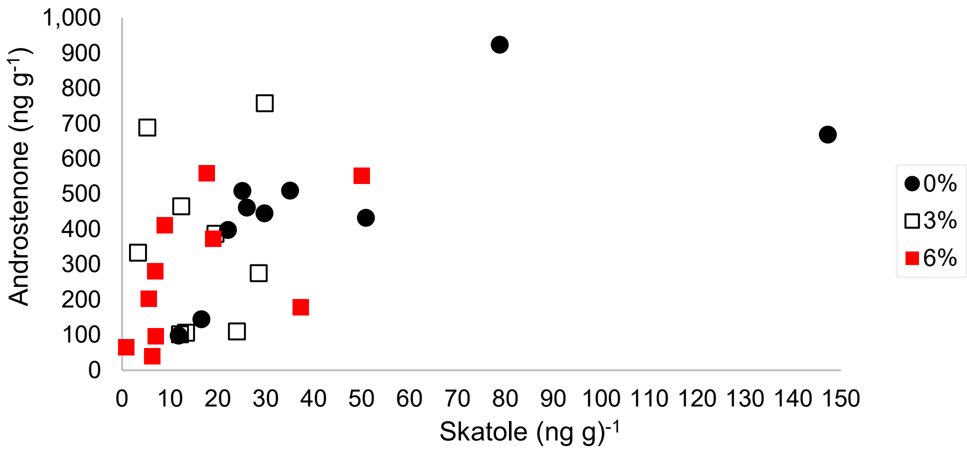

Figure 1. Skatole *vs*. androstenone scatterplot.

Regarding the influence of inulin supplementation on meat quality, no significant differences ($P > 0.05$) between groups were found in pH values, thawing loss, moisture, and intramuscular fat (Table 3). On the other hand, hardness values were found to be significantly different (group P-value was 0.002); the group with 6% of added inulin showed the lowest hardness value, which indicated softer meat. Wang et al. (2019) stated that the dietary inulin supplementation had no significant influence on meat quality, except for the expression of the *MyHC IIb* gene, which was increased by inulin supplementation. Tenderness is affected by fibre type, especially type IIb fibres, and muscles with larger type IIb fibres may be tougher or may have greater hardness (Guo et al., 2019). However, this was not verified in our study, as the increase in inulin led to lower meat hardness.

Table 3. Values (mean ± standard deviation) of physicochemical determinations

Group (added inulin)	Hardness (N)	pH	Thawing loss (%)	Moisture (%)	IMF (%)
0%	6.60 ± 2.24^b	6.01 ± 0.35	4.75 ± 3.09	73.38 ± 1.45	2.28 ± 0,40
3%	6.83 ± 1.99^b	5.87 ± 0.30	6.41 ± 2.95	71.52 ± 3.84	2.29 ± 0.62
6%	5.58 ± 1.86^a	5.91 ± 0.28	5.72 ± 2.29	72.20 ± 4.60	2.60 ± 0.67
P-value	0.002	0.18	0.18	0.30	0.12

^{a,b} Different superscripts within columns indicate significant differences ($p < 0.05$).

Another parameter of meat quality is its colour. Instrumental determination was achieved through the CIELAB system. Results of the parameters L* (brightness), a* (red colour) and b* (yellow colour) are shown in Table 4 and indicate statistically significant differences in red and yellow, particularly in the group supplemented with 3% of inulin. Altmann et al. (2022) measured colour in raw pork loin and found that a change along the b* axis (yellowness) in CIELAB colour space is most discernible, followed by the * axis (redness) and then the L* axis (lightness). However, the difference between the colour coordinate values is minimal, in the order of 1.5 on average, which is too low for a the colour discrimination threshold of the human eye. According to Konica-Minolta (2019), the human eye cannot differentiate some colours even if they are numerically (L*a*b* coordinates) different, depending on the saturation and hue. The L* values showed no significant differences between groups. A similar conclusion was drawn in a study by Gispert et al. (2010). Also, results of the meat colour are inconsistent in the literature since muscle fibre characteristics may depend on genotype as well as environmental factors, such as nutrition, preslaughter and slaughter conditions (Aluwe et al., 2013).

Table 4. Meat colour determinations (mean ± standard deviation)

Group (added inulin)	L*	a*	b*
0%	44.83 ± 4.40	14.18 ± 2.79^{b}	4.64 ± 2.11^{b}
3%	45.53 ± 6.41	12.57 ± 2.26^{a}	3.19 ± 2.23^{a}
6%	44.03 ± 5.53	13.81 ± 2.14^{b}	4.12 ± 2.07^{b}
P-value	0.16	0.00	0.00

^{a,b} Different superscripts within columns indicate significant differences ($p < 0.05$).

Albeit there are statistically significant differences in hardness and colour values (a* and b*), little is known about the influence of inulin on meat quality (Wang et al., 2019). Available studies indicate that inulin addition did not affect meat properties: Grela et al. (2021) reported that the addition of inulin from different sources as prebiotics to pig feed did not have a negative effect on the physicochemical properties of meat; Aluwe et al. (2013) found no significant differences in the meat quality parameters of boars fed with chicory when compared to control. Also, Wang et al. (2019) stated that dietary inulin supplementation had no significant influence on meat quality.

CONCLUSIONS

Commercial feed supplemented with inulin significantly reduced skatole levels present in the entire male pigs' fat, the proportion of 3% of added inulin being sufficient for the purpose. Androstenone values were not altered by the prebiotic. Regarding meat quality, the addition of inulin affected some meat characteristics such as hardness and colour compared to the control group. Feed supplementation with inulin could be considered an alternative to reducing skatole levels and consequent organoleptic improvement.

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REFERENCES

Aaslyng, M.D., Broge, E.H.D., Brockhoff, P.B. & Christensen, R.H. 2015. The effect of skatole and androstenone on consumer response towards streaky bacon and pork belly roll. *Meat Science* **110**, 52–61. doi: 10.1016/j.meatsci.2015.07.001

Altmann, B.A., Gertheiss, J., Tomasevic, I., Engelkes, C., Glaesener, T., Meyer, J., Schäfer, A., Wiesen, R. & Mörlein, D. 2022. Human perception of color differences using computer vision system measurements of raw pork loin. *Meat Science* **188**, 108766. doi: 10.1016/j.meatsci.2022.108766

Aluwe, M., Heyrrnan, E., Theis, S., Sieland, C., Thurman, K. & Millet, S. 2017. Chicory fructans in pig diet reduce skatole in back fat of entire male pigs. *Research in Veterinary Science* **115**, 340–344. doi: 10.1016/j.rvsc.2017.06.016

Aluwe, M., Langendries, K.C.M., Bekaert, K.M., Tuyttens, F.A.M., De Brabander, D.L., De Smet, S. & Millet, S. 2013. Effect of surgical castration, immunocastration and chicory-diet on the meat quality and palatability of boars. *Meat Science* **94**(3), 402–407. doi: 10.1016/j.meatsci.2013.02.015

A.O.A.C. 2016. Association of Official Analytical Chemist, Official Methods of Analysis. 20th Edition. Rockville, USA.

Backus, G.B.C., van den Broek, E., van der Fels, B., Heres, L., Immink, V.M., Knol, E.F., Kornelis, M., Mathur, P.K., van der Peet-Schwering, C., van Riel, J.W., Snoek, H.M., de Smet, A., Tacken, G.M.L., Valeeva, N.I. & van Wagenberg, C.P.A. 2016. Evaluation of producing and marketing entire male pigs. *Njas-Wageningen Journal of Life Sciences* **76**, 29–41. doi: 10.1016/j.njas.2015.11.002

Bilić-Šobot, D., Čandek-Potokar, M., Kubale, V. & Škorjanc, D. 2014. Boar taint: interfering factors and possible ways to reduce it. *Agricultura* **11**, 35–48.

Bonneau, M., Dufour, R., Chouvet, C., Roulet, C., Meadus, W. & Squires, E.J. 1994. The effects of immunization against luteinizing-hormone-releasing hormone of performance, sexual development, and levels of boar taint-related compounds in intact male pigs. *Journal of Animal Science* **72**(1), 14–20. doi: 10.2527/1994.72114x

Bonneau, M. 2006. Factors affecting the level of androstenone. *Acta Veterinaria Scandinavica* **48**(S1). doi: 10.1186/1751-0147-48-s1-s7

Byrne, D.V., Thamsborg, S.M. & Hansen, L.L. 2008. A sensory description of boar taint and the effects of crude and dried chicory roots (Cichorium intybus L.) and inulin feeding in male and female pork. *Meat Science* **79**(2), 252–269. doi: 10.1016/j.meatsci.2007.09.009

Degola, L & Jonkus, D. 2018. The influence of dietary inclusion of peas, faba bean and lupin as a replacement for soybean meal on pig performance and carcass traits. *Agronomy Research* **16**, 389–397.

Doran, E., Whittington, F.W., Wood, J.D. & McGivan, J.D. 2002. Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. *Chemico-Biological Interactions* **140**(1), 81–92. doi: 10.1016/s0009-2797(02)00015-7

EFSA. 2004. Welfare aspects of the castration of piglets. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the castration of piglets (Question No. EFSA-Q-2003-091) *The EFSA Journal* **91**, 1–18, https://doi.org/doi: 10.2903/j.efsa.2004.91

EMA. 2020. EU Veterinary Medicinal Product Database. http://vet.eudrapharm.eu/vet/: European Medicines Agency.

Fredriksen, B., Johnsen, A.M.S. & Skuterud, E. 2011. Consumer attitudes towards castration of piglets and alternatives to surgical castration. *Research in Veterinary Science* **90**(2), 352–357. doi: 10.1016/j.rvsc.2010.06.018

Furnols, M.F.I. & Oliver, M.A. 1999. Production and consumption of pork meat with different levels of boar taint. *Food Science and Technology International* **5**(5), 367–375. doi: 10.1177/108201329900500501

Gispert, M., Oliver, M.A., Velarde, A., Suarez, P., Perez, J. & Furnols, M.F.I. 2010. Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. *Meat Science* **85**(4), 664–670. doi: 10.1016/j.meatsci.2010.03.021

Grela, E.R., Swiatkiewicz, M., Florek, M., Bakowski, M. & Skiba, G. 2021. Effect of Inulin Source and a Probiotic Supplement in Pig Diets on Carcass Traits, Meat Quality and Fatty Acid Composition in Finishing Pigs. *Animals* **11**(8). doi: 10.3390/ani11082438

Guo, X., Qin, B., Yang, X., Jia, J., Niu, J., Li, M., Cao, G. 2019. Comparison of carcass traits, meat quality and expressions of *MyHCs* in muscles between Mashen and Large White pigs. *Italian Journal of Animal Science* **18**(1), 1410–1418. doi:10.1080/1828051x.2019.1674701

Hansen, L.L., Stolzenbach, S., Jensen, J.A., Henckel, P., Hansen-Moller, J., Syriopoulos, K. & Byrne, D.V. 2008. Effect of feeding fermentable fibre-rich feedstuffs on meat quality with emphasis on chemical and sensory boar taint in entire male and female pigs. *Meat Science* **80(**4), 1165–1173. doi: 10.1016/j.meatsci.2008.05.010

Hansen-Moller, J. 1994. Rapid high-performance liquid-chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. *Journal of Chromatography B-Biomedical Applications* **661**(2), 219–230. doi: 10.1016/s0378-4347(94)80049-9.

Heyrman, E., Kowalski, E., Millet, S., Tuyttens, F.A.M., Ampe, B., Janssens, S., Buys, N., Wauters, J., Vanhaecke, L. & Aluwe, M. 2019. Monitoring of behavior, sex hormones and boar taint compounds during the vaccination program for immunocastration in three sire lines. *Research in Veterinary Science* **124**, 293–302. doi: 10.1016/j.rvsc.2019.04.010

Heyrman, E., Millet, S., Tuyttens, F.A.M., Ampe, B., Janssens, S., Buys, N., Wauters, J., Vanhaecke, L. & Aluwe, M. 2018. On farm intervention studies on reduction of boar taint prevalence: Feeding strategies, presence of gilts and time in lairage. *Research in Veterinary Science* **118**, 508–516. doi: 10.1016/j.rvsc.2018.05.008

Honikel, K.O. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Science* **49**(4), 447–457. doi: 10.1016/s0309-1740(98)00034-5

Kjos, N.P., Overland, M., Fauske, A.K. & Sorum, H. 2010. Feeding chicory inulin to entire male pigs during the last period before slaughter reduces skatole in digesta and backfat. *Livestock Science* **134(**1–3), 143–145. doi: 10.1016/j.livsci.2010.06.120

Konica-Minolta. 2019. 'Precise Colour Communication - Part V: Colour discrimination threshold of the human eye.' Available at https://www.konicaminolta.com/instruments/knowledge/color/part5/02.html [Accessed March 24th 2022].

Liu, X., Trautmann, J., Wigger, R., Zhou, G. & Morlein, D. 2017. Fatty acid composition and its association with chemical and sensory analysis of boar taint. *Food chemistry* **231**, 301–308. doi: 10.1016/j.foodchem.2017.03.112

Mathur, P.K., ten Napel, J., Bloemhof, S., Heres, L., Knol, E.F. & Mulder, H.A. 2012. A human nose scoring system for boar taint and its relationship with androstenone and skatole. *Meat. Sci.* **91**(4), 414–422. doi: 10.1016/j.meatsci.2012.02.025

Meyer, D. & Stasse-Wolthuis, M. 2009. The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *European Journal of Clinical Nutrition* **63**(11), 1277–1289. doi:10.1038/ejcn.2009.64

Morlein, D., Schiermann, C., Meier-Dinkel, L., Trautmann, J., Wigger, R., Buttinger, G. & M. Wicke, M. 2015. Effects of context and repeated exposure on food liking: The case of boar taint. *Food Research International* **67**, 390–399. doi: 10.1016/j.foodres.2014.11.037

Morlein, D., Trautmann, J., Gertheiss, J., Meier-Dinkel, L., Fischer, J., Eynck, H.J., Heres, L., Looft, C. & Tholen, E. 2016. Interaction of Skatole and Androstenone in the Olfactory Perception of Boar Taint. *Journal of Agricultural and Food Chemistry* **64**(22), 4556–4565. doi: 10.1021/acs.jafc.6b00355

Overland, M., Kjos, N.K., Fauske, A.K., Teige, J. & Sorum, H. 2011. Easily fermentable carbohydrates reduce skatole formation in the distal intestine of entire male pigs. *Livestock Science* **140**(1–3), 206–217. doi: 10.1016/j.livsci.2011.03.032

Patterson, J.K., Yasuda, K., Welch, R.M., Miller, D.D. & Lei, X.G. 2010. Supplemental Dietary Inulin of Variable Chain Lengths Alters Intestinal Bacterial Populations in Young Pigs. *The Journal of Nutrition* **140(**12), 2158–2161. doi:10.3945/jn.110.130302

Pereira Pinto, R. & Vaz-Velho, M. 2021. A perspective of the Portuguese consumer awareness, beliefs and preferences towards piglet castration methods and its implications on the meat quality. *Agronomy Research* **19**, 1273–1284.

Van Ferneij, J.-P., 2022. The pig castration situation in the European Union. pig333.com - Professional Pigs Community Webpage. Retrieved March 11th 2022 from https://www.pig333.com/articles/the-pig-castration-situation-in-the-european-union_18100/

Vanhonacker, F. & Verbeke, W. 2011. Consumer response to the possible use of a vaccine method to control boar taint v. physical piglet castration with anaesthesia: a quantitative study in four European countries. *Animal* **5**, 1107–1118.

Verplanken, K., Wauters, J., Vercruysse, V., Aluwe, M. & Vanhaecke, L. 2017. Sensory evaluation of boar-taint-containing minced meat, dry-cured ham and dry fermented sausage by a trained expert panel and consumers. *Food Chemistry* **233**, 247–255. doi: 10.1016/j.foodchem.2017.04.105

Wang, W., Chen, D., Yu, B., Huang, Z., Luo, Y., Zheng, P. & He, J. 2019. Effect of Dietary Inulin Supplementation on Growth Performance, Carcass Traits, and Meat Quality in Growing-Finishing Pigs. *Animals* **9(**10), 840. doi:10.3390/ani9100840

Wauters, J., Verplanken, K., Vercruysse, V., Ampe, B., Aluwe, M. &. Vanhaecke, L. 2017. Sensory evaluation of boar meat products by trained experts. *Food Chemistry* **237**, 516–524. doi: 10.1016/j.foodchem.2017.05.128

Weiler, U, Furnols, M.F.I., Fischer, K., Kemmer, H., Oliver, M.A., Gispert, M., Dobrowolski, A. & Claus, R. 2000. Influence of differences in sensitivity of Spanish and German consumers to perceive androstenone on the acceptance of boar meat differing in skatole and androstenone concentrations. *Meat Science* **54**, 297–304.

Weiler, U. & Bonneau, M., 2019. Why it is so difficult to end surgical castration of boars in Europe: Pros and cons of alternatives to piglet castration. In: M. Font i Furnols, B. Lakicevic (ed.): *proceedings of the 60th International Meat Industry Conference (MEATCON)*. Kopaonik (Serbia). Publication No. **333**, IOP Conf. Series: Earth and Environmental Science, 012001

Whittington, F.M., Nute, G.R., Hughes, S.I., McGivan, J.D., Lean, I.J., Wood, J.D. & Doran, E. 2004. Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in Meishan x Large White pigs. *Meat Science* **67**(4), 569–576. doi: 10.1016/j.meatsci.2003.12.010

Zammerini, D., Wood, J.D., Whittington, F.M., Nute, G.R., Hughes, S.I., Hazzledine, M. & Matthews, K. 2012. Effect of dietary chicory on boar taint. *Meat Science* **91**(4), 396–401. doi: 10.1016/j.meatsci.2012.01.020

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https://doi.org/10.15159/AR.22.062

Chlorophyll fluorescence induction method in assessing the efficiency of pre-sowing agro-technological construction of the oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) agrocenosis

Y. Tsytsiura*

Vinnytsia National Agrarian University, Faculty of Agronomy and Forestry, Sonyachna Str., 3, UA21008 Vinnytsia, Ukraine
*Correspondence: yaroslavtsytsyura@ukr.net, yaroslav301974@gmail.com

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Abstract. Chlorophyll fluorescence induction (CFI) is a measure of photosynthetic performance and is widely used by plant physiologists and ecophysiologists. The basic principle of CFI analysis is relatively straightforward. The specified method of analysis during 2015–2020 was applied to assess the optimality of selection of technological sowing parameters such as sowing rate (estimated interval 0.5–4.0 million germinable seeds ha^{-1}), row width (15–30 cm), pre-sowing fertilizer ($N_{0–90}P_{0–90}K_{0–90}$) for three varieties of oilseed radish. The widely tested basic indicators of the CFI curve (F_0, F_{pl}, F_m, F_{st}) were used, as well as possible indices and ratios calculated on their basis in accordance with the CFI analysis methodology.
For the first time, the species characteristics of oilseed radish were investigated by the nature of the CFI curve in relation to spring rape, white mustard, and spring mustard on the 1.5 germinable seeds ha^{-1} (30 cm row width, $N_0P_0K_0$) variant. It was established by the stress sensitivity category of the PSII photosystem that a reliable possibility of using the CFI method for identification studied technological options for sowing. The share of the influence of the technological factor of the sowing method (in %) on the formation of indicators F_0, F_{pl}, F_m, F_{st} in the dispersion scheme of the experiment was consistently 19.3, 8.4, 19.5, 6.3. The influence of the seeding rate factor on the results of F_0, F_{pl}, F_m, F_{st} was (in %) 26.6, 9.5, 42.3, 9.3 and the influence of the fertilizer factor was 13.5, 16.4, 5.7, 12.7, respectively.
The formation of the specified basic indicators of the CFI curve in the resulting interaction of the technological parameters of sowing depended on the hydrothermal conditions of the vegetation of oilseed radish with the share of influence of 20.1, 40.2, 28.1, 30.0, respectively. It was determined that the decrease in the indicator of the hydrothermal coefficient (in the ratio of the increase in the sum of average daily temperatures to the decrease in the amount of precipitation) ensures the following dynamics of changes in the main and derivative indicators of CFI: a decrease F_{pl} 1.3%, F_m 11.8%, ER 8.7%, Lwp 15.9%, R_{Fd} 25.3%, K_{prp} 21.9%, K_{fd} 17.7% and growth F_0 5.1%, F_{st} 7.3%, Q_{ue} 40.4%, K_{ef} 24.0%, V_t 71.3%.
The comparison during the study period of options 4.0 and 0.5 million germinable seeds ha^{-1} determined an averaged decrease in F_0 and F_{st} indicators by 29.5% and 29.1% while increasing F_{pl} and F_m by 2.2% and 38.5%. According to the determined level of CFI indicators for various technological schemes of sowing, an expedient option was recommended, which ensures the highest efficiency of the PSII photosystem of oilseed radish in the range of 1.0–2.0 germinable

seeds ha^{-1} with a fertilization rate of $N_{30–60}P_{30–60}K_{30–60}$ for row sowing and 1.5 germinable seeds ha^{-1} with a fertilization rate of $N_{60–90}P_{60–90}K_{60–90}$ for wide-row sowing.

Key words: chlorophyll fluorescence induction, fertilization rate, oilseed radish, row spacing, seeding rate, yield.

INTRODUCTION

Despite different approaches to the assessment of the physiological state of plants, the basic indicator of the state of plant life is the efficiency of the primary processes of photosynthetic reactions. The indisputable importance of this indicator is determined by both the importance of photosynthetic function in plant life and the high sensitivity of photosynthetic apparatus to changes in environmental factors, especially stress factors. On the other hand, any disruptions in the primary processes of photosynthesis directly affect changes in chlorophyll fluorescence and occur long before visible disturbances in the physiological state of plants, which can be assessed by obvious morphological changes in plant development, the rate of their growth processes and, ultimately, by the productivity level (Suárez et al., 2022).

It is an established fact that the source of fluorescence in the plant cell is light-absorbing pigment molecules, mainly chlorophyll (Kalaji et al., 2017a, 2017b). The effect of stressors of different nature on the plant is overwhelmingly related to the effect on the state of PSII reaction centers (RC), the S II antenna and the light-absorbing complex. The transfer of excitation energy of electrons between the pigments of these complexes and the glow of chlorophyll a reaction centers S II determine the induction curve of chlorophyll a fluorescence (Kalaji et al., 2017b). The shape of this curve is quite sensitive to the changes occurring in the photosynthetic apparatus of plants when adapting it to different environmental conditions, which was the basis for the wide use of the Kautsky effect in the study of plant photosynthesis and assessment of their condition under stress factors (Sloat et al., 2021). Certain interval segments of the chlorophyll fluorescence induction curve (CFI) can be used to analyze the physiological responses of the plant to stressful and induced-technological irritants. At the same time, the curve pattern varies depending on the intensity of exposure and the stress response of the plant itsel (Valcke, 2021).

According to a number of researchers, chlorophyll fluorescence is the fastest, most convenient and informative method among many other experimental methods used for ecological monitoring and assessment of the physiological condition of both individual plants and entire ecosystems (Cendrero-Mateo et al., 2019; Porcar-Castell et al., 2021; Schreiber & Klughammer, 2021).

Today, assessment of chlorophyll fluorescence parameters is used in models of crop productivity formation taking into account their phenotypic development and the corresponding system of successive critical periods of the vegetation (Souza & Yang, 2021), as well as in those that take into account trends in climate change and modern ideas about photosynthetic aspects of plant productivity in these conditions (Gensheimer et al., 2022).

Chlorophyll fluorescence indices are successfully used in assessing the overall stress tolerance of plants (Simeneh, 2020; Legendre et al., 2021; Oláh et al., 2021), plant reactions to heavy metals (Van Zelm et al., 2020; Javed et al., 2022), adaptive response to high and low temperatures (Cetner et al., 2017; Baldocchi et al., 2020; Kim et al., 2021), drought, (Li et al., 2020; Kimm et al., 2021) etc. In recent years, it has been successfully used for early diagnosis of plant diseases and reaction to the degree of damage by pests and herbicides (Hupp et al., 2019; Amri et al., 2021; Sloat et al., 2021).

It also plays an important role in assessing soil nutrition of plants, its balance and efficiency in the formation of biomass and seeds (Larouk et al., 2021; Valcke, 2021).

Proved the possibility of applying the method of CFI as a criterion of optimality of agrotechnological formation of agrocenosis of field crops, especially against the background of different fertilization options (Herritt et al., 2021; Guo et al., 2022).

In addition to this oilseed radish has a wide range of uses in world practice which can be widely used from greening manure and sederation (Tsytsiura, 2019; Ferreira et al., 2021) to biofuel production (Kaletnik et al., 2020, 2021) but there is a lack of study of chlorophyll fluorescence aspects compared to white mustard, spring and winter rape.

Given the above facts, our research hypothesis was to clarify the effectiveness of the CFI method in determining the optimality of pre-sowing design of oilseed radish agrocenosis in the interrelated system of parameters density of standing - row width - level of mineral fertilizer (parameters of pre-sowing agro-technological construction) under the influence of appropriate hydrothermal conditions of its vegetation.

MATERIALS AND METHODS

Experimental conditions

The study of the functioning of the photosystem of oilseed radish plants depending on technological and agroecological factors was carried out during 2015–2020 on the research field (arranged by coordinates N 49°11′31″, E 28°22′16″) located in the zone of the Right-bank Forest-Steppe of Ukraine. The soil cover of the research field is represented by gray forest soils (Luvic Greyic Phaeozem soils (IUSS Working Group WRB, 2015)) with the following agrochemical parameters (for the period of crop rotation): humus 2.02–3.20%, mobile forms of nitrogen 67–92 mg kg^{-1}, phosphorus 149–220 mg kg^{-1}, potassium 92–126 mg kg^{-1} with metabolic acidity of soil solution ($pH_{кcl}$) 5.5–6.0.

The research scheme provided for the use of the spectrum of pre-sowing construction of oilseed radish agrocenosis was presented in a earlier publications (Table 1, Experiment 1). The applied scheme is described by Snedecor (1989) for studying the reaction of plants to a change in the configuration of its individual nutrition area (used by Ukrainian scientists in many studies as the Sinyagin’s scheme (Sinyagin, 1975)). In this scheme the seeding rate (according to the indicator of the total number of plants m^{-2}) and the sowing method (as a criterion for the configuration of the nutrition area and the concretion of plants in the inter-row zone) are separately considered as a factor in the system of ANOVA analysis. Such a dispersion system allows analyzing the impact of sowing rates on the formation of certain indicators by means of a separate analysis based on the configuration of the nutrition area of one plant within the limits of the applied sowing scheme.

For study the peculiarities of the CFI curve formation in comparison with other cruciferous crops, a small plot experiment was carried out (Table 1, Experiment 2) To comply with the rule of uniform abolition, all crops were sown at same time with the same technological variantat (1.5 million pcs. ha^{-1}, inter-row 30 cm, $N_0P_0K_0$). This variant used for all crops in agronomic practice of the research region. All other components of the cultivation technology of the selected group of cruciferous crops were the same.

Table 1. Agrotechnological variants of oilseed radish agrocenosis construction were used in the experiment (Tsytsiura, Ya.H. 2021)

Experiment 1. Specific characteristics CFI of oilseed radish in relation to other cruciferous crops		
Year conditions in the year data expression	Cruciferous crops	Scheme of combinations of variants
Year conditions in the year data expression: 2015–A_1, 2016–A_2, 2017–A_3, 2018–A_4, 2019–A_5, 2020–A_6		
2015–A_1, 2016–A_2, 2016–A_3, 2016–A_4, 2016–A_5, 2016–A_6,	Oilseed radish *(Raphanus sativus* L. var. *oleiformis* Pers.) (B_1) White mustard *(Sinapis alba* L.) (B_2) Spring rape (*Brassica napus* L. var. *oleifera* Metzg.) (B_3) Spring bittercress (*Brassica campestris* var. *oleifera* f. *annua* D.C.) (B_4)	$A_{1–6} \times B_{1–4}$

Experiment 2. Formation of CFI indicators depending on pre-sowing agro-technological construction of the oilseed radish agrocenosis		
Plant placement due to the ratio of the sowing rate (factor B – million germinable seeds ha^{-1}) to the sowing method (factor C – row method (15 cm), wide-row method (30 cm))		Rate of fertilizations (facor D), $kg \cdot ha^{-1}$
4.0 (1.67 (B_1) × 15 (C_1))	2.0 (6.67 (B_1) × 30 (C_2))	$N_0P_0K_0$ (D_1)
3.0 (2.22 (B_2) × 15 (C_1))	1.5 (3.33 (B_2) × 30 (C_2))	$N_{30}P_{30}K_{30}$ (D_2)
2.0 (3.33 (B_3) × 15 (C_1))	1.0 (2.22 (B_3) × 30 (C_2))	$N_{60}P_{60}K_{60}$ (D_3)
1.0 (*6.67 (B_4) × **15 (C_1))	0.5 (1.67 (B_4) × 30 (C_2))	$N_{90}P_{90}K_{90}$ (D_4)
Year conditions in the year data expression: 2015–A_1, 2016–A_2, 2017–A_3, 2018–A_4, 2019–A_5, 2020–A_6		
Scheme of combinations of variants	$A_{1–6} \times B_{1–4} \times D_{1–4}$	The total number of combinations (*N* = 192)

* – distance in a row, cm; ** – width of the row, cm.

Weather conditions

The temperature regime and the humidification regime for the period of research for the stage of formation of oilseed radish yield had significant differences (Fig. 1, Table 2). This allowed to analyze the features of the functioning of the plant photosystem from the standpoint of fluorescence of chlorophyll induction not only in view of agrotechnological variants of agrocenosis, but also from the standpoint of abiotic (agroecological) factors. In particular, the weather conditions in 2015 and 2018 were the most stressful in terms of the impact on plant growth processes and the formation of its assimilation surface in accordance with the values of the hydrothermal coefficient. At the same time, according to the intensity of the increase in average daily temperatures in the absence of atmospheric moisture (Fig. 1) in some years of research, the conditions of 2015 should be considered the most stressful from the standpoint of direct impact from the phenological phase of budding and flowering (Biologische Bundesanstalt, Bundessortenamt and CHemical industry (Test Guidelines…, 2017) (BBCH) 38-64). That is why the accounting indicators of the 2015 experiment were used to detail the effect of temperature and humidity on the induction of chlorophyll fluorescence.

According to the presented data of the stress effect on the growth processes of oilseed radish plants for years of observation 2015–2020 can be placed in the next decrease row 2018–2015–2017–2016–2019–2020.

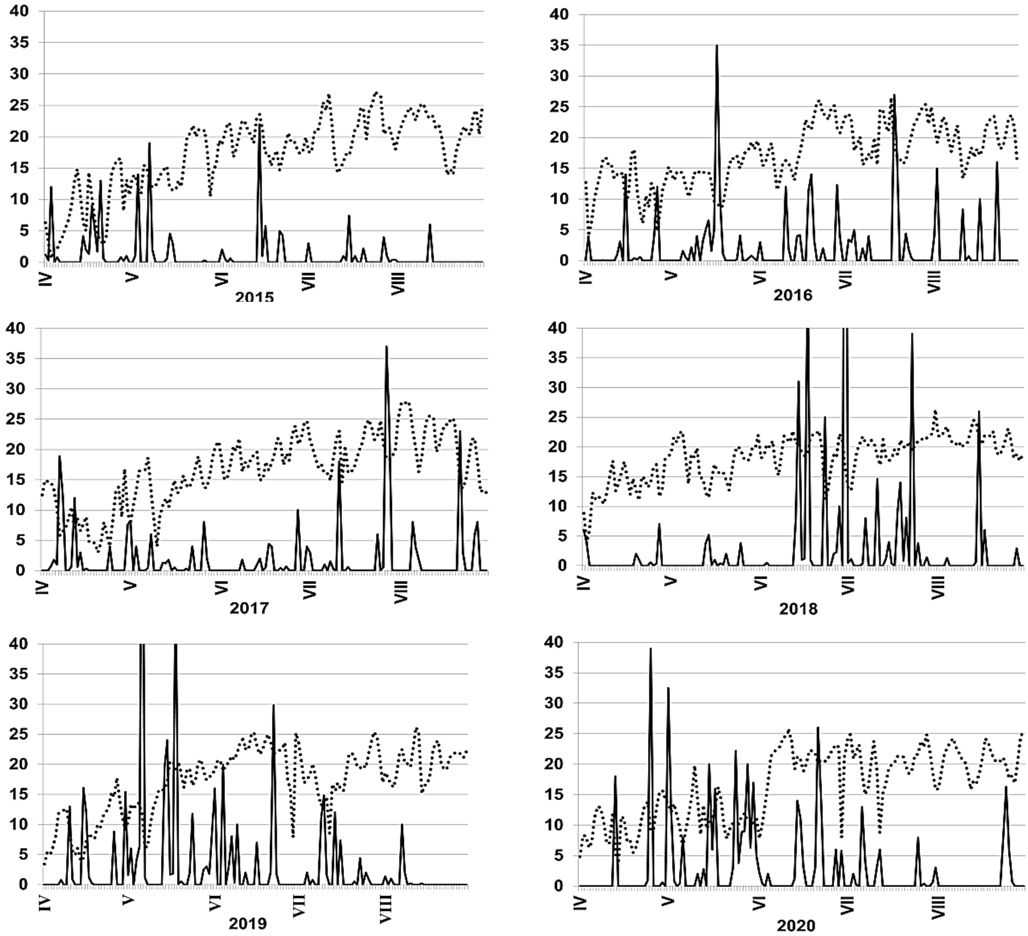


Figure 1. Daily total rainfall and average air temperature during spring triticale growing season, 2015–2020 (dotted line – average daily temperature, °C, continuous line – rainfall, mm) (with added data based Tsytsiura, 2020).

Table 2. Hydrothermal coefficient* for the growing season of oilseed radish (monthly), 2015–2020 (with added data based Tsytsiura, 2020 a)

Year of research	Months					On average for the growing season
	V	VI	VII	VIII	IX	
2015	0.719	0.613	0.230	0.061	0.684	0.430
2016	1.227	0.893	0.682	0.486	0.063	0.663
2017	0.645	0.349	0.806	0.563	1.983	0.824
2018	0.258	3.124	1.349	0.349	0.680	1.179
2019	4.710	1.555	1.003	0.235	0.945	1.690
2020	5.489	1.474	0.649	0.474	1.208	1.859

* – $HTC = \sum R \times (0.1 \times \sum t_{>10})^{-1}$, where the amount of precipitation (ΣR) in mm over a period with temperatures above 10 °C, the sum of effective temperatures (Σt > 10 °C) over the same period, decreased by a factor of 10 (Selyaninov, 1928). Ranking of values: HTC > 1.6 – excessive humidity, HTC 1.3–1.6 – humid conditions, HTC 1.0–1.3 – slightly arid conditions, HTC 0.7–1.0 – arid conditions, HTC 0.4–0.7 – very arid conditions (ranking on the basis: Selyaninov, 1928; Evarte-Bundere & Evarts-Bunders, 2012).

Chlorophyll fluorescence induction method

It was used to measure the parameters of chlorophyll flowering a portable fluorometer 'Floratest'. This fluorometer developed by the scientific and engineering center of microelectronics of the Glushkov Institute of Cybernetics (Romanov et al., 2011). The device is equipped with an LCD screen (128×64 pixels) and a remote optoelectronic sensor with an irradiation wavelength of 470 ± 15 Nm, a spot irradiation area of at least 15 mm^2 and an irradiance within it of at least 2.4 Wm^{-2} (Fig. 2). The spectral range of fluorescence intensity measurements ranges from 670 to 800 nm. The software provided with the device (Portable fluorometer …, 2011). The measurement duration was 4 minutes, the functional period of measurement was 3 minutes.

For measurements, has been used the plant leaf on the phase of the beginning of flowering (BBCH 61-63) from the middle tier by plant height in each series of experiments in the number of 25 for each repetition. Plant leaf was placed in an optical block in the middle part with its upper side toward the light source in. Measurements were taken after leaf adaptation to darkness for 10 min in 4-fold replications for each variant at 90 points with time intervals from 3 microseconds to 300 seconds followed by calculation of relative units for 3 min with registration of changes in chlorophyll fluorescence. The initial measuring point for the 'Floratest' was 1 ms.

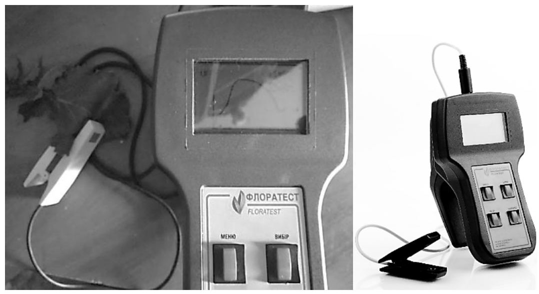


Figure 2. Portable fluorometer 'Floratest' (left) and chlorophyll fluorescence measurements process on oilseed radish leaves (laboratory cultivation).

The measurements were based on the chlorophyll fluorescence induction curve values (Kautsky effect) (Fig. 3) obtained on native leaves.

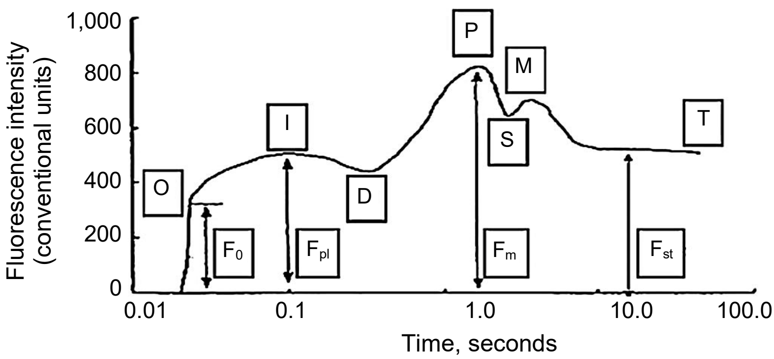


Figure 3. A typical chlorophyll fluorescence induction (CFI) curve (Kautsky curve) (vertical axis – fluorescence intensity (relative units), horizontal axis – time (c)) (Brestic & Zivcak, 2013).

Calculated indicators and statistical analysis

During the experiments, we analyzed the commonly used indices of the curve (Brestic & Zivcak, 2013; Kalaji et al., 2014, 2017a, 2017b), such as:

F_0 – minimal fluorescence (dark) – fluorescence intensity with all PS II reaction centers open while the photosynthetic membrane is in the non-energized state, i.e., dark or low light adapted (O level in the O-I-D-P-T CFI curve);

F_{pl} – value of the 'plateau' fluorescence induction for the time of reaching a temporary slowing down of its signal growth I level in the O-I-D-P-T CFI curve);

F_m – maximal fluorescence (after dark adaptation) – fluorescence intensity with all PSII reaction centers closed all nonphotochemical quenching processes are at a minimum (P level in the O-I-D-P-T CFI curve);

F_{st} – fluorescence in steady state (T level in O–I–D–P–T CFI curve). This is the stationary value of fluorescence induction after the light adaptation of the plant leaf (in our case, its stationary level after 3 min after the start of illumination).

The following parameters were calculated using the standardized CFI curve analysis protocol (Brestic & Zivcak, 2013):

– fluorescence rise according to formula 1:

$$dF_{pl} = F_{pl} - F_0 \tag{1}$$

– maximum variable fluorescence according to formula 2:

$$F_v = F_m - F_0 \tag{2}$$

– index of the effect of exogenous and endogenous factors on the relative number of inactive reaction centers according to formula 3 (Brion et al., 2000; Stirbet & Govindjee, 2011):

$$\frac{dF_{pl}}{F_v} \tag{3}$$

It is also used as an indicator of plant condition regarding the presence of possible phytopathogenic infection, provided this indicator ≥ 0.4–0.5 indicates the presence of infection and significantly increases the probability of detecting viral lesions compared to visual observation (Sarahan, 2011).

– photochemical efficiency or quantum efficiency (EP) according to formula 4, which is an indicator of the impact of exogenous factors and depends on the efficiency of the photochemical reactions of the photosynthetic system II (PS II):

$$EP = \frac{F_v}{F_m} \tag{4}$$

– photochemical quenching (Que) according to formula 5 (Larouk et al., 2021):

$$Q_{ue} = \frac{F_0}{F_v} \tag{5}$$

Leaf water potential (L_{wp}) according to formula 6 (Larouk et al., 2021):

$$L_{wp} = \frac{F_m}{F_0} \tag{6}$$

– plant viability index (according to formula 7), which measures the photosystem II photochemistry efficiency, PS II, and determines the fraction of light absorbed by chlorophyll bound to PS II (photosystem II). It can provide a measure of the rate of linear electron transport and consequently the value of total photosynthesis (Korneev, 2002; Lichtenthaler et al., 2005: Stirbet & Govindjee, 2012; Murchie et al., 2018).

$$RF_d = \frac{F_m - F_{st}}{F_{st}} \tag{7}$$

– indicator of endogenous (stress) factors according to formula 8:

$$K_{ef} = \frac{F_{st}}{F_m} \tag{8}$$

– value of photochemical quenching of fluorescence according to formula 9:

$$QP = \frac{F_m - F_{st}}{F_m - F_0} \tag{9}$$

QP value is affected by both photochemical (CO_2 fixation) and non-photochemical processes (thermal dissipation of energy of the excited state of chlorophyll molecules), – characterizes the adaptability of plants to environmental conditions (Korneev, 2002; Goltsev et al., 2014).

– index of the efficiency of the primary reactions of photosynthesis according to formula 10:

$$K_{prp} = \frac{F_v}{F_0} \tag{10}$$

– fluorescence decay coefficient according to formula 11:

$$K_{fd} = \frac{F_m}{F_{st}} \tag{11}$$

– relative change of fluorescence at time t according to formula 12:

$$V_t = \frac{F_{st} - F_0}{F_m - F_0} \tag{12}$$

To compare the parameters we used a relative value of comparison (%) – the ratio of absolute indicators relating to different objects, but to the same time, calculated according to formula 13 (Rumsey, 2016):

$$k_{\text{comparison}} = \frac{k_1}{k_2} \times 100 \tag{13}$$

where k_1 – indicator of the first object under study, k_2 – indicator of the second object under study.

The seed yield was considered in the format of its biological level by accounting and subsequent calculation of the average seed yield from one plant (in grams) from the general population of plants consisting in the CFI curve indicators accounting.

The studies used varieties (‘Zhuravka’, ‘Raiduha’, ‘Lybid’) of oilseed radish. Given the similarity of the results obtained for the studied of this varieties in the tables and graphics presented only data on the variety ‘Zhuravka’.

The data obtained were analyzed using the analysis of variance (ANOVA) and the GLM procedure. Tukey HSD Test in R (version R statistic i386 3.5.3) was also used. Multiple comparisons of the parameter means computed were performed using the smallest significant difference *LSD* at 5% (95% family-wise confidence level for Tukey HSD Test) for all the parameters studied. Correlation and regression analyzes were performed according to the generally accepted scheme with the statistical software Statistica 10 (StatSoft – Dell Software Company, USA).

RESULTS AND DISCUSSION

Experiment 1. Specific characteristics CFI of oilseed radish in relation to other cruciferous crops

In order to consider the peculiarities of the formation of the basic and calculated indicators indicators of the chlorophyll fluorescence induction curve (CFI) in oilseed radish plants were additionally compared for three species of cruciferous crops, which are widely used in agricultural practice. During the 2015–2020 period of comparative study, a number of significant differences were established in the indicated parameters in oilsed radish (Table 3, Fig. 4).

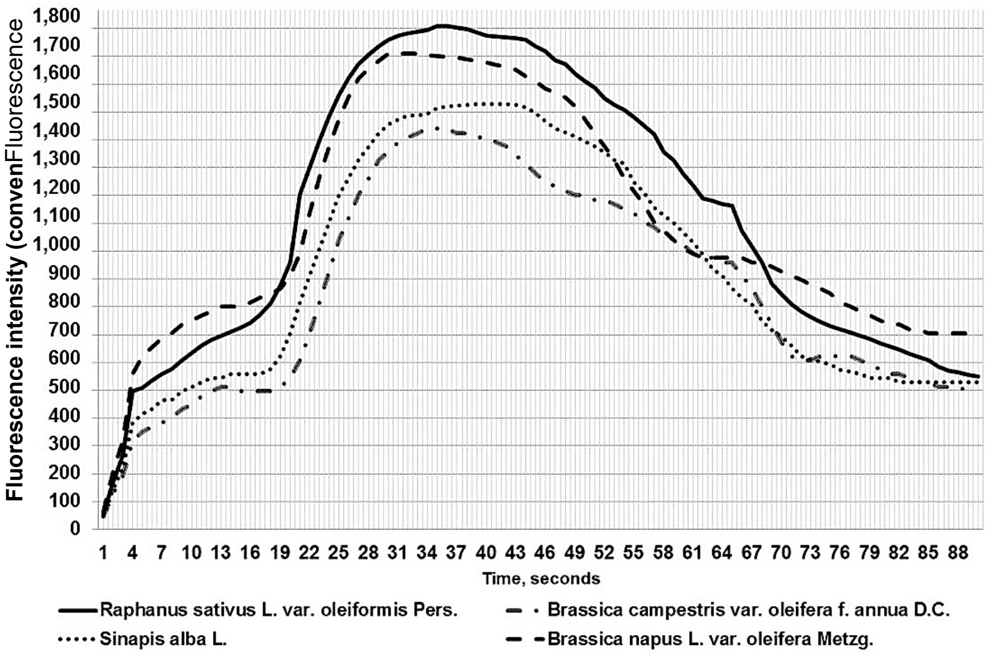


Figure 4. Comparison of CFI curve for four species of cruciferous plants, average average data 2015–2020.

The level of initial fluorescence after dark adaptation F_0 in oilseed radish plants when comparing the average values, was 29.7% higher than in white mustard (*Sinapis alba* L.) and 55.6% higher than in spring bittercress (*Brassica campestris* var. *oleifera* f. *annua* D.C.), however, it is 12.4% lower than that of spring rape (*Brassica napus* L. var. *oleifera* Metzg.).

Such data indicate a specific feature of the transfer of excitation energy from the antenna to the reaction center of the PSII photosystem and the different efficiency of such transfer according to the ergocapacitance indicators of the process. In oilseed radish plants, based on the studies of Kalaji et al. (2016, 2017), Baker & Rosenqvist (2020), Ashrostaghi et al. (2022), the excitation energy transfer system between the pigment molecules of the light-gathering antenna of the PSII cenrer has a high initiation threshold.

Table 3. Basic and calculated values of the CFI curve in selected cruciferous species (relative units of the fluorescence reference for the phase of full flowering of plant species BBCH 65–67), 2015–2020

Plant species	Basic indicators				Estimated indicators and indices											
	F_0	F_{pl}	F_m	F_{st}	dF_{pl}	F_v	dF_{pl}/F_v	EP	L_{wp}	Q_{ue}	RF_d	K_{ef}	QP	K_{prp}	K_{fd}	V_t
Raphanus sativus L. var. oleiformis Pers.	498 ± 56	671 ± 74	1,808 ± 509	551 ± 124	173	1,310	0.132	0.725	3.631	0.380	2.281	0.305	0.960	2.631	3.281	0.040
Brassica campestris var. *oleifera* f. *annua* D.C.	320 ± 42	512 ± 97	1,440 ± 308	496 ± 87	192	1,120	0.171	0.778	4.500	0.286	1.903	0.344	0.843	3.500	2.903	0.157
Sinapis alba L.	384 ± 53	560 ± 114	1,529 ± 314	528 ± 94	176	1,145	0.154	0.749	3.982	0.335	1.896	0.345	0.874	2.982	2.896	0.126
Brassica napus L. var. *oleifera* Metzg.	560 ± 64	800 ± 67	1,711 ± 439	688 ± 122	240	1,151	0.209	0.673	3.055	0.487	1.487	0.402	0.889	2.055	2.487	0.111

*LSD*0.5	F_0	F_{pl}	F_m	F_{st}	The share of influence of experimental factors				
					factors	F_0	F_{pl}	F_m	F_{st}
*LSD*0.5 factor A (year)	8.51	7.67	6.50	5.91	A	19.832	25.862	26.324	28.639
*LSD*0.5 factor B (plant species)	7.57	6.38	5.82	4.84	B	51.321	47.369	53.437	48.251
*LSD*0.5 interaction AB	10.80	11.38	9.59	8.77	AB	28.847	26.769	20.239	23.110

Thus, this is a sign of its stress sensitivity to high and, especially, excessive thickening. It will show a sensitive response to a smaller range of densities than for a species with a low F_0 value, for example, *Brassica campestris* var. *oleifera* f. annua D.C. It is predicted that the reduction of the nutrition area of one plant in oilseed radish will give a more noticeable reaction in the change of the fluorescence induction of chlorophyll than in spring bittercress and white mustard. However, oilseed radish will be more tolerant to changes in the nutrition area than spring rape. This is consistent with findings for other crops in similar studies (Gu et al., 2017; Shomali & Aliniaeifard, 2020; Hosseinzadeh et al., 2021). The conclusion is also consistent with the agrotechnological assessment of the cultivation of the main cruciferous crops and its reaction to changes in the density of placement (Shpaar, 2012; Kayaçetin, 2020).

A similar character was established for the formation of such an indicator as F_{pl} – the value of the fluorescence induction ‘plateau’. For spring mustard and white mustard, its value was significantly lower compared to oilseed radish and spring rape. The maximum value of the indicator of 800 relative units of the fluorescence was noted in spring rapeseed (in total, it is 56.3% more than in spring bittercress, 42.9% than in white mustard, and 19.2% than in oilseed radish). For oilseed radish, there is also a pronounced arc-shaped transition from the maximum value of F_{pl} to the beginning of an intense increase in the induction of chlorophyll. The same character with a pronounced ‘plateau’ was also noted for spring bittercress and with a shift in fixing time for white mustard at the already mentioned lower amplitude. This type of fixation of the F_{pl} indicates differences in the functioning of the studied plant species. In oilseed radish, this confirms its sensitivity to changes in cenosis tension with changes in density, as well as to a possible reaction to changes in the concentration of chlorophyll in case of intensive densification. This is indicated by the findings in the studies and generalisations of Brack & Frank (1998), Brestič et al. (2012), Brestic & Zivcak (2013).

The F_m indicator also had species specificity. The F_m intensity of oilseed radish during the years of study was the highest among the studied species and amounted to 1,018 relative units. For example, this indicator in spring rape was 937 relative units, white mustard – 621, and in spring bittercress – 569. It is known that even with a saturating pulse, F_m depends on the chlorophyll content of the tissue (Brion et al., 2000; Brestic & Zivcak, 2013; Herritt et al., 2020). A decrease in F_m indicates that the object of study is under stress, which means that not all of the PSII electron acceptors can be fully recovered. Considering that the basic stand density for the studied crucifer species of 1.5 million germinable seeds ha^{-1} is one of the recommended in the study area, we assumed about the different stages of physiological state of leaves of different crucifer species at the flowering stage (BBCH 65-67). Given that the studied species in order of prolongation of vegetation can be placed in the following order - spring bittercress - white mustard - spring rape - oilseed radish (Shpaar, 2012) and considering the physiological specificity of crucifers to the maximum reduction of the percentage of leafiness of the stem during plant maturation (Zhang et al., 2020), the indicator F_m indicates that the productive efficiency of the leaf photosystem of spring bittercress is phenostatically shifted to the phenological phase of stemming-budding. Due to this, the age of the leaf at the flowering stage is less than for oilseed radish and spring rape with an intensive peak value of the activity of the assimilation apparatus at the phenological phase of budding-beginning of flowering and a functionally longer period of vital activities of each leaf of plant. For white mustard, an intermediate pattern has been established in the dates indicated. This is confirmed by the leaf water potential (L_{wp}), which is the ratio $F_m F_0^{-1}$. In healthy and young leaves, this ratio is about 4–5, and its value depends on the effect of certain stress factors on the plant, e.g. during drought the ratio can be reduced to 1 (Wang et al., 2016), indicating the destruction of PSII. This is confirmed by our data - the indicator L_{wp} decreases consistently in the row spring bittercress (4.50) - white mustard (3.98) - oilseed radish (3.63) - spring rape (3.05).

Numerous studies confirm that the EP parameter ($F_V F_m^{-1}$) measured in dark adapted plants reflects the potential quantum efficiency of PSII and can be used as a reliable indicator of photochemical activity of the photosynthetic apparatus. For most plants under full development under non-stress conditions the maximum value of this parameter is 0.830 (Björkman & Demmig, 1987; Brestic & Zivcak, 2013; Guidi et al., 2019).

Its decrease means that before the measurement, the plant was subjected to a stress that damaged the S function, resulting in a decrease in electron transport efficiency. This is often observed in plants exposed to various stress factors (Kalaji et al., 2017b; Petjukevics & Skute, 2022; Feria-Gómez et al., 2022). The change in EP parameter value is considered the most sensitive indicator of the effect of photo-inhibition (the phenomenon of suppression of photosynthesis and damage to the photosynthetic apparatus at high light intensity) (He et al., 1996). The EP parameter can also be used as an indicator of the degradation of D_1-protein (an important element of PSII), which has been damaged during stress, e.g., light stress, or modified for other reasons, leading to inactivation of FS reaction centres (Rintamäki et al., 1995). However, during prolonged stress, there is a significant reduction in EP, which is also accompanied by a decrease in photosynthetic intensity (Flexas e al., 2004; Posudin & Bogdasheva, 2010; Brestič et al., 2012; Brestic & Zivcak, 2013; Stirbe et al., 2014; Kalaji et al., 2017b; Keller et al., 2019; McAusland et al., 2019; Pérez-Bueno et al., 2019; Casto et al., 2021). Thus, taking into account that the EP is an effective indicator of the optimality of growth and physiological processes of plants associated with their photo-assimilating activity, the agro-technological parameters of 1.5 million pcs. ha^{-1} of germinable seeds on an unfertilized variant are the most optimal in such a reduction series of species - spring bittercress - white mustard - oilseed radish - spring rape. This is also indicated by other calculated coefficients of intensity and quality of chlorophyll fluorescence process, in particular Q_{ue}, R_{fd}, V_t.

It should be noted that a special area on the CFI curve in the period of 62–65 seconds of fixation of the device. This segment of the curve is noted in all years of research and well expressed in oilseed radish. According to Korneev (2002), such a transition on the segment of the F_m–F_{st} curve indicates physiological mechanisms of preadaptation of the PSII photosystem during the transition to stationary fluorescence. According to Flexas et al. (2002) this characterizes the stress sensitivity of the species in the area of transition from the active expression of maximum fluorescence to its stationary level. The low severity of this area on the CFI curve in white mustard is apparently due to the higher rate of physiological aging of leaves than in other cruciferous species in the analyzed group, as indicated in the publications Hasanuzzaman (2020).

In our study the parameter F_{st} should also be mentioned separately. This parameter characterises the total intensity of chlorophyll fluorescence emitted by photosynthetic objects under steady-state light conditions. After dark adaptation, it takes about 3–5 minutes for CFI curve to reach stationary state (F_{st} level). At this point, there is an equilibrium between the assimilative force production in photochemical reactions

(ATP and NADPH molecules) and the enzymatic reactions that use these molecules in the dark phase. Any disruption of photosynthetic reactions (e.g. caused by stress factors) delays reaching stationary state (F_{st}) (Korneev, 2002; Kalaji et al., 2017, 2017a). It is noted (Ruban & Murchie, 2012; Mathur et al., 2014; Magney et al., 2020) that under intensive stress as well as natural leaf ageing, the F_{st} value can be significantly higher than the initial F_0 value and vice versa under optimum conditions. Under excessive stress factor conditions, the time to reach F_{st} is intensively shortened compared to normal or optimum growth and development conditions for the plant species. The results of our studies in the case of the value of F_{st} for the studied plant species confirmed the findings regarding the phenostasis of leaf development and photosynthetic system of different cruciferous species at the flowering date, and also allow us to indicate the specificity of photosystems of oilseed radish and other studied species, for which the difference between F_0 and F_{st} parameters was 53 relative units, and for spring bittercress with a similar index this value was 176 relative units. Thus, leaf apparatus of oilseed radish is highly stress-sensitive due to the possibility of using the difference index F_0 and F_{st} to assess the overall level of adaptivity of the plant's photosystem. These conclusions are confirmed by similar studies on a number of other plant species (Liu et al., 2017; Baba et al., 2019; Maguire et al., 2020; Dechant et al., 2020).

Should also note that according to the value of photochemical quenching fluorescence (QP), which characterizes the overall adaptability of plants to environmental conditions (Korneev, 2002), the studied species according to their QP values can be placed in the following order: oilseed radish (0.960) - spring rape (0.889) - white mustard (0.874) - spring bittercress (0.843).

The species viability index (R_{Fd}), which, when applied to the calculated indexes of the CFI curve, shows the threshold level of the environmental stressor (Korneev, 2002; Gururani et al., 2015), this series has a different character according to our estimates: oilseed radish (0.695) - spring bittercress (0.656) - white mustard (0.655) - spring rape (0.598).

It can be stated, based on the analysis of the basic and derivative indices of the CFI curve, that oilseed radish has both a high level of adaptability and also capable of long-term resistance to high levels of exogenous stress factors, unlike spring rape, in which the adaptive potential has limitations relative to the level of stress factor value.

The adaptability of the species is also characterised by the relative fluorescence variable at time t - index V_t. According to received data, which are also confirmed by Brestic & Zivcak (2013), a lower level of V_t indicates that the photosynthetic and photochemical processes in the plants are optimal against the respective hydrothermal regimes of the environment, determining the smoothness of the threshold indicative points of the CFI curve. For oilseed radish in particular, this indicator was 69.5% lower than the average value for the other species during the study period.

The significance of the above mentioned species-specific differences in the main parameters of the CFI curve is confirmed by the results of analysis of variance of the experiment data, where the interval of values within the limits of specific indicators was 47–53%, and their formation was significantly influenced by hydrothermal conditions of vegetation in the corresponding interval of the influence share 19–29%. Given similar studies (Naidu & Long, 2004; Murata et al., 2007; Kuhlgert et al., 2016; Østrem et al., 2018; Cong et al., 2022), such a level of dependence classifies cruciferous crops as edaphic sensitive species and the chlorophyll fluorescence induction method itself as a reliable indicator of optimal or stressful agrocenosis formation of these crops. Thus, the identified features of the formation of basic and indicative indicators of the CFI curve confirmed the possibility of their use to identify the level of stress both environmental conditions and agrotechnological factors of cultivation technology, which can be considered as systemic stress factors in the system of assessment of photosynthetic activity of plants. These conclusions are confirmed by a number of studies in the field of chlorophyll fluorescence for the analysis of stress factors of agrocenoses of various agricultural crops, in particular Arnetz (1997), Raza et al. (2017), Baker & Rosenqvist (2020), Żelazny & Lukáš (2020), Viljevac et al. (2022).

Experiment 2. Formation of CFI indicators depending on pre-sowing agro-technological construction of the oilseed radish agrocenosis

The values of the CFI curve (Table 4) give reason to affirm that the density of oilseed radish plants, sowing method and the mineral fertilizers was stress factors that affect plant growth and development, the formation of corresponding plant mass and corresponding levels of photosynthetic activity. It was determined that the value of the F_0 criterion decreases with the reduction of the area of plant nutrition.

A constant tendency to decrease F_0 within the studied options and the minimum values of this indicator for the maximum area of plant nutrition (variant 0.5 million germinable seeds ha^{-1}) was established. The indicative reaction of oilseed radish plants to the change in the density of the agrocenosis in the reaction of photosynthetic processes was confirmed.

Given the steady downward trend in F_0 within the studied variants and the minimum values of this indicator at minimum seeding rates for both sowing methods, oilseed radish should be attributed to species with a highly sensitive response to its agrocenosis density, which will be indirectly expressed in photosynthetic processes as well. The percentage reduction in F_0 in comparing the seeding rates thresholds was 24.0% for row sowing and 19.7% for wide-row sowing.

The percentage decrease in the value of F_0 for the averaged fertilizer group in the comparison of marginal technological options for row sowing was 24.0%, and for the same interval of wide-row sowing 19.7%. In fact, the studied options detailed the configuration of the area of nutrition of oilseed radish plants. With the row method of sowing, it approaches a square shape, and with wide-row sowing, it approaches a rectangular shape. That is, the change in the configuration of the power supply area affected the CFI indicators. Judging by the dynamics of oilseed radish, it was tolerant to higher levels of density in a row, provided that the inter-row distance is optimized accordingly. According to the value of F_0 on variants of 30 cm row spacing, the value of reducing the distance between plants in a row within 5% of the technologically appropriate will not significantly affect the intensity of photosynthetic reactions of the PSII photosystem of plants. At the same time, the use of mineral fertilizers had a twofold effect. Fertilization in the application rate of $N_{90}P_{90}K_{90}$ in variants with a minimum plant nutrition area (1.67×15.0 cm) ensured an increase in F_0 by an average of 17.0%, creating the prerequisites for plant miniaturization and the formation of depressed architecture (noted in the studies of Cui et al. (2003)). At the same time and also enhances competitive physiological growth plant processes, causes its idiotipic depression, signs of decreased chlorophyll content (by colour change) and acceleration of the intensity of phenological staging of the assimilating plant surface.

Table 4. Baseline and estimated indicators of the CFI curve in the oilseed radish variety 'Zhuravka' depending on technological variants of pre-sowing agro-technological construction its agrocenosis, 2015–2020 (in relative units for flowering phase BBCH 65)

Plant placement	Fertilizer	Basic indicators				Estimated indicators and indices											
		F_0	F_{pl}	F_m	F_{st}	dF_{pl}	F_v	dF_{pl}/F_v	EP	L_{wp}	Q_{ue}	RF_d	K_{ef}	QP	K_{prp}	K_{fd}	V_t
4.0 million, row	1	565	662	1,420	615	97	855	0.113	0.602	2.51	0.661	1.309	0.433	0.942	1.513	2.309	0.058
	2	581	684	1,571	637	103	990	0.104	0.630	2.70	0.587	1.466	0.405	0.943	1.704	2.466	0.057
	3	624	677	1,512	684	53	888	0.060	0.587	2.42	0.703	1.211	0.452	0.932	1.423	2.211	0.068
	4	661	697	1,478	725	36	817	0.044	0.553	2.24	0.809	1.039	0.491	0.922	1.236	2.039	0.078
3.0 million, row	1	522	612	1,509	557	90	987	0.091	0.654	2.89	0.529	1.709	0.369	0.965	1.891	2.709	0.035
	2	536	630	1,611	584	94	1,075	0.087	0.667	3.00	0.499	1.759	0.363	0.955	2.006	2.759	0.045
	3	546	632	1,644	598	86	1,098	0.078	0.668	3.01	0.497	1.749	0.364	0.953	2.011	2.749	0.047
	4	561	625	1,608	617	64	1,047	0.061	0.651	2.87	0.536	1.606	0.384	0.947	1.866	2.606	0.053
2.0 million, row	1	492	641	1,735	526	149	1,243	0.120	0.716	3.53	0.396	2.298	0.303	0.973	2.526	3.298	0.027
	2	500	662	1,791	543	162	1,291	0.125	0.721	3.58	0.387	2.298	0.303	0.967	2.582	3.298	0.033
	3	503	681	1,819	552	178	1,316	0.135	0.723	3.62	0.382	2.295	0.303	0.963	2.616	3.295	0.037
	4	514	675	1,824	567	161	1,310	0.123	0.718	3.55	0.392	2.217	0.311	0.960	2.549	3.217	0.040
1.0 million, row	1	461	655	1,824	488	194	1,363	0.142	0.747	3.96	0.338	2.738	0.268	0.980	2.957	3.738	0.020
	2	464	674	1,902	490	210	1,438	0.146	0.756	4.10	0.323	2.882	0.258	0.982	3.099	3.882	0.018
	3	463	692	1,944	484	229	1,481	0.155	0.762	4.20	0.313	3.017	0.249	0.986	3.199	4.017	0.014
	4	459	711	1,978	477	252	1,519	0.166	0.768	4.31	0.302	3.147	0.241	0.988	3.309	4.147	0.012
2.0 million, wide-row	1	505	642	1,690	576	137	1,185	0.116	0.701	3.35	0.426	1.934	0.341	0.940	2.347	2.934	0.060
	2	515	667	1,718	592	152	1,203	0.126	0.700	3.34	0.428	1.902	0.345	0.936	2.336	2.902	0.064
	3	543	672	1,704	624	129	1,161	0.111	0.681	3.14	0.468	1.731	0.366	0.930	2.138	2.731	0.070
	4	562	663	1,694	651	101	1,132	0.089	0.668	3.01	0.496	1.602	0.384	0.921	2.014	2.602	0.079
1.5 million, wide-row	1	498	671	1,808	551	173	1,310	0.132	0.725	3.63	0.380	2.281	0.305	0.960	2.631	3.281	0.040
	2	505	694	1,876	559	189	1,371	0.138	0.731	3.71	0.368	2.356	0.298	0.961	2.715	3.356	0.039
	3	509	709	1,952	561	200	1,443	0.139	0.739	3.83	0.353	2.480	0.287	0.964	2.835	3.480	0.036
	4	511	703	1,935	571	192	1,424	0.135	0.736	3.79	0.359	2.389	0.295	0.958	2.787	3.389	0.042

Table 4 (continued)

1.0 million, wide-row	1	459	659	1,847	503	200	1,388	0.144	0.751	4.02	0.331	2.672	0.272	0.968	3.024	3.672	0.032
	2	457	674	1,894	492	217	1,437	0.151	0.759	4.14	0.318	2.850	0.260	0.976	3.144	3.850	0.024
	3	455	692	1,929	484	237	1,474	0.161	0.764	4.24	0.309	2.986	0.251	0.980	3.240	3.986	0.020
	4	452	703	1,987	497	251	1,535	0.164	0.773	4.40	0.294	2.998	0.250	0.971	3.396	3.998	0.029
0.5 million, wide-row	1	432	668	1,956	481	236	1,524	0.155	0.779	4.53	0.283	3.067	0.246	0.968	3.528	4.067	0.032
	2	428	683	2,028	475	255	1,600	0.159	0.789	4.74	0.268	3.269	0.234	0.971	3.738	4.269	0.029
	3	425	711	2,104	465	286	1,679	0.170	0.798	4.95	0.253	3.525	0.221	0.976	3.951	4.525	0.024
	4	421	719	2,189	457	298	1,768	0.169	0.808	5.20	0.238	3.790	0.209	0.980	4.200	4.790	0.020

$LSD_{0.5}$	F_0	F_{pl}	F_m	F_{st}	The share of influence of experimental factors				
					factors	F_0	F_{pl}	F_m	F_{st}
$LSD_{0.5}$ factor A (year)	4.81	5.74	5.90	3.70	A	20,116	40,244	28,095	29.970
$LSD_{0.5}$ factor B (sowing method)	2.77	3.31	3.40	2.14	B	19,330	8,447	19,545	6,330
$LSD_{0.5}$ factor C (seeding rate)	3.92	4.68	4.82	3.02	C	26.595	9.473	42.346	39.300
LSD_{05} factor D (fertilizer)	3.92	4.68	4.82	3.02	D	13.462	16.431	5.662	12.670
$LSD_{0.5}$ interaction AB	6.80	8.11	8.34	5.24	AB	0.020	0.009	0.076	0.023
$LSD_{0.5}$ interaction AC	9.61	11.47	11.79	7.41	AC	0.028	0.010	0.164	5.240
$LSD_{0.5}$ interaction AD	9.61	11.47	11.79	7.41	AD	0.014	0.017	0.022	0.010
$LSD_{0.5}$ interaction BC	5.55	6.62	6.81	4.28	BC	11,908	21.323	2.579	1.224
$LSD_{0.5}$ interaction BD	5.55	6.62	6.81	4.28	BD	2.613	0.240	0.038	0.732
$LSD_{0.5}$ interaction CD	7.85	9.37	9.63	6.05	CD	2.380	2,762	0.496	4.333
$LSD_{0.5}$ interaction ABC	13.59	16.22	16.68	10.47	ABC	0.013	0.022	0.010	0.005
$LSD_{0.5}$ interaction ABD	13.59	16.22	16.68	10.47	ABD	0.003	0.001	0.001	0.003
$LSD_{0.5}$ interaction ACD	19.22	22.94	23.59	14.81	ACD	0.003	0.003	0.002	0.016
$LSD_{0.5}$ interaction BCD	11.10	13.25	13.62	8.55	BCD	3,511	1.017	0.960	0.143
$LSD_{0.5}$ interaction ABCD	27.19	32.44	33.36	20.95	ABCD	0.004	0.001	0.004	0.001

A factor – year conditions. Fertilizer options consistently: 1 – $N_0P_0K_0$; 2 – $N_{30}P_{30}K_30$; 3 – $N_{60}P_{60}K_{60}$; 4 – $N_{90}P_{90}K_{90}$.

The analysis of the F_0 indicator for other technological options of the plant nutrition area allowed to determine the optimal rates of fertilizers. It was $N_{30}P_{30}K_{30}$ for the interval 3.0–4.0 million germinable seeds ha^{-1} and $N_{60}P_{60}K_{60}$ for the interval 1.5–2.0 million germinable seeds ha^{-1}. For the remaining technological variantss, it can be increased to $N_{60}P_{60}K_{60}$. These results are also confirmed by the values of the other two basic parameters of the CFI curve, Fpl and Fm, which demonstrated similar dynamics of changes within the studied variants.

It was established that under conditions of saturated light intensity, the maximum value of fluorescence on the induction curve was due to the dynamic balance between the processes of fluorescence, photochemistry, and thermal dissipation. It is believed that at point Fm, under conditions of maximum fluorescence, photosynthesis is at a minimum level.

This index is the most variable of all others due to adaptive changes in the structure of the pigment complex (Nesterenko et al. (2006). When there is insufficient light, both light-gathering and aerial chlorophylls increase, which is accompanied by an increase in the F_m level (Brestic & Zivcak, 2013). These are the reasons that lead to the largest range of F_m values, both when evaluating the formation of CFI curve indicators in different types of cruciferous crops (Table 3) and its value for the studied variants (Table 4).

The dynamics of formation of Fm was similar to the change of the F_0 indicator. It was established that the optimal variants of the plant nutrition area also had a higher F_m index. This is explained by the reaction of the F_{pl}–F_m section of the CFI curve to factors such as changes in plant illumination, damage to the photosystem by various types of infections and pests, chlorophyll concentration, and the level of mineral nutrition. On the basis of this, the optimal agrotechnological intervals for the nutrition area of oilseed radish plants was similar to the determination based on the evaluation of the F_0 index. Similar conclusions were made in other studies (Janušauskaite & Feiziene, 2012; Tikkanen et al., 2014; Kalaji et al., 2017 and 2017b; Tsai et al., 2019; Hou et al., 2021; Tomaškin et al., 2021; Valcke, 2021).

We should also note a certain specificity of fluorescence formation of the 'plateau' zone F_{pl}, the value of which had a more significant response to the fertilizer system than to the variants of seeding rate and seeding method. This is indicated by the values of the factor influence results in the multifactor dimensional analysis system, where the latter accounted for 8.4–9.5% and the fertilizer for 16.4%. We assume that this dependence can be explained by the nature of the F_{pl} value. The fluorescence index at the F_{pl} level is due to rapid energy saturation by reaction centres (RC) that do not transfer energy to the electron-transport chain (they do not recover the primary acceptor QA and thus there are reaction centres that do not recover the electron-transport chain). The F_{pl} index expresses the slowing down stage of the CFI curve and determines certain species-specific features of the levels of plant photosystem organization. The species specificity in this case is characterized by a rather narrow interval spectrum of F_{pl} values within the range of 610–720 relative units with predominance of even narrower interval of 630–660 relative units. Additional mineral nutrition due to the optimization of the photosystem and the increase in the concentration of chlorophyll (which was noted in the studies of Nesterenko et al. (2006) and Kalaji et al. (2017 and 2017a)) provided higher F_{pl} values.

It should be noted that the EP (F_v/F_m) index we mentioned earlier, known in the literature as the 'maximum quantum efficiency' of PSII (Kalaji et al., 2017b) is a measure sensitive to photosynthetic productivity and effective in assessing photoinhibition.

Researchers experimentally determined for other plants that this indicator heads towards 1 when the physiological state of the plant is normal (Korneev, 2002; Kalaji et al., 2017a). According to these statements, the possibility of significantly optimizing the functioning of the photosystem of oilseed radish plants in such options as 1.0 million germinable seeds ha^{-1} (row spacing 15 cm, $N_{90}P_{90}K_{90}$) and 0.5 million germinable seeds ha^{-1} (row spacing 30 cm, $N_{90}P_{90}K_{90}$) was established. For these variants, the EP indicator was 0.768 and 0.808, respectively.

The defined variants are characterized by the highest plant viability index (RF_d), high level of leaf water potential (L_{wp}), which essentially indicates the physiological activity of leaves and the level of their watering (Lei et al., 2006; Evans, 2009). Other positive features of these variants are the reduced photochemical quenching (Q_{ue}) and the significantly higher photosynthetic primary reaction efficiency (K_{prp}) and the lowest endogenous stress factor level (K_{ef}). When comparing the variants 4.0 million pcs. ha^{-1} of germinable seeds and 0.5 million pcs. ha^{-1} of germinable seeds, we should note a 23.8% decrease in the intensity of photochemical reactions. According to Valcke (2021) this pattern of ratio makes the variant of oilseed radish cultivation by row seeding with the rate of 4.0 million pcs. ha^{-1} of germinable seeds to be clearly stressful with formation of plants with reduced vitality morphological and physiological features and ultimately forms a significantly lower biomass and seed productivity of the cenosis.

The conclusions made above and the results of the evaluation of criterion F_{st} are confirmed. It is worth noting that the degree of decrease in chlorophyll fluorescence from maximum (F_m) to steady-state (F_{st}) is often used as an integral indicator of the activity of the photosynthetic apparatus of plants. It was noted (Nesterenko et al., 2006; Logan et al., 2014; Jonathan, 2017) that F_{st} index the amount of chlorophyll not involved in energy transfer to PSII reaction centres. An increase in this index indicates an inhibition of the outflow of reduced photoproducts from reaction centres as a result of unfavourable environmental factors. It is noted, that the value of this index can serve to diagnose the intensity of the stress factor by estimating the time of its achievement on the CFI curve, the ratio of its value between the initial chlorophyll fluorescence induction (F_0) and its maximum value, and by comparing the difference between the value of F_0 and F_{st} (Roháček, 2002; Nesterenko et al., 2012; Schreiber & Klughammer, 2013; Murchie et al., 2018). Moreover, Flexas et al. (2002) recommend the use of steady-state fluorescence to indicate the water status of plants by the ratio of F_{st} and F_0. For oilseed radish was established that the value of the steady-state fluorescence Fst is higher than the value of its initial level F_0. This difference amounted to 27–71 conventional fluorescence units.

It is important to note, that chloroplasts of oilseed radish leaves in the variants of higher area of plant nutrition with a maximal fertilization rate are characterized by a considerable drop of chlorophyll fluorescence from F_m to stationary level F_{st}, that is a sign of intensive course of dark photochemical reactions, which is an evidence of optimization of vitamin tactics and physiological photochemical processes of plants. Certain aspects of this were confirmed by research Sepúlveda & Johnstone (2019), Schuback et al. (2021).

It is installed that the F_{st} value for variants with maximum density of oilseed radish plants had significantly higher values than in the variants with minimum density. When applied to the F_{st} index, the optimality of the technological variant can be assessed by comparing the F_0 and F_{st} indexes. Closeness of their values against the background of considerably according to F_m index criterion results in decrease of the indicator of endogenous (stress) factors K_{ef}, the value of which characterizes the total intensity of stress on edaphic conditions (according to Schreiber & Klughammer, 2013; Ruban et al., 2016) and increases the value of photochemical quenching of fluorescence QP (according to Schreiber et al., 1995; Maxwell & Johnson, 2000; Korneev, 2002; Larouk et al., 2021)). Thus, in comparing the variants 4.0 and 0.5 million pcs. ha^{-1} of germinable seeds the value of K_{ef} was 47.6% lower for the lower limit variant of nutrition area. The level of QP was higher on the average by 3.8% on the background the reduction of the rate of relative variable fluorescence (V_t) by 57.7% and increasing of the rate of photosynthetic primary reactions (K_{prp}) by 2.8 times.

Specific information about the physiological state of plants within the studied experimental variants was obtained by analyzing the ratio $dF_{pl}\ F_v^{-1}$. However, under these excitation light conditions, this ratio can characterise the infectivity of the plants with viruses (according to Korneev, 2002; Bonfig et al., 2006; Christen et al., 2007; Romanov et al., 2010; Burling et al., 2011; Amri et al., 2021). In particular, in research Kirik et al. (2011) an excess of dF_{pl}/F_v over 0.4 at excitation light intensities that do not saturate the pigment matrix in energy indicates a high probability of plant viral infection.

In our studies, this coefficient was in the range 0.044–0.170 within the variants, which may indicate that the plants are not infected with viral infection. Still, the tendency for its value to increase with decreasing seeding rate and, consequently, the density of the plants, indicates an increase in the potential for plant infections in oilseed radish by optimizing growth processes, an intensive increase in vegetative mass, and as was shown earlier before the increase of leaf watering by the water potential indicator L_{wp}.

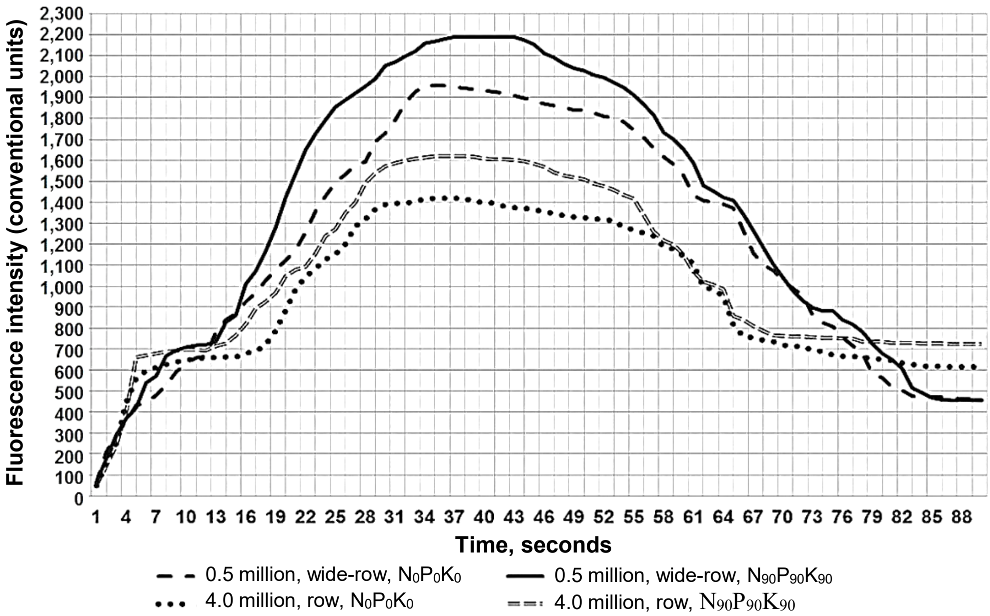
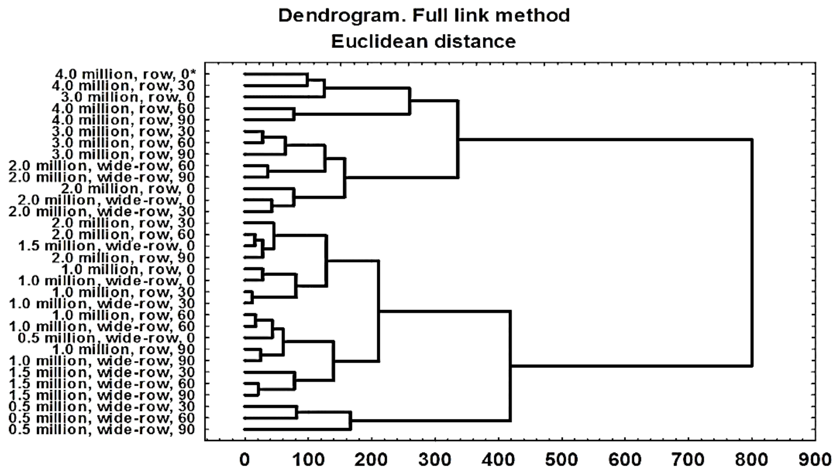


Figure 5. Induction changes of CFI curves in oilseed radish variety 'Zhuravka' under different variants of research, average data 2015–2020.

Visualization of the peculiarities of the formation of the parameters of the CFI curve (Fig. 5) clearly confirmed the described results. The overall stressfulness of the variant with 4.0 million pcs. ha^{-1} of germinable seeds according to the main accounting basic parameters has been confirmed. This interval as stressful can also be identified by the characteristic part of the CFI curve (62–65 seconds of registration) of beveled plateau-like character, which at a lower plant nutrition area had a shorter fixation period and less pronounced character with a smoother transition to steady fluorescence (F_{st}). This is confirmed by the results of the visual assessment of the CFI curves for the different plant species carried out in the studies by Lootens et al. (2004), Lichtenthaler et al. (2005), Baležentienė & Šiuliauskienė (2007), Brestic & Zivcak (2013), Murchie & Lawson (2013), Kalaji et al. (2017b).

Our conclusions are also supported by the results of the summary cluster analysis of the baseline indicators of the CFI curve (Fig. 6). By value of Euclidean distance, variants with seeding rates of 4.0 and 3.0 million pcs. ha^{-1} of germinable seeds in row sowing have essential criterial distance in values of induction indices in relation to variants with seeding rates in the interval of 0.5–1.0 million pcs. ha^{-1} of germinable seeds. As for seeding methods, the nature of the Euclidean distance indicates that the intensity of the decrease in the values in the cluster system for string sowing covers agrotechnological variants from 2.0 million pcs. ha^{-1} of germinable seeds without fertilizer to 3.0–4.0 million pcs. ha^{-1} of similar seeds for all variants. application of fertilizer. For wide-row sowing, this interval has a smaller technological range and includes variants from 1.5 million pcs. ha^{-1} of germinable seeds without fertilizer to 2.0 million pcs. ha^{-1} of germinable seeds against all fertilizer options.



* the application rate of fertilizers in kg ha^{-1}.

Figure 6. Dendrogram of cluster analysis (full link method) for baseline indicators of CFI curve in the context of the studied technological variants, average data 2015–2020.

Based on this, it was concluded that from the position of providing the optimization of photosynthetic apparatus activity of oilseed radish plants the configuration of plant nutrition area is essential for oilseed radish, and the change of row spacing allows to shift the expediency of applied seeding rate to higher level of predicted calculation of plant productivity increase its biological level. That is to say with reference of Driever et al. (2014) the combination of appropriate levels of plant photoassimilation with predicted productivity of 1 m^2 of accounting area was achieved.

The results of summary clustering of data allow and confirm the stress of application of high rates of fertilizers with an increase in seeding rate over 2.0 million pcs. ha^{-1} of germinable seeds for row sowing of oilseed radish and over 1.5 million pcs. ha^{-1} of germinable seeds for wide-row sowing variant. This indicates the closeness of Euclidean distances for variants with the absence of fertilizer and variants with a fertilization rate of $N_{90}P_{90}K_{90}$.

This is confirmed by the closeness of the Euclidean distances for variants with a fertilizer rate of 0 and 90 kg ha^{-1}. With an increase in the area of plant nutrition, paired systems of combinations of Euclidean distances were noted in the variants of technological pairs of 60–90 kg ha^{-1} for close technological groups in terms of the area of plant nutrition.

More detailed and deep analysis of the features of CFI curve indication is performed by their correlation in a single system, which, according to Larouk et al. (2021) allows to determine the combinatorics of its influence in the physiological process of photoassimilation and plant photosystem functioning. Based on the values, the strongest correlations are found for the maximum fluorescence (F_m) with high marginal correlation values, either directly or inversely related to other indicators in a pairwise correlation (Table 5). Accordingly, the least significant correlation among the correlation pairs of the comparison is found for the 'plateau' zone fluorescence indicator (F_{pl}). In our opinion, this can be explained by the nature of the role of these indicators in the dynamic processes and energetics of the CFI curve formation. The Fm value is the main indicator of the fluorescence process, which determines the overall intensity of the process and the psolidating nature of its attenuation rates. The range of correlation coefficients is intrinsic to the system of such analyses, as observed in both the earlier (Korneev, 2002), and more recent (Bussotti et al., 2020; Larouk et al., 2021; Rao et al., 2021) publications. It is noted that the value of the correlation can change both in magnitude and direction.

Table 5. Range of the correlation coefficients of the CFI curve (range of values during 2015–2020)

	F_{pl}	F_m	F_{st}	F_v	EP	L_{wp}	Q_{ue}	ISP
F_0	0.634–0.732***	-0.468**–-0.844***	0.394*–0.768***	-0.387*–-0.792***	-0.454**–-0.846***	-0.602**–-0.945***	0.396*–0.946***	-0.381*–-0.601**
F_{pl}		0.342*–0.487**	-0.076^{ns}–-0.275*	0.243*–0.334*	0.090^{ns}–0.262*	0.094^{ns}–0.264*	0.053^{ns}–-0.264*	0.426**–0.569**
F_m			-0.643**–-0.807***	0.762***–0.992***	0.731***–0.963***	0.728***–0.964***	-0.730***–-0.964***	0.628**–0.839***
F_{st}				-0.642**–-0.840***	-0.680**–-0.910***	-0.681**–-0.910***	0.680**–0.911***	-0.505**–-0.641**
F_v					0.739***–0.986***	0.727***–0.981***	-0.718***–-0.977***	0.678**–0.818***
EP						0.763***–0.987***	-0.732***–-0.985***	0.451**–0.773***
L_{wp}							-0.755***–-0.989***	0.449***–0.779***
Q_{ue}								-0.459**–-0.775***

Legend: ISP – Individual Seed Productivity (g plant^{-1}); ns not significant at 5%; *, **, *** Significant at 5%, 1%, 0.1% level probability, respectively.

This process is primarily influenced by stress factors such as drought, high or low temperatures, plant diseases or viral infection. In our case, we observed only a significant decrease in the indicator across a range of dependencies, often in the range of two or threefold decrease, as Table 5 clearly shows. It should be noted that the tightness of the relationship increased as the stress of the year increased and, conversely, tended to decrease as the parameters optimised. However, cases of directional changes in dependence were rare. According to the findings of Bussotti et al. (2020), this indicates that the baseline indicators of the CFI curve for oilseed radish can be used in a system for assessing the adaptive strategy in terms of its vitality tactics.

An indicator of seed productivity of plants was introduced into the system of correlations, as a result criterion of the optimality of the area of plant nutrition.

Analysis of the seed yield of oilseed radish plants within the studied agrotechnological variants (Fig. 7) showed significant differences between them in the value of the existing indicator of the biological level of individual seed productivity over a multi-year evaluation period.

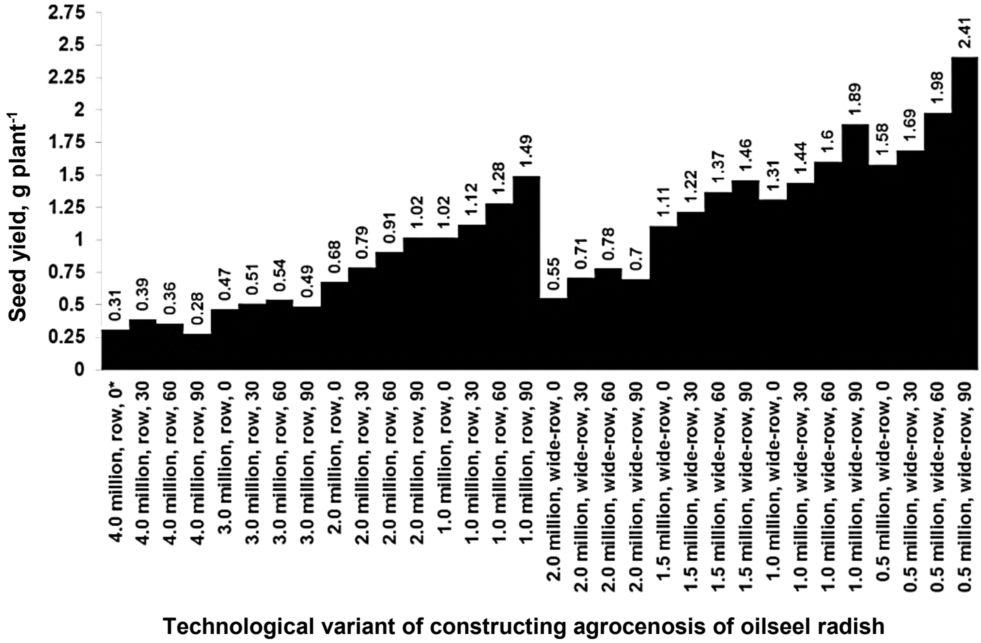


Figure 7. Individual seed productivity of oilseed radish plants (biological level) of 'Zhuravka' variety in various variants of experiment 2, average data 2015–2020.

(For factors A – year, B – sowing method, C – seeding rate, D – fertilizer: *LSD*$_{05}$: A – 0.08, B – 0.05, C – 0.06, D – 0.06, AB – 0.11, AC – 0.16, AD – 0.16, BC – 0.09, BD – 0.09, CD – 0.13, ABC – 0.22, ABD – 0.22, ACD – 0.32, BCD – 0.18, ABCD – 0.45. Fraction of the resultant effect of experience factors on the resultant trait (%): A 21.75; B 14.10; C 34.73; D 12.25; AB 1.89; AC 2.31; AD 1.00; BC 4.96; BD 0.75; CD 4.17; ABC 0.84; ABD 0.14; ACD 0.47; BCD 0.40; ABCD 0.25).

Noted the steady growth of seed yield from a plant for the growth of the nutrition of plant. The overall average growth in the comparison of variants of row-crop and wide-row sowing was 1.82. This means that a change in the configuration of the feeding area has a positive effect on the formation of the reproductive effort of plants and its assimilative activity. Fertilizers in general had a positive effect on the level of seed yield from the plant, providing an average increase of 9.6% to the control. The effect of fertilizer application was significantly different. Within the variants of row sowing, increases from growth of fertilizers rate to 60 kg ha^{-1} (nitrogen, phosphorus and potassium) at the level of 5–11% were considered for variants 3.0–4.0 million pcs. ha^{-1} of germinable seeds. An increase in the fertilizers rate up to 90 kg ha^{-1} (nitrogen, phosphorus and potassium) for these variants did not form the subsequent growth, but on the contrary had the opposite effect in comparison with the fertilizers rate of 60 kg ha^{-1} (nitrogen, phosphorus and potassium). In general, we noted a pattern of increase in growth from the use of fertilizer in comparison with the previous version of the fertilizer with an increasing pattern for both increasing the width of row spacing and reducing the seeding rate. Thus, the average growth rate of fertilizer for row sowing was 7.1% for the period of research, and for wide-row sowing - 11.9%. The minimum increase of 4.0–5.0% was recorded in variants with seeding rate of 3.0–4.0 million pcs. ha^{-1} of germinable seeds, and the maximum increase of 16.4–21.7% in variants with 1.0 million pcs. ha^{-1} of germinable seeds for row sowing and 0.5 million pcs. ha^{-1} of germinable seeds for wide-row sowing. At the same time, the performance of individual seed production was significantly influenced by the weather conditions of the growing season, with the factor share of the impact of agri-environmental factors of the year in the system of studied variants was 21.75%. Based on the climatic resources of the research region the yield of oilseed radish seeds of the studied genotypes varies from 1.1 to 2.5 t ha^{-1} and corresponds to the potential of the studied varieties of oilseed radish (Tsytsiura, 2019, 2020), and the yield is the integration of standing density and individual productivity of one plant, the most appropriate variant should be considered in the interval of 1.0–2.0 million pcs. ha^{-1} of germinable seeds at row sowing with the fertilizers rate 30–60 kg ha^{-1} (nitrogen, phosphorus and potassium) of fertilization and 1.5 million pcs. ha^{-1} of germinable seeds at row sowing with the fertilizers rate 60–90 kg ha^{-1} (nitrogen, phosphorus and potassium).

Although these variants do not exhibit the effective-optimal level of the CFI curve for a number of indicators (emphasis added above), the level achieved in the experiment, combined with factors of the appropriate reproductive architectonics of the plants, is sufficient to obtain the maximum yield in the system of variants of the experiment. This is also confirmed by the results of the correlation analysis (Table 5). According to the obtained values of the correlation coefficients, the basic indicators of the CFI curve can be used in predicting the yield of oilseed radish seeds. This possibility is confirmed for a number of cruciferous crops in studies Guo et al. (2005), Jamil & Rha (2013), Athar et al. (2015), Kalaji et al. (2018), Bukhat et al. (2020), Ayyaz et al. (2021), De Canniére & Jonard (2021).

The magnitude of the correlation coefficients associated with the ISP indicator proved a significant negative correlation between the yield seeds of oilseed radish and such indicators of the CFI curve as F_0, F_{st}. The direct significant correlation was determined for F_{pl}, F_m, F_v, EP, L_{wp} and Q_{ue}. Given the variation in closeness of relationship and the recommendations of Arkes (2019), the most reliable criteria for predicting individual levels of seed production of oilseed radish plants will be F_m, F_v and their ratios. Similar results, given the biological and physiological specificity of cruciferous plants, were reported in studies with rapeseed and white mustard by Jamil & Rha (2013) and Kalaji et al. (2018). However, the evaluation period of these authors was shorter or compared to typical and stressed years.

The role of weather conditions in the formation of indicators of the CFI curve

The aspect of significant influence on chlorophyll fluorescence hydrothermal regime of the year is not new. A number of recent generalized publications (Brestic & Zivcak, 2013; Abid et al., 2016; Kalaji et al., 2017b; Jedmowski et al., 2015; Hamann, 2021; Larouk et al., 2021; Moore et al., 2021; Lin et al., 2022; Wang et al., 2022) emphasize both the influence on the magnitude and expression of the basic criteria of the CFI curve of temperature and moisture regime, and their systemic impact on the overall nature of photosynthetic activity of the assimilative surface of plants. These conclusions was supported by the results of the assessment of baseline and estimated CFI curve indicators for the most stressful conditions in 2015 (Table 2, Fig. 1) presented in Table 6.

Changes were assessed against a total six-year study period in which three years was stressful (aridity, high average daily temperatures) in terms of HTC (2015, 2016, 2017) and three with near-optimal temperature regimes against excessive moisture (2018–2020). (Table 2). It allowed to use the six-year test cycle as a statistically valid comparison to the most stressful year according to McDonald (2014). The obtained results indicate an overall decrease in the aggregate system of agro-technological options as a percentage comparison with the average annual cycle of counts for the period 2015–2020: F_{pl} by 1.3%, F_m by 11.8%, EP by 8.7%, L_{wp} by 15.9%, RF_d by 25.3%, K_{prp} by 21.9%, K_{fd} by 17.7%. At the same time, there was an increase in the following indicators: F_0 by 5.1%, F_{st} by 7.3%, Q_{ue} by 40.4%, K_{ef} by 24.0%, V_t by 71.3%. Thus, for oilseed radish in general, with a significant decrease in moisture supply against the background of an intensive increase in average daily temperatures is characterized by a general decrease in the amplitude of the curve CFI against the background of increasing fluorescence 'plateau' zone (F_{pl}) and the value of steady-state fluorescence (F_{st}).

This results in an overall increase in overall plant stress and a decrease in plant vitality according to the given reduction dynamics of the criteria L_{wp}, RF_d, K_{prp} and K_{fd}. Against this background the intensity of the relative variable fluorescence at time t (V_t) increases significantly, indicating an increase in the reaction rate in the F_m–F_{st} part of the curve with an earlier shift of 3–4 seconds in the F_{st}-achieving point. This is clearly confirmed by the data in Fig. 8 within the limits of the technological variants studied in 2015.

In addition, for the stressful 2015 of the lines of the CFI curve had a pronounced microrelief oscillating character with a weakly pronounced 'plateau zone' in the range of 62–65 seconds, especially in the case of maximum density of agrocenosis of oilseed radish. At the same time, the maximum reduction of indicators by the specified general tendency was observed for seeding rates in the range of 3.0–4.0 million germinable seeds ha^{-1} at row sowing with maximum effect in variants of application fertilizing rate of $N_{30–60}P_{30–60}K_{30–60}$ and rates in the range of 1.5–2.0 million germinable seeds ha^{-1} with fertilizing rate of $N_{90}P_{90}K_{90}$ at wide-row sowing. However, there was a significant difference in plant stress response between row sowing and wide-row sowing with an averaging factor of 1.117 in the wide-row/row sowing system.

Table 6. Baseline and estimated indexes of the CFI curve in the oil radish variety 'Zhuravka' depending on technological variants of pre-sowing design of its agrocenosis, 2015 (in relative units of the fluorescence on the phase of flowering BBCH 65)

Plant placement	Fertilizer	Basic indicators				Estimated indicators and indices											
		F_0	F_{pl}	F_m	F_{st}	dF_{pl}	F_v	dF_{pl}/F_v	EP	L_{wp}	Q_{ue}	RF_d	K_{ef}	QP	K_{prp}	K_{fd}	V_t
4.0 million, row	1	579	655	1,160	629	76	581	0.131	0.501	2.00	0.997	0.844	0.542	0.914	1.003	1.844	0.086
	2	590	671	1,284	654	81	694	0.117	0.540	2.18	0.850	0.963	0.509	0.908	1.176	1.963	0.092
	3	638	689	1,127	707	51	489	0.104	0.434	1.77	1.305	0.594	0.627	0.859	0.766	1.594	0.141
	4	687	711	1,057	755	24	370	0.065	0.350	1.54	1.857	0.400	0.714	0.816	0.539	1.400	0.184
3.0 million, row	1	533	603	1,348	594	70	815	0.086	0.605	2.53	0.654	1.269	0.441	0.925	1.529	2.269	0.075
	2	548	621	1,409	625	73	861	0.085	0.611	2.57	0.636	1.254	0.444	0.911	1.571	2.254	0.089
	3	568	628	1,307	642	60	739	0.081	0.565	2.30	0.769	1.036	0.491	0.900	1.301	2.036	0.100
	4	590	618	1,265	665	28	675	0.041	0.534	2.14	0.874	0.902	0.526	0.889	1.144	1.902	0.111
2.0 million, row	1	511	633	1,568	556	122	1,057	0.115	0.674	3.07	0.483	1.820	0.355	0.957	2.068	2.820	0.043
	2	521	648	1,602	578	127	1,081	0.117	0.675	3.07	0.482	1.772	0.361	0.947	2.075	2.772	0.053
	3	535	668	1,529	596	133	994	0.134	0.650	2.86	0.538	1.565	0.390	0.939	1.858	2.565	0.061
	4	551	661	1,495	618	110	944	0.117	0.631	2.71	0.584	1.419	0.413	0.929	1.713	2.419	0.071
1.0 million, row	1	478	645	1,633	517	167	1,155	0.145	0.707	3.42	0.414	2.159	0.317	0.966	2.416	3.159	0.034
	2	486	661	1,744	524	175	1,258	0.139	0.721	3.59	0.386	2.328	0.300	0.970	2.588	3.328	0.030
	3	492	677	1,655	526	185	1,163	0.159	0.703	3.36	0.423	2.146	0.318	0.971	2.364	3.146	0.029
	4	501	691	1,639	539	190	1,138	0.167	0.694	3.27	0.440	2.041	0.329	0.967	2.271	3.041	0.033
2.0 million, wide-row	1	527	636	1,504	609	109	977	0.112	0.650	2.85	0.539	1.470	0.405	0.916	1.854	2.470	0.084
	2	535	665	1,588	631	130	1,053	0.123	0.663	2.97	0.508	1.517	0.397	0.909	1.968	2.517	0.091
	3	541	668	1,516	672	127	975	0.130	0.643	2.80	0.555	1.256	0.443	0.866	1.802	2.256	0.134
	4	617	659	1,461	708	42	844	0.050	0.578	2.37	0.731	1.064	0.485	0.892	1.368	2.064	0.108
1.5 million, wide-row	1	518	664	1,714	579	146	1,196	0.122	0.698	3.31	0.433	1.960	0.338	0.949	2.309	2.960	0.051
	2	529	687	1,776	594	158	1,247	0.127	0.702	3.36	0.424	1.990	0.334	0.948	2.357	2.990	0.052
	3	544	699	1,763	615	155	1,219	0.127	0.691	3.24	0.446	1.867	0.349	0.942	2.241	2.867	0.058
	4	569	690	1,669	636	121	1,100	0.110	0.659	2.93	0.517	1.624	0.381	0.939	1.933	2.624	0.061
1.0 million, wide-row	1	474	652	1,750	531	178	1,276	0.139	0.729	3.69	0.371	2.296	0.303	0.955	2.692	3.296	0.045
	2	481	663	1,811	539	182	1,330	0.137	0.734	3.77	0.362	2.360	0.298	0.956	2.765	3.360	0.044
	3	491	678	1,795	552	187	1,304	0.143	0.726	3.66	0.377	2.252	0.308	0.953	2.656	3.252	0.047
	4	509	683	1,728	570	174	1,219	0.143	0.705	3.39	0.418	2.032	0.330	0.950	2.395	3.032	0.050

Table 6 (continued)

0.5 million, wide-row	1	443	660	1,898	498	217	1,455	0.149	0.767	4.28	0.304	2.811	0.262	0.962	3.284	3.811	0.038
	2	448	674	1,962	500	226	1,514	0.149	0.772	4.38	0.296	2.924	0.255	0.966	3.379	3.924	0.034
	3	454	699	1,955	502	245	1,501	0.163	0.768	4.31	0.302	2.894	0.257	0.968	3.306	3.894	0.032
	4	456	704	1,971	509	248	1,515	0.164	0.769	4.32	0.301	2.872	0.258	0.965	3.322	3.872	0.035

LSD$_{0.5}$	F_0	F_{pl}	F_m	F_{st}	The share of influence of experimental factors				
					factors	F_0	F_{pl}	F_m	F_{st}
LSD$_{0.5}$ factor A (sowing method)	3.24	4.36	9.61	6.58	A	14.38	13.26	42.63	5.19
LSD$_{0.5}$ factor B (seeding rate)	4.58	6.16	13.60	9.30	B	64.97	13.23	51.25	77.98
LSD$_{0.5}$ factor C (fertilizer)	4.58	6.16	13.60	9.30	C	12.71	27.63	2.78	12.01
LSD$_{0.5}$ interaction AB	6.48	8.71	19.23	13.16	AB	2.46	39.92	2.03	0.20
LSD$_{0.5}$ interaction AC	6.48	8.71	19.23	13.16	AC	0.31	0.36	0.49	0.27
LSD$_{0.5}$ interaction BC	9.17	12.32	27.19	18.61	BC	4.77	2.78	0.70	4.27
LSD$_{0.5}$ interaction ABC	12.97	17.42	38.46	26.31	ABC	0.40	2.81	0.12	0.09

Fertilizer options consistently: 1 – $N_0P_0K_0$; 2 – $N_{30}P_{30}K_{30}$; 3 – $N_{60}P_{60}K_{60}$; 4 – $N_{90}P_{90}K_{90}$.

The obtained data are positively correlated with a number of recent publications in this field of research such as Guidi et al. (2019), Baldocchi et al. (2020), Hamann (2021), Larouk et al. (2021), Ashrostaghi et al. (2022), Guo et al. (2022).

With similar tendencies in the basic and derived indicators of the CFI curve for oilseed radish under stress (water deficit and high average daily temperatures), certain peculiarities was we also established. The following features of the formation of the CFI curve in the conditions of the stress effect of the hydrothermal regime on the vegetation of oilseed radish were determined, in particular fixation of the parameter F_{pl} on the dynamic section of the CFI curve (no classical 'plateau' for the increase of environmental stressors), the long fixation period of the same F_m values, which creates a plateau effect in the fixation area of maximum fluorescence, the long fixation period and equal values of the stationary fluorescence (F_{st}).

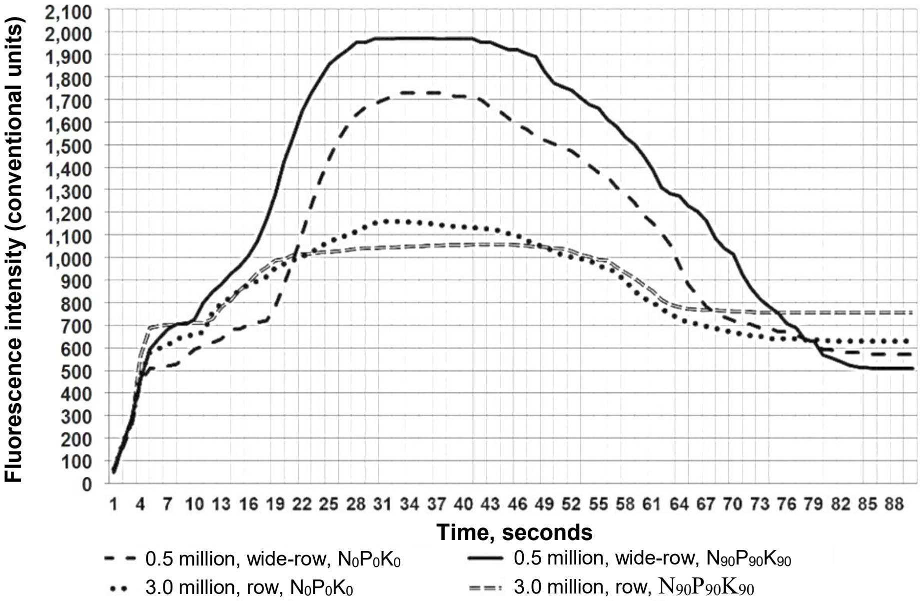


Figure 8. Induction changes of CFI curves in oilseed radish variety 'Zhuravka' under different variants for conditions of the most stressful hydrothermal regime, 2015.

Given the defined approaches for assessing plant stress responses in the general kinetics of the CFI curve according to Thach et al. (2007) and Brestic & Zivcak (2013) and Stirbet et al. (2018) oilseed radish were classified as a sensitive plant for both moisture and temperature stress parameters. This is confirmed by the simultaneous increase in F_0 and F_{st} with a disproportionate decrease in F_m against the background of the combined stress index of the hydrothermal coefficient (HTC) used in the analysis.

Interpretation of statistical evaluation of CFI curve indicators

In addition to the classical statistical data processing (ANOVA analysis), the Tukey HSD Test in R statistic was applied to the data averaged in repetitions over the period 2015–2020. Table 7 presents the results of the conducted statistical evaluation, which, in comparing successive pairs of variants, is typologically similar to the criterion '$LSD_{0.5}$

interaction BCD' (the resulting indicator of the interaction of factors in the general scheme ANOVA analysis (Table 1)).

Table 7. Tukey HSD Test results for baseline CFI curve, 2015–2020

	Df	Sum Sq	Mean Sq	F value	Pr(> F)
	F_0 index				
RE	31	154,038	4,969	11.82	< 2e-16***
Residuals	96	40,342	420		
	F_{pl} index				
RE	31	150,021	4,839	11.92	< 2e-16***
Residuals	96	38,963	406		
	F_m index				
RE	31	4,406,222	142,136	1,145	< 2e-16***
Residuals	96	11,914	124		
	F_{st} index				
RE	31	573,166	18,489	233.5	< 2e-16 ***
Residuals	96	7,602	79		

Signifscant codes: '***' 0.001; '**' 0.01; '*' 0.05; '.' 0.1; ' ' 1.

On the basis of the Tukey HSD Test, a significance matrix was constructed comparing pairs of possible combinations of research factors (Fig. 9). The specified matrix made it possible to place the basic indicators of the CFI curve of oilseed radish in relation to the reliable response of its values to the investigated agrotechnological factors in such a dynamic growth row F_0 (302 non-significant pairs of comparisons in the general scheme of factor combinations) < F_{pl} (297 non-significant pairs) < F_{st} (93 non-significant pairs) < F_m (25 non-significant pairs). The presented series corresponded to the level of physiological sensitivity of the CFI curve indicators to changes in the area of plant nutrition and its configuration under different rates of fertilizers. On the other hand, this nature of its formation in view of the statement regarding the variation of physiologically constant plant traits ensured the increasing nature of the variation of the CFI curve indexes at each point of its fixation with extrema in the sections of the curve F_0–F_{pl} and F_m–F_{st}. These statistical results, taking into account the significance of the differences between the marginal technological variants of the general experimental system for the F_0 and F_{pl} indicators, as well as the increase in the significance of the difference for technologically close variants for the F_m and F_{st} indexes, proved the possibility of using the chlorophyll fluorescence induction method for selecting the optimal variants for the nutrition area and the corresponding fertilization of oilseed radish plants.

Cstatistically confirms the conclusions made and the value of the $LSD_{0.5}$ criterion of the interaction of BCD factors according to the classical scheme of the ANOVA test (Tables 4, 6). The significance of the dimension of this criterion corresponded to the significance of the difference between pairs of comparisons for the Tukey HSD test. However, the clarity of the Tukey test is beyond doubt.

No	Ncv	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	$B_1C_1D_1$	–	c,d	c,d	c,d	c,d	a,b,c,d	a,b,c	a,b,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
2	$B_1C_1D_2$	c,d	–	c,d	c,d	c,d	a,b	a,b	a,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b, c,d	a,b, c,d	a,b,c,d
3	$B_1C_1D_3$	a,b,c,d	a,b,c,d	–	c,d	d	c,d	c,d	c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	c,d	a,b,c,d	a,b,c,d	a,b,c,d
4	$B_1C_1D_4$	a,b,c,d	a,b,c,d	c,d	–	a,b,c,d	c,d	c,d	a,c,d	a,c,d	a,c,d	a,b,c,d	a,c,d	c,d	a,c,d	a,b,c,d	a,b,c,d
5	$B_2C_1D_1$	c,d	c,d	c,d	c,d	–	c,d	c,d	c,d	c,d	a,b,c	a,b,c	a,b,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
6	$B_2C_1D_2$	c,d	c,d	c,d	c,d	c,d	–	c,d	c,d	c,d	a,b,c,d	a,b,c,d	a,b,c	c,d	a,b,c,d	a,b,c,d	a,b,c,d
7	$B_2C_1D_3$	c,d	c,d	c,d	c,d	c,d	c	–	c,d	c,d	a,b,c,d	a,b,c,d	a,b,c,d	c,d	a,b,c,d	a,b,c,d	a,b,c,d
8	$B_2C_1D_4$	c,d	c,d	c,d	c,d	c,d	d	c	–	c,d	a,b	a,b,c	a,b	c,d	a	a,b	a,b
9	$B_3C_1D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	–	c,d	c,d	c,d	d	a,b	a,b	a,b
10	$B_3C_1D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	–	c,d	c,d	d	c,d	c,d	a
11	$B_3C_1D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,b	c	–	c,d	a,b,d	c,d	c,d	c,d
12	$B_3C_1D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,b	c,d	d	–	a,d	c,d	c,d	c,d
13	$B_4C_1D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	–	c,d	c,d	c,d
14	$B_4C_1D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	–	c,d	c,d
15	$B_4C_1D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,b,c	c	–	c,d
16	$B_4C_1D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,b,c	c	c	–
17	$B_1C_2D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	c,d	c,d	a,b,c,d	a,b,c,d
18	$B_1C_2D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	d	c,d	c,d	c,d	c,d	c,d	c,d	a,c,d
19	$B_1C_2D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,c,d
20	$B_1C_2D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,c,d	a,c,d
21	$B_2C_2D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
22	$B_2C_2D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
23	$B_2C_2D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
24	$B_2C_2D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
25	$B_3C_2D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	c	c,d
26	$B_3C_2D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	c
27	$B_3C_2D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,b,c	c	c,d	c
28	$B_3C_2D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,b,c	c	c	d
29	$B_4C_2D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	c,d	c,d	c,d
30	$B_4C_2D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	c,d	c,d	c,d
31	$B_4C_2D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
32	$B_4C_2D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d

No	Ncv	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	$B_1C_1D_1$	a,b,c,d	a,b,c,d	a,b,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
2	$B_1C_1D_2$	a,b,c,d	a,b,c,d	a,b,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
3	$B_1C_1D_3$	c,d	a,b,c,d	a,b,c,d	c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
4	$B_1C_1D_4$	c,d	c,d	a,c,d	c,d	b,c,d	c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,c,d	a,b,c,d	c,d	c,d	a,b,c,d	a,b,c,d
5	$B_2C_1D_1$	b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c	a,b,c,d	a,b,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
6	$B_2C_1D_2$	c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
7	$B_2C_1D_3$	c,d	a,b,c	a,b,c,d	c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
8	$B_2C_1D_4$	c,d	a,b,c,d	a,b,c	c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,c,d	a,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c,d
9	$B_3C_1D_1$	a,c,d	c,d	c,d	c,d	c,d	a,b,c,d	b,c,d	a,b,c,d	c,d	a,c,d	a,b,c,d	a,c,d	a,c,d	c,d	b, c,d	a,b,c,d
10	$B_3C_1D_2$	c	c	c,d	c,d	c	c	c	c,d	c,d	c,d	c,d	a,c,d	c,d	c,d	c,d	c,d
11	$B_3C_1D_3$	c,d	c,d	c,d	c,d	c	c	c	c,d	c,d	c,d	c,d	a,c,d	c,d	c,d	c,d	c,d
12	$B_3C_1D_4$	c,d	c,d	c,d	c,d	c	c	c	c	d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
13	$B_4C_1D_1$	c,d	c,d	c,d	c,d	d	c,d	a,b,c,d	a,c	c,d	c,d	c,d	c,d	c,d	c	b,c	a,b,c
14	$B_4C_1D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	-	c	c	c
15	$B_4C_1D_3$	c,d	c,d	c,d	c,d	c,d	c,d	d	d	c,d	c,d	c,d	c,d	d	c	c	c
16	$B_4C_1D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	d	c	c	c
17	$B_1C_2D_1$	–	c,d	c,d	c,d	c,d	c	c,d	c,d	c,d	c,d	a,c,d	b,c,d	c,d	c,d	c,d	a,b,c,d
18	$B_1C_2D_2$	c	–	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
19	$B_1C_2D_3$	d	d	–	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
20	$B_1C_2D_4$	d	c,d	d	–	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	a,b,c,d
21	$B_2C_2D_1$	c,d	c,d	c,d	c,d	–	c,d	c,d	c,d	c,d	c,d	c,d	a,c,d	c,d	c,d	c,d	c,d
22	$B_2C_2D_2$	c,d	c,d	c,d	c,d	c	–	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d
23	$B_2C_2D_3$	c,d	c,d	c,d	c,d	c	c	–	c,d	c,d	c,d	c,d	c,d	d	c,d	c,d	c,d
24	$B_2C_2D_4$	c,d	c,d	c,d	c,d	c	c	-	–	c,d	c,d	c,d	c,d	d	c,d	c,d	c,d
25	$B_3C_2D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	–	c,d	c,d	c,d	c,d	c,d	c,d	c
26	$B_3C_2D_2$	c,d	c,d	c,d	c,d	c,d	d	c,d	c,d	c	–	c,d	c,d	c,d	c,d	c,d	c
27	$B_3C_2D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	d	c	c	–	c,d	c,d	c	c	c
28	$B_3C_2D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	c	c	–	c,d	c,d	c,d	c
29	$B_4C_2D_1$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	–	c,d	c,d	c,d
30	$B_4C_2D_2$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	–	c,d	c,d
31	$B_4C_2D_3$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	–	c,d
32	$B_4C_2D_4$	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c,d	c	c	–

Ncv – combination of research factors according to the scheme (Table 1); a,b,c,d – comparable pairs of variants with a significant difference in terms of F_0, F_{pl}, F_m and F_{st}, respectively (at the 95% family−wise confidence level).

Figure 9. Reliability of matching pairs of the combination of research factors according to Tukey HSD test.

In addition to a comprehensive assessment of the CFI curve parameters in oilseed radish, it is important to establish the variability of their fixation within a certain time period. Such an analysis was also important from the point of view of the predicted increasing value of the variation of the CFI curve indicators. According to Posudin et al. (2010), Guo & Tan (2015), Derks et al. (2015), Goltsev et al (2016), this is allowed to estimate and determine the internal variability of plant assimilation, determined, among others, by agrotechnological factors of formation of agrocenosis of respective crop. Using basic variability parameters and mean error estimates (McDonald, 2014), we developed a graphical interpretation of the statistical evaluation of the CFI curve records (Figs 10–11). The formation of the mean values at each point of the instrumental fixation of the CFI curve indicators by a graphical interpretation of the standard deviation indicates a consistent increase of the standard error of the mean at the curve segment in the interval F_{pl}–F_m.

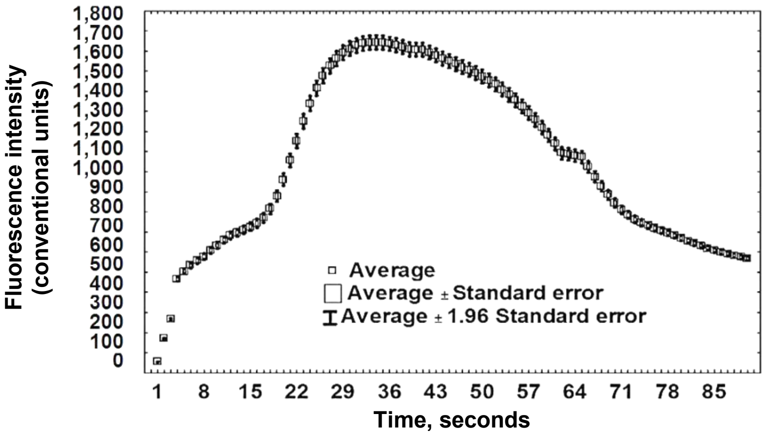


Figure 10. Graph of the average CFI curve of oilseed radish variety 'Zhuravka' in the average interval of different variants according to the combined general data 2015–2020.

The high values of the mean error retain their numerical values with some amplitude of oscillation until an intense decline towards the stationary fluorescence level F_{st}. This pattern of dynamic changes is confirmed by the graphical interpretation of the standard deviation of the CFI curve data (Fig. 11).

It should note that with increasing standard deviation and increasing total error of the mean, the oscillation coefficient indicates a low variation component in the CFI curve, namely the section covering the period from 23 to 41 seconds of recording, followed by a slow increase until the steady-state fluorescence F_{st} stage. The maximum values of the oscillation coefficient were recorded exactly in the F_0–F_{pl} interval. Similar results were obtained in Korneev (2002), Lamote et al. (2007), Brestic & Zivcak (2013), Ni et al. (2019), Khan et al. (2021), which observed the highest spreads of maximum fluorescence F_0 in different plant species within the reference group.

However, it is pointed out that in relation to the average, this deviation from the average does not form high rates of variation, unlike the sections of the CFI curve in the F_0–F_{pl} segment and the F_m–F_{st} segment. In contrast, studies (Flexas et al., 2002; Henriques, 2009; Bresson et al., 2015) showed and calculated minimal variation both in the F_0–F_{pl} sections and when reaching the F_{st} point.

The variable component of the basic indicators of the CFI curve is also determined by the hydrothermal conditions of the oilseed radish vegetation. This is confirmed by the results of the factor analysis in the ANOVAe estimation scheme of the experiment variants. It was determined that the share of influence of hydrothermal conditions of the year in the formation of the basic indicators of the CFI curve was in the range of

20.11–40.24%. Accordingly, the highest impact was for the F_{pl} indicator and the lowest for the F_0 indicator. Based on this, it was concluded that the indexes of the CFI curve depended on weather conditions, which additionally act as regulators of the positive or negative effect of the studied agrotechnological factors on the optimization of the post-sowing formation of the agrocenosis of oilseed radish.

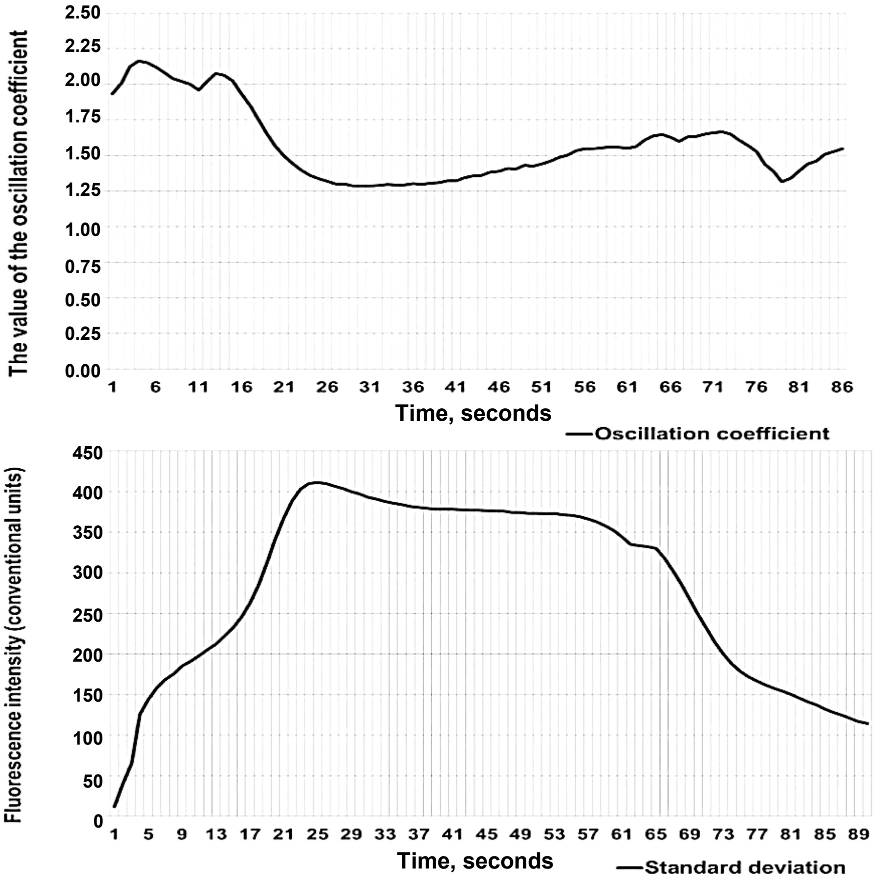


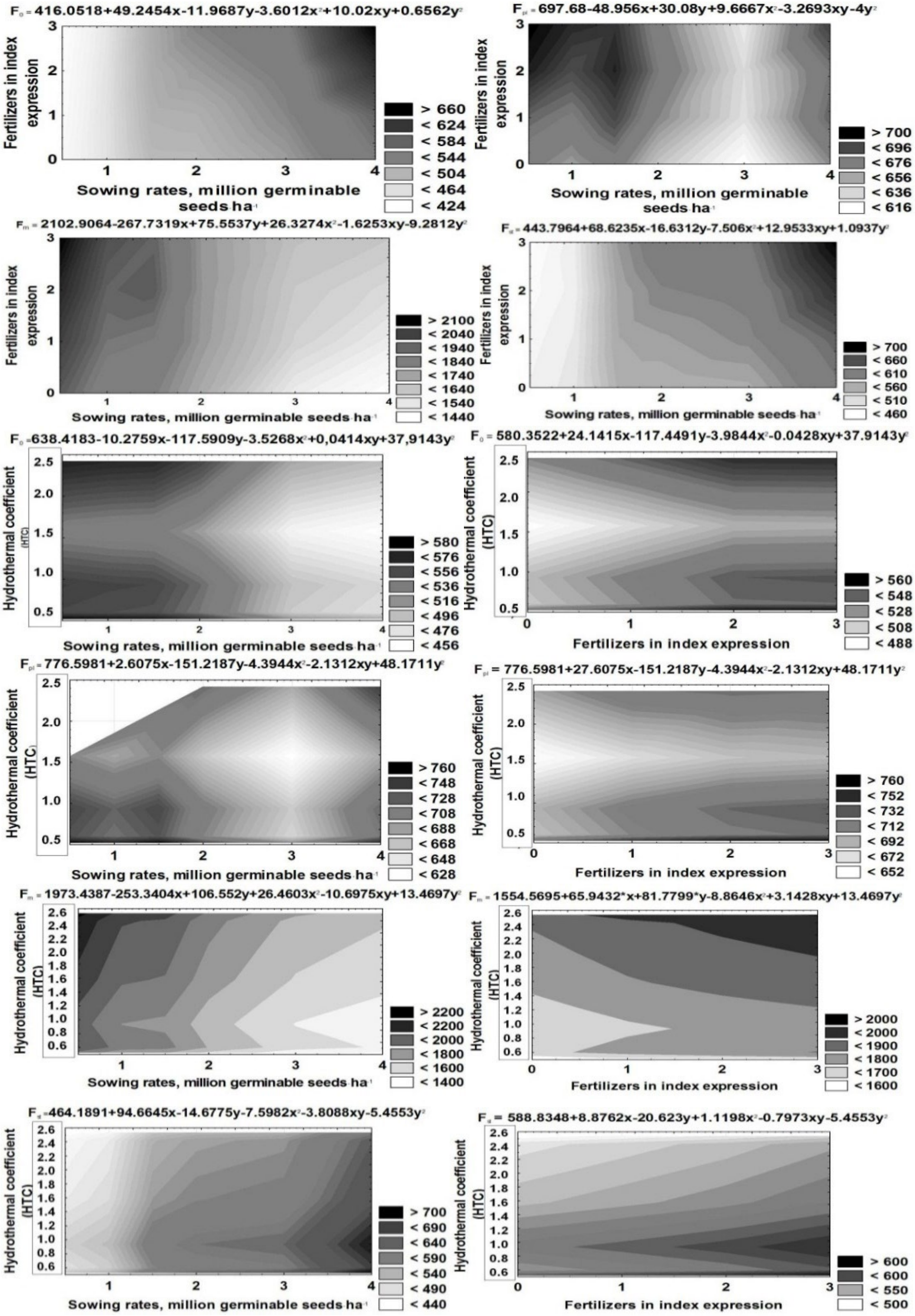
Figure 11. Statistical parameters of CFI curve of oilseed radish variety 'Zhuravka' in the average interval of different variants according to the combined general data 2015–2020.

As a result, generalizations made earlier on dynamic and complex nature of interrelations formation between the system of experience factors, basic indicative indices of CFI curve and indices of hydrothermal regime of environment during vegetation period of oilseed radish plants are shown by regression dependence data in Table 8 and Fig. 12. The obtained statistically reliable multiple regression coefficients indicated a sufficiently close dependence of complex combinatorial nature between the basic indicators of the CFI curve and agrotechnological factors in the system of experience variants. The established regression dependence in the equations of the second stage in accordance with Arkes (2019) indicates the presence of reciprocal extremes for the corresponding variants at the situational combination of factors in the system of dependencies.

Table 8. Regression dependences of the main indicators of the CFI curve on technological factors of oilseed radish agrocenosis design and the hydrothermal regime of the growing season (based on the data 2015–2020)

CFI curve indicator	Equation components x	Equation components y	Regression equation of dependence	Multiple regression coefficient R/R^2	Statistical significance criteria R
F_0	Seeding rates, million germinable seeds ha^{-1}	Fertilizers in index expression	$F_0 = 416.0518+49.2454x-11.9687y-3.6012x^2+10.02xy+0.6562y^2$	0.942^{***} /0.887	$F/SS_{total} = 50.3$ (p = .000000), $t_{05} = 47.72$ (p = 0.0000)
F_{pl}			$F_{pl} = 697.68-48.956x+30.08y+9.6667x^2-3.2693xy-4y^2$	0.656^{***} /0.430	$F/SS_{total} = 10.96$ (p = .000284), $t_{05} = 75.20$ (p = 0.0000)
F_m			$F_{max} = 2102.9064-267.7319x+75.5537y+26.3274x^2-1.6253xy-9.2812y^2$	0.955^{***} /0.912	$F/SS_{total} = 65.86$ (p = .0000000), $t_{05} = 84.79$ (p = 0.0000)
F_{st}			$F_{st} = 443.7964+68.6235x-16.6312y-7.506x^2+12.9533xy+1.0937y^2$	0.895^{***} /0.801	$F/SS_{total} = 25.42$ (p = .000000), $t_{05} = 32.43$ (p = 0.0000)
F_0	Seeding rates, million germinable seeds ha^{-1}	Hydrothermal coefficient (HTC)	$F_0 = 638.4183-10.2759x-117.5909y-3.5268x^2+0.0414xy+37.9143y^2$	0.727^{***} /0.592	$F/SS_{total} = 48.48$ (p = .000000), $t_{05} = 113.85$ (p = 0.0000)
F_{pl}			$F_{pl} = 871.8071-62.8073x-166.7862y+10.8176x^2+3.9494xy+50.5807y^2$	0.425^{**} /0.180	$F/SS_{total} = 7.03$ (p = .000001), $t_{05} = 101.17$ (p = 0.0000)
F_m			$F_{max} = 1973.4387-253.3404x+106.552y+26.4603x^2-10.6975xy+13.4697y^2$	0.920^{***} /0.846	$F/SS_{total} = 238.4$ (p = .000000), $t_{05} = 118.46$ (p = 0.0000)
F_{st}			$F_{st} = 464.1891+94.6645x-14.6775y-7.5982x^2-3.8088xy-5.4553y^2$	0.885^{***} /0.783	$F/SS_{total} = 155.9$ (p = .000000), $t_{05} = 74.64$ (p = 0.0000)
F_0	Fertilizers in index expression	Hydrothermal coefficient (HTC)	$F_0 = 580.3522+24.1415x-117.4491y-3.9844x^2-0.0428xy+37.9143y^2$	0.349^{*} /0.121	$F/SS_{total} = 6.0$ (p = .000005), $t_{05} = 81.04$ (p = 0.0000)
F_{pl}			$F_{pl} = 776.5981+27.6075x-151.2187y-4.3944x^2-2.1312xy+48.1711y^2$	0.523^{**} /0.274	$F/SS_{total} = 12.0$ (p = .000000), $t_{05} = 105.0$ (p = 0.0000)
F_m			$F_{max} = 1554.5695+65.9432x+81.7799y-8.8646x^2+3.1428xy+13.4697y^2$	0.551^{**} /0.304	$F/SS_{total} = 18.8$ (p = .000000), $t_{05} = 49.76$ (p = 0.0000)
F_{st}			$F_{st} = 588.8348+8.8762x-20.623y+1.1198x^2-0.7973xy-5.4553y^2$	0.445^{**} /0.198	$F/SS_{total} = 10.7$ (p = .000000), $t_{05} = 49.77$ (p = 0.0000)

*, **, *** Significant at 5%, 1%, 0.1% level probability, respectively.



Consistently left and right dependencies for parameters: F_0, F_{pl}, F_m, F_{st}, F_0, F_0, F_{pl}, F_{pl}, F_m, F_m, F_{st}, F_{st}).

Figure 12. Projection surfaces of dependences of the main indicators of the CFI curve on technological factors of oilseed radish agrocenosis design and hydrothermal regime of vegetation (based on the 2015–2020 consolidated data set).

For all possible combinations in the regression scheme presented, the tightness of the multiple dependence on the basic criteria of the CFI curve in order of decreasing significance and approximation (according to R^2 criteria) with respect to experience and environmental factors can be placed in the following row: $F_m > F_{st} > F_0 > F_{pl}$. At the same time, the highest tightness of mutual influence on the formation of these indicators was noted for the use of seeding rates and fertilizer as influence factors, and, respectively, the lowest - for fertilizer and hydrothermal coefficient for the period from the beginning of the growing season to the date of recording the indicators of the CFI curve.

Thus, the confirmed stress role of fertilizers in the variants of increased seeding rates in interaction with the hydrothermal features of the environment complicates the nature of the direct expression of the impact both by combining the stress of a certain fraction of the influence of year conditions factors in the system of ANIOVA (Table 4), and by the dual role of fertilizers in a number of the variants studied (stimulation of growth - stress through increased competition in oilseed radish agrocenoses with with the smallest plant nutrition area).

As for the hydrothermal coefficient, to ensure the most harmonized CFI curve of oilseed radish plants, based on the nature of the reaction surfaces (Fig. 11) its value in the range 1.0–1.5 with tolerances from 0.8 to 1.7 is desirable to realize maximum crop bio-productivity of oiseed radish plant. It has been confirmed that this interval corresponds to the physiological desirability for optimising the growth and development of cruciferous crops (Hasanuzzaman, 2020; Bakhshandeh & Mohsen, 2021).

The reaction surfaces of the corresponding regression triads presented in Fig. 11 also allowed to identify the optimal variants for pre-sowing agro-technological construction of oilseed radish agrocenosis, which guarantee the harmonization of the basic indicators of the CFI curve in the study area and ensure the implementation of appropriate bioproductivity of 1 m^2 of sown area. Such agrotechnological parameters, taking into account the determined values of indicators F_0, F_{pl}, F_m and F_{st}, were the application of seeding rates in the interval of 1.0–2.0 million germinable seeds ha^{-1} (row sowing) and fertilization rate of $N_{30–60}P_{30–60}K_{30–60}$ and 1.5 million germinable seeds ha^{-1} (wide-row sowing) with a fertilization rate of $N_{60–90}P_{60–90}K_{60–90}$.

CONCLUSIONS

Thus, by the value of the main indices of the CFI curve F_0, F_{pl}, F_m, F_{st} and possible ratios, indices and coefficients calculated on their basis in comparison with such species as white mustard, spring bittercress and spring rape, oilseed radish should be considered as species with a wide range of adaptability to stress factors with a sensitive indicative response of the plant assimilative photosystem structures.

This allowed the application of the chlorophyll fluorescence induction method in the identification of optimal agro-technological solutions for its cultivation in order to maximise its photo-assimilative potential as a species.

Based on the results of the main recommended indicators in the analysis of the CFI curve within the studied options of pre-sowing design of oilseed radish agrocenosis by the parameters of row spacing, seeding rate and fertilizer application in the agro-climatic zone of research of such agricultural options: row sowing with seeding rate in the range of 1.0–2.0 million germinable seeds ha^{-1} with the fertilization rate of 30–60 kg N ha^{-1}, 30–60 kg P ha^{-1}, and 30–60 kg K ha^{-1} and wide-row sowing with seeding rate of million germinable seeds ha^{-1} with the fertilization rate of 60–90 kg N ha^{-1}, 60–90 kg P ha^{-1}, and 60–90 kg K ha^{-1}.

Certain variants allowed to combine low values of F_0, and F_{st} parameters in comparison group at the level of 450–570 relative units of fluorescence with high values of maximum fluorescence (F_m) in the range of 1,700–2,000 relative units against the background of certain level of correlation dependence of direct and inverse character between the specified parameters and individual seed productivity of plants in the range of 0.560–0.992 ($p < 0.95$–0.99) and value of multiple regression coefficient between 0.349–0.955 ($p < 0.95$–0.99). This provided obtaining of appropriate indexes of CFI curve that certify both a sufficient leve of photoassimilative activity of oilseed radish plants and a high level of their viability. This made it possible to form a productive architecture of oilseed radish plants, which ensured the maximum seed yield among the studied variants.

REFERENCES

Amri, M., Abbes, Z., Trabelsi, I., Ghanem, M.E., Mentag, R. & Kharrat, M. 2021. Chlorophyll content and fluorescence as physiological parameters for monitoring *Orobanche foetida* Poir. infection in faba bean. *PLoS ONE* **16**(5), e0241527.

Arkes, J. 2019. *Regression Analysis A Practical Introduction*. 1st Edition. Routledge, 362 pp.

Arnetz, B.B. 1997. Technological stress: psychophysiological aspects of working with modern information technology. *Scandinavian Journal of Work, Environment & Health* **23**, 97–103.

Ashrostaghi, T., Aliniaeifard, S., Shomali, A., Azizinia, S., Abbasi Koohpalekani, J., Moosavi-Nezhad, M. & Gruda, N.S. 2022. The Role Player in Cucumber Response to Cold Stress. *Agronomy* **12**, 201.

Athar, H.U.R., Zafar, Z.U. & Ashraf, M. 2015. Glycinebetaine improved photosynthesis in canola under salt stress: evaluation of chlorophyll fluorescence parameters as potential indicators. *Journal of Agronomy and Crop Science* **201**, 428–442.

Ayyaz, A., Farooq, M.A., Dawood, M., Majid, A., Javed, M., Athar, H.U.R., Bano, H. & Zafar, Z.U. 2021. Exogenous melatonin regulates chromium stress-induced feedback inhibition of photosynthesis and antioxidative protection in Brassica napus cultivars. *Plant Cell Reports* **40**, 2063–2080.

Baba, W., Kompała-bąba, A., Zabochnicka-świątek, M., Luźniak, J., Hanczaruk, R., Adamski, A. & Kalaji, H.M. 2019. Discovering trends in photosynthesis using modern analytical tools: More than 100 reasons to use chlorophyll fluorescence. *Photosynthetica* **57**(2), 668–679.

Baker, N.R. & Rosenqvist, E. 2020 Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany* **71**(4), 1647.

Bakhshandeh, M.E. & Mohsen, J.J. 2021. Halothermal and hydrothermal time models describe germination responses of canola seeds to aging. *Plant Biology* **23**(4), 621–629.

Baldocchi, D.D., Ryu, Y., Dechant, B., Eichelmann, E., Hemes, K., Ma, S., Sanchez, C.R., Shortt, R., Szutu, D., Valach, A., Verfaillie, J., Badgley, G., Zeng, Y. & Berry, J.A. 2020. Outgoing near-infrared radiation from vegetation scales with canopy photosynthesis across a spectrum of function, structure, physiological capacity, and weather. *Journal of Geophysical Research: Biogeosciences* **125**, e2019JG005534.

Baležentienė, L. & Šiuliauskienė, D. 2007. Chlorophyll fluorescence estimation of fodder galega (*Galega orientalis* Lam.) in situ and dependence on different leaf rank and cultivars. *Agronomy Research* **5**(1), 3–12.

Björkman, O. & Demmig, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* **170**(4), 489–504.
Bonfig, K.B., Schreiber, U., Gabler, A., Roitsch, T. & Berger, S. 2006. Infection with virulent and avirulent P. syringae strains differentially affects photosynthesis and sink metabolism in Arabidopsis leaves. *Planta* **225**, 1–12.
Brack, W. & Frank, H. 1998. Chlorophyll a fluorescence: a tool for the investigation of toxic effects in the photosynthetic apparatus. *Ecotox-icology and Environmental Safety* **l40**(1–2), 34–41.
Bresson, J., Vasseur, F., Dauzat, M., Koch, G., Granier, C. & Vile, D. 2015. Quantifying spatial heterogeneity of chlorophyll fluorescence during plant growth and in response to water stress. *Plant methods* **11**, 23.
Brestič, M., Živčák, M., Kalaji, H.M., Carpentier, R. & Allakhverdiev, S.I. 2012. Photosystem II thermostability in situ: Environmentally induced acclimation and genotype-specific reactions in *Triticum aestivum* L. *Plant Physiology and Biochemistry* **57**, 93–105.
Brestic, M & Zivcak, M. 2013. PSII fluorescence techniques for measurement of drought and high temperature stress signal in plants: protocols and applications. In: *Rout GR, Das AB (eds.) Molecular stress physiology of plants*. Springer Dordrecht, 87–131.
Brion, O.V., Korneev, D.Yu., Snegur, O.I. & Kitaev, O.O. 2000. *Instrumental study of the photosynthetic apparatus by induction of chlorophyll fluorescence: Guidelines for students of the Faculty of Biology*. Kyiv: Kyiv University Publishing and Printing Center, 15 pp. (in Ukrainian).
Bukhat, S., Manzoor, H., Zafar, Z.U., Azeem, F. & Rasul, S. 2020. Salicylic acid induced photosynthetic adaptability of Raphanus sativus to salt stress is associated with antioxidant capacity. *Journal of Plant Growth Regulation* **39**, 809–822.
Burling, K., Hunsche, M. & Noga, G. 2011. Use of blue-green and chlorophyll fluorescence measurements for differentiation between nitrogen deficiency and pathogen infection in winter wheat. *Journal of Plant Physiology* **168**, 1641–1648.
Bussotti, F., Gerosa, G., Digrado, A. & Pollastrini, M. 2020. Selection of chlorophyll fuorescence parameters as indicators of photosynthetic efciency in large scale plant ecological studies. *Ecological Indicators* **108**, 105686.
Casto, A., Schuhl, H., Schneider, D., Wheeler, J., Gehan, M. & Fahlgren, N. 2021. Analyzing chlorophyll fluorescence images in PlantCV PT. *Earth and Space Science*, AID 10.1002/essoar.10508322.2.
Cendrero-Mateo, M.P., Wieneke, S., Damm, A., Alonso, L., Pinto, F., Moreno, J., Guanter, L., Celesti, M., Rossini, M., Sabeter, N., Cogliati, S., Julitta, T., Rascher, U., Goulas, Y., Aasen, H., Pacheco-Labrador, J. & Arthur, A.M. 2019. Sun-Induced Chlorophyll Fluorescence III: Benchmarking Retrieval Methods and Sensor Characteristics for Proximal Sensing. *Remote Sensing* **11**, 962.
Cetner, M.D., Kalaji, H.M., Goltsev, V., Aleksandrov, V., Kowalczyk, K., Borucki, W. & Jajoo, A. 2017. Effects of nitrogendeficiency on efficiency of light-harvesting apparatus in radish. *Plant Physiology and Biochemistry* **119**, 81–92.
Christen, D., Schonmann, S., Jermini, M., Strasser, R.J. & Défago, G. 2007. Characterization and early detection of grapevine (*Vitis vinifera*) stress responses to esca disease by in situ chlorophyll fluorescence and comparison with drought stress. *Environmental and Experimental Botany* **60**, 504–514.
Cong, W., Yang, K. & Wang, F. 2022. Canopy Solar-Induced Chlorophyll Fluorescence and Its Link to Transpiration in a Temperate Evergreen Needleleaf Forest during the Fall Transition. *Forests* **13**, 74.
Cui, X., Tang, Y., Gu, S., Nishimura, S., Shi, S. & Zhao, X. 2003. Photosynthetic depression in relation to plant architecture in two alpine herbaceous species. *Environmental and experimental botany* **50**(2), 125–135.

De Canniére, S. & Jonard, F. 2021. Tracking Water Limitation in Photosynthesis with Sun-Induced Chlorophyll Fluorescence. 2021 *IEEE International Geoscience and Remote Sensing Symposium IGARSS*, 6367–6370.

Dechant, B., Ryu, Y., Badgley, G., Zeng, Y., Berry, J.A., Zhang, Y., Goulas, Y., Li, Z., Zhang, Q., Kang, M., Li, J. & Moya, I. 2020. Canopy structure explains the relationship between photosynthesis and sun-induced chlorophyll fluorescence in crops. *Remote Sensing of Environment* **241**, 111733.

Derks, A., Schaven, K. & Bruce, D. 2015. Diverse mechanisms for photoprotection in photosynthesis. Dinamic regulation of photosystem II excitation in response to rapin environmental change. *Biochimica et Biophysica Acta – Bioenergetics* **1847**(4–5), 468–485.

Driever, S., Lawson, T., Andralojc, P., Raines, C. & Parry, M. 2014. Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes. *Journal of Experimental Botany* **65** (17), 4959–4973.

Evans, J.R. 2009. Potential errors in electron transport rates calculated from chlorophyll fluorescence as revealed by a multilayer leaf model. *Plant and Cell Physiology* **50**, 698–706.

Evarte-Bundere, G. & Evarts-Bunders, P. 2012. Using of the Hydrothermal coefficient (HTC) for interpretation of distribution of non-native tree species in Latvia on example of cultivated species of genus Tilia. *Acta Biologica Universitatis Daugavpiliensis* **12**, 135–148.

Feria-Gómez, D.F., Londoño-Puerta, D.A. & Córdoba-Gaona, O. de J. 2022. Relationship between the chlorophyll a fluorescence and the yield in banana (Musa AAA Simmonds cv. Cavendish). *Revista Colombiana de Ciencias Hortícolas* **16**(1), e13313.

Ferreira, L.C., Moreira, B.R.A., Montagnolli, R.N., Prado, E.P., Viana, R.D.S., Tomaz, R.S., Cruz, J.M., Bidoia, E.D., Frias, Y.A. & Lopes, P.R.M. 2021. Green Manure Species for Phytoremediation of Soil With Tebuthiuron and Vinasse. *Frontiers in Bioengineering and Biotechnology* **8**, 613–642.

Flexas, J., Escalona, J.M., Evain, S., Gulías, J., Moya, I., Osmond, C.B. & Medrano, H. 2002. Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO_2 assimilation and stomatal conductance during water-stress in C3 plants. *Physiologia Plantarum* **114**(2), 231–240.

Flexas, J., Bota, J., Loreto, F., Cornic, G. & Sharkey, T.D. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology* **6**, 269–279.

Gensheimer, J., Turner, A.J., Köhler, P., Frankenberg, C. & Chen, J. 2022. A convolutional neural network for spatial downscaling of satellite-based solar-induced chlorophyll fluorescence (SIFnet). *Biogeosciences* **19**, 1777–1793.

Goltsev, V.N., Kaladzhi, M.Kh., Kuzmanova, M.A. & Allakhverdiev, S.I. 2014. *Variable and delayed fluorescence of chlorophyll a – theoretical foundations and practical applications in plant research*. Moscow-Izhevsk: Institute for Computer Research. 220 pp. (in Russian).

Goltsev, V.N., Kalaji, H.M., Paunov, M. & Baba, W. 2016. Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. *Russian Journal of Plant Physiology* **63**, 869–893.

Gu, J., Zhou, Z., Li, Z., Chen, Y., Wang, Z., Zhang, H. & Yang, J. 2017. Photosynthetic properties and potentials for improvement of photosynthesis in pale green leaf rice under high light conditions. *Frontiers in Plant Science* **8**, 1082.

Guidi, L., Lo Piccolo, E. & Landi, M. 2019. Chlorophyll Fluorescence, Photoinhibition and Abiotic Stress: Does it Make Any Difference the Fact to Be a C3 or C4 Species? *Frontiers in Plant Science* **10**,174.

Guo, Y.P., Guo, D.P., Zhaoa, J.P., Liua, H., Penga, Y., Wanga, Q.M., Chenb, J.S. & Rao, G.Z. 2005. Photosynthetic responses of radish (*Raphanus sati*vus var. *longipinnatus*) plants to infection by turnip mosaic virus. *Photosynthetica* **43**, 457–462.

Guo, Y. & Tan, J. 2015. Recent advances in the application of chlorophyll a fluorescence from Photosystem II. Photochem. *Photobiology* **91**, 1–14.

Guo, X., Li, G., Ding, X., Zhang, J., Ren, B., Liu, P., Zhang, S. & Zhao, B. 2022. Response of Leaf Senescence, Photosynthetic Characteristics, and Yield of Summer Maize to Controlled Release Urea-Based Application Depth. *Agronomy* **12**, 687.

Gururani, M.A., Venkatesh, J. & Tran, L.S.P. 2015. Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant* **8**, 1304–1320.

Hamann, F.A. 2021. *Potential and limitations for the practical use of fluorescence sensors to detect physiological adaptations of crops*. PhD Thesis. Bonn. Institute for Crop Science and Resource Conservation, 116 pp.

Hasanuzzaman, M. 2020. *The Plant Family Brassicaceae Biology and Physiological Responses to Environmental Stresses*. Springer, Singapore. 531 pp.

He, J., Chee, C.W. & Goh, C.J. 1996. 'Photoinhibition' of Heliconia under natural tropical conditions: the importance of leaf orientation for light interception and leaf temperature. *Plant, Cell & Environment* **19**(11), 1238–1248.

Henriques, F.S. 2009. Leaf Chlorophyll Fluorescence: Background and Fundamentals for Plant Biologists. *Botanical Review* **75**, 249–270.

Herritt, M.T., Long, J.C., Roybal, M.D., Moller Jr, D.C., Mockler, T.C., Pauli, D. & Thompson, A.L. 2021. FLIP:FLuorescence imaging pipeline for field-based chlorophyll fluorescence images. *SoftwareX*. **14**, Article 100685.

Herritt, M.T., Pauli, D., Mockler, T.C. & Thompson, A.L. 2020. Chlorophyll fluorescence imaging captures photochemical efficiency of grain sorghum (*Sorghum bicolor*) in a field setting. *Plant Methods* **16**, 109.

Hosseinzadeh, M., Aliniaeifard, S., Shomali, A. & Didaran, F. 2021. Interaction of light intensity and CO_2 concentration alters biomass partitioning in Chrysanthemum. *Journal of Horticultural Research* **29**, 45–56.

Hou, W.H., Zhang, Y.X., Wang, H.J., Zhang, Q.X., Hou, M.L., Cong, B.M. & Du, X. 2021. Effects of nitrogen application level on leaf photosynthetic characteristics and chlorophyll fluorescence characteristics of *Leymus chinensis*. *Acta Agrestia Sinica* **29**, 532–536.

Hupp, S., Rosenkranz, M., Bonfig, K., Pandey, C. & Roitsch, T. 2019. Noninvasive Phenotyping of Plant-Pathogen Interaction: Consecutive In Situ Imaging of Fluorescing Pseudomonas syringae, Plant Phenolic Fluorescence, and Chlorophyll Fluorescence in Arabidopsis Leaves. *Frontiers in Plant Science* **10**, 1239.

IUSS Working Group. 2015. *WRB: World Reference Base for Soil Resources. World Soil Resources Reports* 106. FAO. Rome, pp. 85–90.

Jamil, M. & Rha, E. 2013. NaCl Stress-Induced Reduction in Grwoth, Photosynthesis and Protein in Mustard. *Journal of Agricultural Science* **5**(9), 114–127.

Janušauskaite, D. & Feiziene, D. 2012. Chlorophyll fluorescence characteristics throughout spring triticale development stages as affected by fertilization. *Acta Agriculturae Scandinavica, Section B. Soil and Plant Science* **62**, 7–15.

Javed, M., Ashraf, M., Iqbal, M., Farooq, M.A., Zafar, Z.U. & Athar, H.-ur-R. 2022. Chlorophyll fluorescence, ion uptake, and osmoregulation are potential indicators for detecting ecotypic variation in salt tolerance of Panicum antidotale Retz. *Arid Land Research and Management* **36**(1), 84–108.

Jedmowski, C., Ashoub, A., Momtaz, O.A., & Brüggemann, W. 2015. Impact of Drought, Heat, and Their Combination on Chlorophyll Fluorescence and Yield of Wild Barley (*Hordeum spontaneum*). *Journal of Botany*. Article ID 120868, 1–9.

Jonathan, M.B. 2017. Continuous excitation chlorophyll fluorescence parameters: a review for practitioners. *Tree Physiology* **37**(8), 1128–1136.

Kalaji, H.M., Jajoo, A., Oukarroum, A. & Brestic, M. 2016. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum* **38**, 102–114.

Kalaji, H., Schansker, G., Ladle, R., Goltsev, V., Bosa, K., Allakhverdiev, S., Brestic, M., Bussotti, F. & Calatayud, A. 2014. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research* **122**, 121–158.

Kalaji, H.M., Schansker, G., Brestic, N., Bussotti, F., Calatayud, A., Ferroni, L. & Goltsev, V. 2017. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynthesis Research* **132**, 13–66.

Kalaji, H.M., Bąba, W., Gediga, K., Goltsev, V., Samborska, I., Cetner, M.D., Dimitrova, S., Piszcz, U., Bielecki, K., Karmowska, K., Dankov, K. & Kompała-Bąba, A. 2017a. Chlorophyll fluorescence as a tool for nutrient status identification in rapeseed plants. *Photosynthesis Research* **136**, 329–343.

Kalaji, H.M., Goltsev, V.N., Żuk-Golaszewska, K., Zivcak, M. & Brestic, M. 2017b. *Chlorophyll Fluorescence. Understanding Crop Performance: Basics and Applications*. CRC Press, Boca Raton, 222 pp.

Kalaji, H.M., Bąba, W., Gediga, K., Goltsev, V., Samborska, I.A., Cetner, M.D., Dimitrova, S., Piszcz, U., Bielecki, K., Karmowska, K., Dankov, K. & Kompała-Bąba, A. 2018. Chlorophyll fluorescence as a tool for nutrient status identification in rapeseed plants. *Photosynthesis Research* **136**(3), 329–343.

Kaletnik, G., Pryshliak, N. & Tokarchuk, D. 2021. Potential of production of energy crops in Ukraine and their processing on solid biofuels. *Ecological Engineering and Environmental Technology* **22**(3), 59–70.

Kaletnik, G., Honcharuk, I. & Okhota, Y. 2020. The Waste-free production development for the energy autonomy formation of Ukrainian agricultural enterprises. *Journal of Environmental Management and Tourism* **11**(3), 513–522.

Kayaçetin, F. 2020. Botanical characteristics, potential uses, and cultivation possibilities of mustards in turkey: A review. *Turkish Journal of Botany* **44**, 101–127.

Keller, B., Matsubara, S., Rascher, U., Pieruschka, R., Steier, A., Kraska, T. & Muller, O. 2019. Genotype specific photosynthesis × environment interactions captured by automated fluorescence canopy scans over two fluctuating growing seasons. *Frontiers in Plant Science* **10**, 1482.

Khan, N., Essemine, J., Hamdani, S., Qu, M., Lu, M.-J.A., Parveen, S., Stirbet, A., Govindjee, G. & Zhu, X.-G. 2021. Natural variation in the fast phase of chlorophyll a fluorescence induction curve (OJIP) in a global rice minicore panel. *Photosynthesis Research* **150**(1–3), 137–158.

Kim, J., Ryu, Y., Dechant, B., Lee, H., Kim, H.S., Kornfeld, A. & Berry, J.A. 2021 Solar-induced chlorophyll fluorescence is non-linearly related to canopy photosynthesis in a temperate evergreen needleleaf forest during the fall transition *Remote Sensing of Environment* **258**, 112362.

Kimm, H., Guan, K., Burroughs, C.H., Peng, B., Ainsworth, E.A., Bernacchi, C.J., Moore, C.E., Kumagai, E., Yang, X., Berry, J.A. & Wu, G. 2021. Quantifying high-temperature stress on soybean canopy photosynthesis: the unique role of sun-induced chlorophyll fluorescence. *Global Change Biology* **27**, 2403–2415.

Kirik, M.M., Taranuho & Yu.M. 2011. Diagnosis of viral infection of black currant and raspberry by the method of induction of chlorophyll fluorescence of leaves. *Bulletin of Agrarian Science: Coll. Science. works* **10**, 26–28 (in Ukrainian).

Korneev, D.Yu. 2002. *Informational capabilities of the method of chlorophyll fluorescence induction*. Kiev: Alterpress. 188 pp. (in Ukrainian).

Kuhlgert, S., Austic, G., Zegarac, R., Osei-Bonsu, I., Hoh, D., Chilvers, M.I., Roth, M.G., Bi, K., TerAvest, D. & Weebadde, P. 2016. MultispeQ Beta: a tool for large-scale plant phenotyping connected to the open PhotosynQ network. *Royal Society Open Science* **3**(10), 160592.

Lamote, M., Johnson, L.E. & Lemoine, Y. 2007. Interspecific differences in the response of juvenile stages to physical stress: fluorometric responses of fucoid embryos to variation in meteorological conditions. *Journal of Phycology* **43**(6), 1164–1176.

Larouk, C., Gabon, F., Kehel, Z., Djekoun, A., Nachit, M. & Amri, A. 2021. Chlorophyll Fluorescence and Drought Tolerance in a Mapping Population of Durum Wheat. *Contemporary Agriculture* **70**(3–4), 123–134.

Legendre, R., Basinger, N.T. & van Iersel, M.W. 2021. Low-Cost Chlorophyll Fluorescence Imaging for Stress Detection. *Sensors* **21**(6), 2055.

Lei, S., Xiping, D. & Li, Y. 2006. Effects of water deficit on fluorescence parameters of wheat with different ploidies. *Xibei Zhiwu Xuebao* **26**(2), 343–347.

Li, Z., Zhang, Q., Li, J., Yang, X., Wu, Y., Zhang, Z., Wang, S., Wang, H. & Zhang, Y. 2020. Solar-induced chlorophyll fluorescence and its link to canopy photosynthesis in maize from continuous ground measurements *Remote Sensing of Environment* **236**, 111420.

Lichtenthaler, H.K., Buschmann, C. & Knapp, M. 2005. How to Correctly Determine the Different Chlorophyll Fluorescence Parameters and the Chlorophyll Fluorescence Decrease Ratio RFd of Leaves with the PAM Fluorometer. *Photosynthetica* **43**, 379–393.

Lin, J., Shen, Q., Wu, J., Zhao, W. & Liu, L. 2022. Assessing the Potential of Downscaled Far Red Solar-Induced Chlorophyll Fluorescence from the Canopy to Leaf Level for Drought Monitoring in Winter Wheat. *Remote Sensing* **14**, 1357.

Liu, L., Guan, L. & Liu, X. 2017. Directly estimating diurnal changes in GPP for C3 and C4 crops using far-red sun-induced chlorophyll fluorescence. *Agricultural and Forest Meteorology* **232**, 1–9.

Logan, B.A., Demmig-Adams, B., Adams, W.W. & Bilger, W. 2014. Context, quantification, and measurement guide for non-photochemical quenching of chlorophyll fluorescence, in *Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria*, eds B. Demmig-Adams, G. Garab, W.W. Adams, Govindjee (Dordrecht: Springer), pp. 187–201.

Lootens, P., Van Waes, J. & Carlier, L. 2004. Effect of a short photoinhibition stress on photosynthesis, chlorophyll a fluorescence, and pigment contents of different maize cultivars. can a rapid and objective stress indicator be found? *Photosynthetica* **42**, 187–192.

Magney, T.S., Barnes, M.L. & Yang, X. 2020. On the covariation of chlorophyll fluorescence and photosynthesis across scales. *Geophysical Research Letters* **47**, e2020GL091098.

Maguire, A.J., Eitel, J.U.H., Griffin, K.L., Magney, T.S., Long, R.A., Vierling, L.A., Schmiege, J.S., Jennewein, W.A., Weygint, N.T. & Bruner, B.S.G. 2020. On the functional relationship between fluorescence and photochemical yields in complex evergreen needleleaf canopies. *Geophysical Research Letters* **47**, e2020GL087858.

Mathur, S., Agrawal, D. & Jajoo, A. 2014. Photosynthesis: Responses to High Temperature Stress. *Journal of Photochemistry and Photobiology* **137**, 116–126.

Maxwell, K. & Johnson, G.N. 2000. Chlorophyll fuorescence – a practical guide. *Journal of Experimental Botany* **51**(345), 659–668.

McAusland, L., Atkinson, J.A., Lawson, T. & Murchie, E.H. 2019. High throughput procedure utilising chlorophyll fluorescence imaging to phenotype dynamic photosynthesis and photoprotection in leaves under controlled gaseous conditions. *Plant Methods* **15**, 109.

McDonald, J.H. 2014. *Handbook of Biological Statistics*, 3rd ed. Sparky House Publishing, Baltimore, Maryland. 291 pp.

Moore, C.E., Meacham-Hensold, K., Lemonnier, P., Slattery, R.A., Benjamin, C., Bernacchi, C.J., Lawson, T. & Cavanagh, A.P. 2021. The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. *Journal of Experimental Botany* **72**(8), 2822–2844.

Murata, N., Takahashi, S., Nishiyama, S. & Allakhverdiev, S.I. 2007. Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta* **1767**, 414–421.

Murchie, E.H., Kefauver, S., Araus, J.L., Muller, O., Rascher, U., Flood, P.J. & Lawson, T. 2018. Measuring the dynamic photosynthome. *Annals of Botany* **122**(2), 207–220.

Murchie, E.H. & Lawson, T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany* **64**, 3983–3998.

Naidu, S.L. & Long, S.P. 2004. Potential mechanisms of low-temperature tolerance of C4 photosynthesis in Miscanthusgiganteus: an in vivo analysis. *Planta* **220**, 145–155.

Nesterenko, T.V., Tikhomirov, A.A. & Shikhov, V.N. 2006. Ontogenetic approach to the assessment of plant resistance to prolonged stress using chlorophyll fluorescence induction method. *Photosynthetica* **44**(3), 321–332.

Nesterenko, T.V., Tikhomirov, A.A. & Shikhov, V.N. 2012. Influence of excitation light intensity and leaf age on the slow chlorophyll fluorescence transiet in radish. *Biophysics* **57**(4), 614–620 (in Russian).

Ni, Z., Lu, Q., Huo, H. & Zhang, H. 2019. Estimation of Chlorophyll Fluorescence at Different Scales: A Review. *Sensors* **19**(13), 1–22.

Oláh, V., Hepp, A., Irfan, M. & Mészáros, I. 2021. Chlorophyll Fluorescence Imaging-Based Duckweed Phenotyping to Assess Acute Phytotoxic Effects. *Plants* **10**, 2763.

Østrem, L., Rapacz, M., Larsen, A., Marum, P. & Rognli, O.A. 2018. Chlorophyll a Fluorescence and Freezing Tests as Selection Methods for Growth Cessation and Increased Winter Survival in ×Festulolium. *Frontiers in Plant Science* **9**, 1200.

Pérez-Bueno, M.L., Pineda, M. & Barón, M. 2019. Phenotyping plant responses to biotic stress by chlorophyll fluorescence imaging. *Frontiers in Plant Science* **10**, 1135.

Petjukevics, A. & Skute, N. 2022. Chlorophyll fluorescence changes, as plant early state indicator under different water salinity regimes on invasive macrophyte *Elodea canadensis* (Michx., 1803). *ARPHA Preprints*, e82408.

Portable fluorometer 'Floratest': Operating instructions. Institute of Cybernetics of V.M. Elushkova NAS of Ukraine, 2011. 27 pp. (in Ukraine).

Porcar-Castell, A., Malenovský, Z., Magney, T., Van Wittenberghe, S., Fernández-Marín, B., Maignan, F., Zhang, Y., Maseyk, K., Atherton, J., Albert, L.P., Robson, T.M., Zhao, F., Garcia-Plazaola, J.-I., Ensminger, I., Rajewicz, P.A., Grebe, S., Tikkanen, M., Kellner, J.R., Ihalainen, J.A., Rascher, U. & Logan, B. 2021. Chlorophyll a fluorescence illuminates a path connecting plant molecular biology to Earth-system science. *Natural Plants* **7**, 998–1009.

Posudin, Y.I., Godlevska, O.O., Zaloilo, I.A. & Kozhem'yako, Ya.V. 2010. Application of portable fluorometer for estimation of plant tolerance to abiotic factors. *Agrophysics* (**24**)4, 363–368.

Posudin, Y.I. & Bogdasheva, O.V. 2010. Fluorescent analysis of pea sown Pisum sativum during development and under the influence of external factors. *Scientific reports of National University of Life and Environmental Sciences of Ukraine* **5**(21), 123–128 (in Ukrainian).

Rao, L., Li, S. & Cui, X. 2021. Leaf morphology and chlorophyll fluorescence characteristics of mulberry seedlings under waterlogging stress. *Scientific Reports* **11**, 13379.

Raza, M.A.S., Shahid, A.M., Saleem, M.F., Khan, I.H., Ahmad, S., Ali, M. & Iqbal, R. 2017. Effects and management strategies to mitigate drought stress in oilseed rape (*Brassica napus* L.): a review. *Zemdirbyste-Agriculture* **104**(1), 85–94.

Rintamäki, E., Salo, R., Lehtonen, E. & Aro, E.-M. 1995. Regulation of D1-protein degradation during photoinhibition of photosystem II *in vivo*: Phosphorylation of the D1 protein in various plant groups. *Planta* **195**(3), 379–386.

Roháček, K. 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica* **40**, 13–29.

Romanov, V.O., Artemenko, D.M. & Braiko, Y.O. 2011. Family of portable devices 'Floratest': preparation for serial production. *Computer tools, networks and systems* **10**, 85–93.

Romanov, V.O., Starodub, M.F. & Galelyuka, I.B. 2010. Biosensors for express-diagnostics of photosynthesis and acute viral infections. *Proceedings: Measurements Control Computers in Economy and Environment Protection* **3**, 3–7.
Ruban, A.V. 2016. Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. *Plant Physiology* **170**, 1903–1916.
Ruban, A.V. & Murchie, E.H. 2012. Assessing the photoprotective effectiveness of non-photochemical chlorophyll fluorescence quenching: a new approach. *Biochimica et Biophysica Acta* **1817**, 977–982.
Rumsey, D.J. 2016. *Statistics for Dummies*. 2nd Edition. John Wiley & Sons Inc. 408 pp.
Sarahan, E.V. 2011. Features of practical application of portable biosensor devices of the 'Floratest' family. *Computer tools, networks and systems* **10**, 94–103 (in Ukrainian).
Schreiber, U., Hormann, H., Neubauer, C. & Klughammer, C. 1995. Assessment of photosystem II photochemical quantum yield by chlorophyll fluorescence quenching analysis. *Functional Plant Biology* **22**, 209–220.
Schreiber, U. & Klughammer, C. 2013. Wavelength-dependent photodamage to Chlorella investigated with a new type of multi-color PAM chlorophyll fluorometer. *Photosynthesis Research* **114**, 165–177.
Schreiber, U. & Klughammer, C. 2021. Evidence for variable chlorophyll fluorescence of photosystem I in vivo. *Photosynthesis Research* **149**, 213–231.
Schuback, N., Tortell, P.D., Berman-Frank, I., Campbell, D.A., Ciotti, A., Courtecuisse, E., Erickson, Z.K., Fujiki, T., Halsey, K., Hickman, A.E., Huot, Y., Gorbunov, M.Y., Hughes, D.J., Kolber, Z.S., Moore, C.M., Oxborough, K., Prášil, O., Robinson, C.M., Ryan-Keogh, T.J. & Varkey, D.R. 2021. Single-turnover variable chlorophyll fluorescence as a tool for assessing phytoplankton photosynthesis and primary productivity: opportunities, caveats and recommendations. *Frontiers in Marine Science* **8**, 1–24, 690607.
Selyaninov, G.L. 1928. About the agricultural evaluation of the climate. *Trudy GGO* **20**, 177–185 (in Russian).
Sepúlveda, P.M. & Johnstone, D. 2019. A Novel Way of Assessing Plant Vitality in Urban Trees. *Forests*. **10** (1), 2.
Shomali, A. & Aliniaeifard, S. 2020. Overview of signal transduction in plants under salt and drought stresses. In *Salt and Drought Stress Tolerance in Plants*. Springer: Berlin/Heidelberg, Germany, pp. 231–258.
Shpaar, D. 2012. *Rapeseed and colza: cultivation, harvesting, use*. Publisher: Zerno, 368 pp.
Simeneh, T.A. 2020. Photosynthesis limiting stresses under climate change scenarios and role of chlorophyll fluorescence. *Cogent Food & Agriculture* **6**, 1–18.
Sinyagin, I.I. 1975. *Plant nutrition areas*. Moscow, Rosselkhozizdat, 383 pp. (in Russian).
Sloat, L.L., Lin, M., Butler, E.E., Johnson, D., Holbrook, N.M., Huybers, P.J., Lee, J.E. & Mueller, N.D. 2021. Evaluating the benefits of chlorophyll fluorescence for in-season crop productivity forecasting. *Remote Sensing of Environment* **260**, 112478.
Snedecor, G.W. 1989. *Statistical Methods* 8th Edition. Iowa State University Press. 503 pp.
Souza, A. & Yang, Y. 2021. High-Throughput Corn Image Segmentation and Trait Extraction Using Chlorophyll Fluorescence Images. *Plant Phenomics* **2021**, Article ID 9792582, 15 pp.
Stirbet, A. & Govindjee. 2011. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and photosystem II: Basis and applications of the OJIP fluorescence transient. *Journal of Photochemistry and Photobiology B* **104**, 236–257.
Stirbet, A. & Govindjee. 2012. Chlorophyll a fluorescence induction: a personal perspective of the thermal phase, the J-I-P rise. *Photosynthesis Research* **113**, 15–61.
Stirbet, A., Lazár, D., Kromdijk, J. & Govindjee 2018. Chlorophyll a fluorescence induction: can just a one-second measurement be used to quantify abiotic stress responses? *Photosynthetica* **56**, 86–104.

Stirbet, A., Riznichenko, G.Y., Rubin, A.B. & Govindjee. 2014. Modeling chlorophyll a fluorescence transient: relation to photosynthesis. *Biochemistry-Moscow*+ **79**, 291–323.
Suárez, J.C., Vanegas, J.I., Contreras, A.T., Anzola, J.A., Urban, M.O., Beebe, S.E., Rao, I.M. 2022. Chlorophyll Fluorescence Imaging as a Tool for Evaluating Disease Resistance of Common Bean Lines in the Western Amazon Region of Colombia. *Plants* **11**, 1371.
Test Guidelines for the conduct of tests for distinctness. uniformity and stability of Fodder Radish (*Raphanus sativus* L. var. *oleiformis* Pers.). Geneva. 2017. 19 p.
Thach, L.B., Shapcott, A., Schmidt, S. & Critchley, C. 2007. The OJIP fast fluorescence rise characterizes Craptophyllum species and their stress responses. *Photosynthesis Research* (**94**)2, 423–436.
Tikkanen, M., Mekala, N.R. & Aro, E.M. 2014. Photosystem II photoinhibition-repair cycle protects photosystem I from irreversible damage. *Biochimica et Biophysica Acta* **1837**, 210–215.
Tomaškin, J., Tomaškinová, J. & Theuma, H. 2021. Chlorophyll Fluorescence as a Criterion for the Diagnosis of Abiotic Environmental Stress of Miscanthus x Giganteus Hybrid. *Polish Journal of Environmental Studies* **30**(4), 3269–3276.
Tsai, Y.C., Chen, K.C., Cheng, T.S., Lee, C., Lin, S.H. & Tung, C.W. 2019. Chlorophyll fluorescence analysis in diverse rice varieties reveals the positive correlation between the seedlings salt tolerance and photosynthetic efficiency. *MC Plant Biology* **19**, 403.
Tsytsiura, Y.H. 2019. Evaluation of the efficiency of oil radish agrophytocoenosis construction by the factor of reproductive effort. *Bulgarian Journal of Agricultural Science* **25**(6), 1161–1174.
Tsytsiura, Y.H. 2020. Modular-vitality and ideotypical approach in evaluating the efficiency of construction of oilseed radish agrophytocenosises (*Raphanus sativus* var. *oleifera* Pers.). *Agraarteadus* **31**(2), 219–243.
Tsytsiura, Y. 2020a. Formation and determination of the individual area of oilseed radish leaves in agrophytocenosises of different technological construction. *Agronomy Research* **18**(3), 2217–2244.
Tsytsiura, Ya.H. 2021. Matrix quality variability of oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) and features of its formation in technologically different construction of its agrophytocenosis. *Agronomy Research* **19**(1), 300–326.
Valcke, R. 2021. Can chlorophyll fluorescence imaging make the invisible visible? *Photosynthetica* **59**(SI), 381–398.
Van Zelm, E., Zhang, Y. & Testerink, C. 2020. Salt tolerance mechanisms of plants. *Annual Review of Plant Biology* **71**, 1–31.
Viljevac Vuleti´c, M., Mihaljevi´c, I., Tomaš, V., Horvat, D., Zduni´c, Z. & Vukovi´c, D. 2022. Physiological Response to Short-Term Heat Stress in the Leaves of Traditional and Modern Plum (*Prunus domestica* L.) Cultivars. *Horticulturae* **8**, 72.
Wang, S., Huang, C., Zhang, L., Lin, Y., Cen, Y. & Wu, T. 2016. Monitoring and assessing the 2012 drought in the Great Plains: Analyzing satellite-retrieved solar-induced chlorophyll fluorescence, drought indices, and gross primary production. *Remote Sensing* **8**, 61.
Wang, C., Gu, Q., Zhao, L., Li, C., Ren, J. & Zhang, J. 2022. Photochemical Efficiency of Photosystem II in Inverted Leaves of Soybean [*Glycine max* (L.) Merr.] Affected by Elevated Temperature and High Light. *Frontiers in Plant Science* **12**, 772644.
Żelazny, W.R. & Lukáš, J. 2020. Drought Stress Detection in Juvenile Oilseed Rape Using Hyperspectral Imaging with a Focus on Spectra Variability. *Remote Sensing* **12**(20), 3462.
Zhang, J., Zhao, B., Yang, C., Shi, Y., Liao, Q., Zhou, G., Wang, C., Xie, T., Jiang, Z., Zhang, D., Yang, W., Huang, C. & Xie, J 2020. Rapeseed Stand Count Estimation at Leaf Development Stages With UAV Imagery and Convolutional Neural Networks. *Frontiers in Plant Science* **11**, 617.

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Doddapaneni, T.R.K.C., Praveenkumar, R., Tolvanen, H., Rintala, J. & Konttinen, J. 2018. Techno-economic evaluation of integrating torrefaction with anaerobic digestion. *Applied Energy* **213**, 272–284. doi: 10.1016/j.apenergy.2018.01.045

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Ritchie, M.E. & Olff, H. 2020. Herbivore diversity and plant dynamics: compensatory and additive effects. In: Olff, H., Brown, V.K. & Drent R.H. (eds) *Herbivores between plants and predators. Proc. Int. Conf. The 38th Symposium of the British Ecological Society*, Blackwell Science, Oxford, UK, pp. 175–204.

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