Agronomy Research **21**(S2), 560–576, 2023
https://doi.org/10.15159/AR.23.023

Measuring and alleviating drought stress in pea and lentil

O. Prysiazhniuk[1,*], O. Maliarenko[1], L. Biliavska[2], V. Voitovska[1], L. Kononenko[3], N. Klymovych[3], N. Poltoretska[3], O. Strilets[1] and L. Voievoda[3]

[1]Institute of Bioenergy Crops and Sugar Beet National Academy of Agrarian Sciences of Ukraine, 25 Klinichna Str., UA03110 Kyiv, Ukraine
[2]Poltava State Agrarian University, 1/3 Skovorody Str., UA36003 Poltava, Ukraine
[3]Uman National University of Horticulture, 1 Instytutska Str., UA20305 Uman, Ukraine
[*]Correspondence: ollpris@gmail.com

Received: January 31st, 2023; Accepted: April 17th, 2023; Published: April 22nd, 2023

Abstract. Water deficit in the soil can cause drought stress in plants and drastically affect plant growth and crop yield. Therefore, early detection of drought stress in plants followed by the timely application of agronomic measures to alleviate plant conditions is crucial. This research aimed to study the agronomic practices that could reduce the sensitivity of pea and lentil to drought stress. The practices included (i) soil amendment with moisture retainer (hydrogel), (ii) seed treatment with a growth regulator to promote root formation, (iii) application of a biological formulation to boost soil mycorrhizal biota, and (iv) foliar application of micro fertilisers. The research was carried out in Ukraine in 2015−2020. Drought stress in plants was detected by measuring chlorophyll fluorescence with a portable fluorometer Floratest and calculating the ratio of variable to maximum fluorescence Fv/Fm of the photosystem. The content of proline, high values of which in vegetative organs point out to stress in plants, was determined by colorimetric analysis using ninhydrin.

In pea, the incorporation of hydrogel (Aquasorb) and growth regulator (Mycofriend) combined with seed treatment (Kelpak SC) and foliar application of micro fertiliser (Biovit or Freya-Aqua Legumes) at BBCH 14 led to obtaining Fv/Fm values from 0.81 to 0.82. Similarly in lentil, the maximum value of Fv/Fm (0.67) was obtained with the application of all studied agronomic practices, with the correlation coefficient between yield and Fv/Fm at the flowering stage (BBCH 61) $r = 0.97$. In pea, the correlation between yield and Fv/Fm at the budding stage (BBCH 51) was $r = 0.99$. The content of proline in photosynthetic plant organs was species-specific; however, in the control treatment, where plants were exposed to drought, its maximum value was 1.10 μmol g^{-1} in pea and 1.40 μmol g^{-1} in lentil, while with the application of the proposed agronomic practices proline content was only 0.56 μmol g^{-1} in pea and 0.36 μmol g^{-1} in lentil. Obtained strong correlation between proline content in plant vegetative organs and the ratio of variable to maximum fluorescence Fv/Fm of the plant photosystem indicates that measurement of Fv/Fm with portable fluorometer might be an effective method of early identification of drought stress in pea and lentil.

Key words: chlorophyll fluorescence, portable fluorometer, hydrogel, growth regulator, micro fertiliser.

INTRODUCTION

Drought stress is the main cause of considerable yield losses in many crops, including leguminous lentil (*Lens culinaris*) and pea (*Pisum sativum*) traditionally grown in Ukraine (Prysiazhniuk et al., 2020). Water deficit causes considerable inhibition of plant growth and development and negatively affects root and leaf formation resulting in yield shortage. Drought stress is a major cause of the low yield of lentil in many regions of the world (Zeroual et al., 2023). For example, in the Mediterranean region, lentil yield can be highly affected by fluctuations in seasonal precipitation, as the intensive rainfalls occur in winter, while in the period from March to May, plants are exposed to drought and high temperatures (Choukri et al., 2020).

Water deficit in the critical stages of growth and development of pea and lentil may result in falling buds, flowers and fruits, low seed weight, and, consequently, low yield (Coyne et al., 2020). Drought stress negatively affects the majority of C3 crops that do not have mechanisms for alleviating the negative impact of drought (Guidi et al., 2019; Marchin et al., 2020; Zhuang et al., 2020; Mihaljevic et al., 2021). While we can provide plants with water at the beginning of vegetation by adjusting the timing of seedbed preparation and sowing (Sen et al., 2016), in the rest of the vegetation season, the plants are defenceless against drought.

Crop resistance to drought can be increased through a breeding approach, i.e., the development of drought-tolerant varieties (Ghanem et al., 2015; Larouk et al., 2021; Snowdon et al., 2021). Another approach to alleviating drought stress in plants may be agronomic, i.e., application of certain agronomic practices, for example, conservation tillage, seed treatment, proper fertilisation, application of growth regulators, plant-promoting rhizobacteria and arbuscular mycorrhizal fungi that have proven to be useful in diminishing the adverse effects of drought stress (Rosa et al., 2023). A well-developed root system is crucial for efficient water uptake from the soil, while a weak root system may be a reason for slow growth and development of plants. A weak root system makes plants vulnerable to the drying up of the soil layer. Our previous research demonstrated that seed treatment with growth regulators can help prevent the issue of a weak root system (Prysiazhniuk et al., 2020). However, a limited number of research on such practices in lentil and pea has been reported.

Agronomic practices will be successful if they are applied at the right time; this is especially true in the case of growth regulators. To determine the right time, portable fluorometers may be used. Such devices detect changes occurring in the plant photosynthetic system by determining the fluorescence state of the plant photosystem and transforming it into an electrical signal with subsequent processing of the signal (Tsai et al., 2019; Suárez et al., 2022; Tsytsiura, 2022). Drought stress in C3 plants can be identified by the Templer protocol - calculating the ratio of variable to maximum fluorescence Fv/Fm of plant photosystem (Templer et al., 2017). Another (and the only alternative) method that can provide reliable results in the case of moderate drought stress is the determination of Fs/Fo (Flexas et al., 2000; Flexas et al., 2002). However, this method is effective only under moderate drought stress and is not suitable for most crops. Measuring Fv/Fm has been proven to identify even severe drought stress in plants (Arrobas et al., 2016). Interestingly, chlorophyll fluorescence can be used not only to assess plant resistance to abiotic stress factors (Simeneh, 2020; Legendre et al., 2021; Larouk et al., 2021; Moore et al., 2021; Oláh et al., 2021; Lin et al., 2022; Wang et al., 2022), specifically water deficit (Li et al., 2020; Kimm et al., 2021) and high or low temperatures (Baldocchi et al., 2020; Kim et al., 2021) but also to detect plant diseases and pests (Hupp et al., 2019; Amri et al., 2021; Sloat et al., 2021), even pollution by heavy metals (Van Zelm et al., 2020; Javed et al., 2022).

One of the conventional methods of detecting drought stress in plants is determining proline content in plant vegetative organs (Ain-Lhout et al., 2001; Al-Khayri, 2002). Its accumulation is species-specific and is considered a stress reaction but not an indicator of tolerance to drought stress (Liu & Zhu, 1997; Hoai & Shim, 2003). Proline also influences cell proliferation and initiates plant recovery after stress; therefore, it can be found in plants even at the stage of their recovery from stress (Yamada et al., 2005; Valliyodan & Nguyen, 2006; Szabados & Savoure, 2009). Contrary to the proline method, express analysis with the use of a portable fluorometer can detect stress in plants more selectively and precisely (Larouk et al., 2021).

The purpose of the research was to develop a method of early detection of drought stress in lentil and pea with the use of a portable fluorometer and study the efficiency of proposed agronomic practices to alleviate plant conditions.

MATERIALS AND METHODS

Place and crop rotation

Field experiments on lentil and pea were carried out in the Uladivske-Liulyntsi Experimental Breeding Station (49°34'30.7"N 28°22'39.5"E) of the Institute of Bioenergy Crops and Sugar Beet National Academy of Agrarian Sciences of Ukraine in 2015−2020 (lentil) and 2015−2019 (pea). Pea and lentil were grown in conventional grain and beet crop rotation with the following crop alternation: leguminous crops - winter wheat - sugar beet - maize. The total area of the field with leguminous crops was 70 ha. The field was divided into two equal parts for the cultivation of pea and lentil (3,220 m^2 each). In the crop rotation, the place of leguminous crops did not change; therefore, the effect of preceding crops can be neglected.

Soil conditions

The field experiment was established in deep medium-loamy chernozem with the humus content (by the Tyurin and Kononova method) of 3.9%, nitrate nitrogen of 16.4 mg kg^{-1}, ammonium nitrogen of 38.7 mg kg^{-1}, mobile phosphates (by the Chirikov method) of 83 mg kg^{-1} and exchangeable potassium (by the Chirikov method) of 103 mg kg^{-1}. The reaction of the soil solution was slightly acidic, close to neutral. The availability of mineral nitrogen (nitrate + ammonium) was medium, phosphorus low and exchangeable potassium high.

Weather conditions

In April−July 2015–2020, the air temperature was higher compared to the average long-term data, and precipitation was uneven. May of 2020 was colder by 2.6 °C compared to long-term (20 years) data (Fig. 1). The hottest vegetation seasons were in 2015, 2016, 2018 and 2019. The lack of precipitation was observed in April and July in all years of the experiment. The driest years were 2015 and 2017, while 2019 and 2020 had alternate dry and rainy periods. Therefore, it can be concluded that weather conditions were favourable for the purpose of studying drought stress in crops.

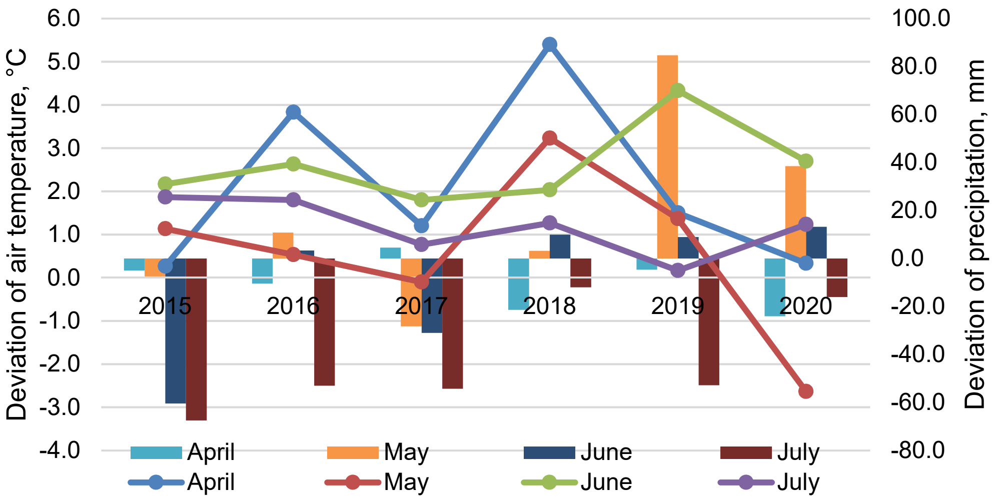


Figure 1. Deviation of monthly average temperature and precipitation from long-term average data.

Experimental design and treatments

Field experiments were carried out in four replications at the randomized block design. The area of each assessed plot was 35 m^2. Total area of lentil was 3,220 m^2 and pea 3,220 m^2.

Some treatments were the same for both pea and lentil - application of hydrogel Aquasorb (200 kg ha^{-1}), seed treatment with Kelpac SC and application of mycorrhizal bio formulation Mycofriend, while micro fertilisers were different due to the different needs of crops. Specifically, in lentil sowings, Reakom-SP-Legumes (3 L ha^{-1}, BBCH 14) and Quantum Legumes (1.0 L ha^{-1}, BBCH 14) fertilisers were used, while in pea sowings, Biovit (7 L ha^{-1}, BBCH 14) and Freya-Aqua Legumes (1.5 L ha^{-1}, BBCH 14) were used. The complete set of experimental treatments is presented in Table 1.

Hydrogel Aquasorb was incorporated into the soil in the process of early spring tillage using Amazone ZA-TS 3200 spreader. Mycorrhizal bio formulation Mycofriend (1 L ha^{-1}) was applied before soil cultivation with a hinged field sprayer Amazone UF at a rate of 200 L ha^{-1}.

Measurement of chlorophyll fluorescence

We used a portable fluorometer Floratest (developed at the Institute of Cybernetics National Academy of Sciences and the Institute of Bioenergy Crops and Sugar Beet National Academy of Agrarian Sciences of Ukraine). The device operates by generally recognized algorithms for the determination of the fluorescence intensity of chlorophyll and F_v/F_m of the photosystem (Maxwell & Johnson, 2000; Prysiazhniuk et al., 2017).

Plant chlorophylls absorb light energy in the photosynthetic active radiation range from 390 to 730 nm with the maxima in the ranges from 400 to 500 nm and 600 to 700 nm (Buschmann, 2007; Buschmann, 2008). Some design features of the fluorometer sensors impose some restrictions on determining the activity of the photosystem of plants with small leaves and tendrils (such as lentil and pea) (Cavender-Bares & Fakhri, 2004). For accurate determination of chlorophyll fluorescence in leaves of such plants we used a method described by Gitelson et al. (1999) which allows analysing the most reliable range of chlorophyll measurement by narrowing the measurement diapason to 700−735 nm. Limiting the spectrum range of red radiation made it possible to significantly increase the accuracy of the chlorophyll measurements compared to the methods commonly used to measure stress in plants (George et al., 2006). It was only necessary to change the settings of the microprocessor program of the device (Prysiazhniuk et al., 2017).

Table 1. Design of the lentil and pea experiments on the agronomic practices to increase drought tolerance at early stages of plant growth and development

Moisture-retainer	Mycorrhizal bio formulation	Growth regulator (seed treatment)	Micro fertilisers lentil	Micro fertilisers pea	Treatment No
Control	Control	Control	Control	Control	1
			Reakom-SP-Legumes	Biovit	2
			Quantum Legumes	Freya-Aqua Legumes	3
		Kelpak SC	Control	Control	4
			Reakom-SP-Legumes	Biovit	5
			Quantum Legumes	Freya-Aqua Legumes	6
	Mycofriend	Control	Control	Control	7
			Reakom-SP-Legumes	Biovit	8
			Quantum Legumes	Freya-Aqua Legumes	9
		Kelpak SC	Control	Control	10
			Reakom-SP-Legumes	Biovit	11
			Quantum Legumes	Freya-Aqua Legumes	12
Aquasorb	Control	Control	Control	Control	13
			Reakom-SP-Legumes	Biovit	14
			Quantum Legumes	Freya-Aqua Legumes	15
		Kelpak SC	Control	Control	16
			Reakom-SP-Legumes	Biovit	17
			Quantum Legumes	Freya-Aqua Legumes	18
	Mycofriend	Control	Control	Control	19
			Reakom-SP-Legumes	Biovit	20
			Quantum Legumes	Freya-Aqua Legumes	21
		Kelpak SC	Control	Control	22
			Reakom-SP-Legumes	Biovit	23
			Quantum Legumes	Freya-Aqua Legumes	24

Measurements and statistical analysis

The measurement of plant biometric parameters was carried out by sampling 50 plants per replication. The yield was determined in a plot-by-plot manner, and the grain moisture was adjusted accordingly.

The free proline content was determined by the method of colorimetric analysis using ninhydrin. To this end, plant material was homogenized. Extraction was carried out with a solution of ethanol and water in a ratio of 70:30. After that, a reaction mixture (1% ninhydrin in acetic acid with ethanol) was added, and the resulting mixture was incubated in a water bath at 95 °C for 30 min. Then, the tubes were cooled down and centrifuged. After that, the optical density of the ninhydrin-proline solution was determined using a spectrophotometer at a wavelength of 520 nm. The calibration graph was plotted using L-proline (Carillo & Gibon, 2011).

Statistical processing of the experimental data was performed using the analysis of variations (ANOVA) and correlation-regression analysis (Marques de Sá, 2007) using the software Statistica 12 (Rumsey, 2016). MS Excel 2019 was used for the visualization of the regression equations, obtained and verified in Statistica 12.

RESULTS AND DISCUSSION

Field experiments were carried out in the conditions of the unstable water content of soil; therefore, it was important to assess the water content available to plants in the 0−20 cm layer. In 2015, at the time of sowing, water content was 35 mm, while with the use of the hydrogel Aquasorb, it increased to 38 mm (satisfactory). Similarly, water content was assessed as satisfactory in 2016, 2019, and 2020 and good in 2017, and 2018. In 2015, at the time of flowering, water content decreased to 4–7 mm (unsatisfactory). Similarly unsatisfactory water content of the soil was also in 2017, while in 2016, 2018, 2019, and 2020 years it was satisfactory (Tables 2 and 3). Application of hydrogel provided an additional 3 mm of water available to plants in the 0–20 cm layer, as granules of hydrogel trap condensed water (dew) and capillary water in the upper soil layer.

Table 2. The water content of soil (mm) in pea sowings under the application of hydrogel Aquasorb at an application rate of 200 kg ha^{-1} (2015–2019)

Treatment	Stage of measurement					
	BBCH 01		BBCH 61		BBCH 91	
	Soil layer (cm)					
	0–20	0–100	0–20	0–100	0–20	0–100
2015						
Without moisture retainer	34	199	3	59	5	36
With moisture retainer	37	202	6	62	8	39
2016						
Without moisture retainer	32	198	21	151	25	88
With moisture retainer	35	201	24	154	28	91
2017						
Without moisture retainer	49	245	9	42	0	22
With moisture retainer	52	248	12	45	3	25
2018						
Without moisture retainer	41	203	38	138	34	112
With moisture retainer	44	206	41	141	37	115
2019						
Without moisture retainer	27	164	23	101	10	83
With moisture retainer	30	167	25	104	13	86

Plants undergo drought stress when the water content of the soil is limited or when transpiration is intensive (Sperdouli & Moustakas, 2014). In the literature, we found that a decrease in the water content of the soil to 70% of the soil capacity had a negative effect on the growth and development of pea (Moisa et al., 2019), while a decrease to 80% caused a decrease in the concentration of chlorophylls *a* and *b* and the maximum quantum efficiency of the photosystem II (Fv/Fm) in pea and lentil (Arafa et al., 2021; Suprasanna et al., 2016) along with an increase in the proline content (Meena et al., 2019).

Table 3. The water content of soil (mm) in lentil sowings under the application of hydrogel Aquasorb at a dose of 200 kg ha^{-1} (2015–2020)

Treatment	Stage of measurement					
	BBCH 01		BBCH 61		BBCH 91	
	Soil layer (cm)					
	0–20	0–100	0–20	0–100	0–20	0–100
2015						
Without moisture retainer	35	200	4	60	5	37
With moisture retainer	38	203	7	63	8	40
2016						
Without moisture retainer	33	198	20	150	24	87
With moisture retainer	36	201	23	153	27	90
2017						
Without moisture retainer	52	243	10	44	1	23
With moisture retainer	55	246	13	47	4	26
2018						
Without moisture retainer	42	205	37	137	34	112
With moisture retainer	45	208	40	140	37	115
2019						
Without moisture retainer	29	166	24	101	11	83
With moisture retainer	32	169	27	104	14	86
2020						
Without moisture retainer	26	140	20	94	7	48
With moisture retainer	29	143	23	97	10	51

In our research, we chose the ratio F_v/F_m as an indicator of drought stress state in pea (Table 4) and lentil plants (Table 5). The data on the pea photosystem efficiency (Table 4) show that in the control treatment, the plants were largely affected by drought at the budding stage (BBCH 51). This does not mean that they died since the drought in the years of research was not so severe but the indicator F_v/F_m was low - 0.33, while with the application of hydrogel, it was 0.43.

In our experiments, pea responded well to the application of all agronomic practices that increased the efficiency of the photosynthetic apparatus. The most efficient was the combined application of all practices, i.e., incorporation of hydrogel and mycorrhizal bio formulation, seed treatment with Kelpak SC, and foliar application of micronutrients Biovit or Freya-Aqua Legumes (BBCH 14). F_v/F_m values in such treatments were at the level of 0.81−0.82. A strong correlation was found between the pea yield and F_v/F_m of the photosystem at the budding stage (BBCH 51), with r = 0.99. The regression dependence is shown in Fig. 2.

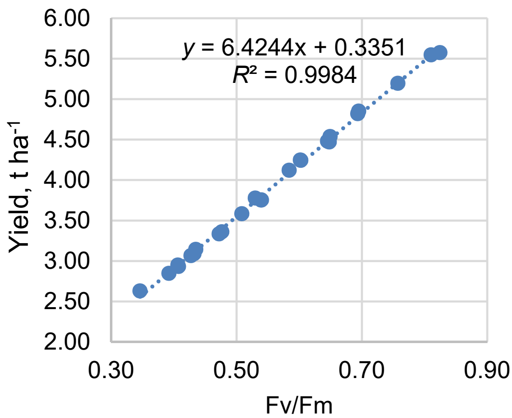


Figure 2. Regression between pea yield and F_v/F_m.

Table 4. F_v/F_m of pea at the budding stage (BBCH 51)

Treatment No	2015	2016	2017	2018	2019
1	0.33	0.37	0.34	0.36	0.33
2	0.38	0.43	0.40	0.42	0.39
3	0.36	0.41	0.36	0.43	0.40
4	0.42	0.45	0.42	0.45	0.43
5	0.45	0.49	0.47	0.50	0.48
6	0.43	0.47	0.45	0.52	0.49
7	0.40	0.42	0.39	0.42	0.41
8	0.42	0.46	0.42	0.44	0.42
9	0.41	0.45	0.40	0.44	0.43
10	0.46	0.53	0.51	0.50	0.54
11	0.51	0.57	0.54	0.52	0.57
12	0.50	0.55	0.53	0.53	0.59
13	0.56	0.53	0.46	0.59	0.51
14	0.63	0.61	0.52	0.66	0.59
15	0.60	0.58	0.48	0.67	0.60
16	0.70	0.63	0.56	0.71	0.64
17	0.70	0.69	0.63	0.76	0.70
18	0.69	0.66	0.60	0.80	0.71
19	0.65	0.58	0.52	0.67	0.60
20	0.67	0.64	0.58	0.71	0.62
21	0.69	0.63	0.55	0.72	0.64
22	0.79	0.74	0.67	0.80	0.79
23	0.79	0.82	0.73	0.87	0.85
24	0.83	0.79	0.72	0.91	0.88
$LSD_{0.05}$	0.04	0.05	0.03	0.04	0.03

Table 5. F_v/F_m of lentil at the flowering stage (BBCH 61)

Treatment No	2015	2016	2017	2018	2019	2020
1	0.29	0.37	0.39	0.30	0.31	0.33
2	0.30	0.38	0.39	0.30	0.33	0.35
3	0.32	0.38	0.39	0.31	0.32	0.36
4	0.37	0.39	0.41	0.32	0.33	0.35
5	0.41	0.39	0.40	0.32	0.33	0.34
6	0.40	0.39	0.40	0.32	0.34	0.34
7	0.36	0.39	0.40	0.35	0.35	0.38
8	0.39	0.39	0.40	0.34	0.33	0.38
9	0.41	0.38	0.40	0.35	0.35	0.39
10	0.42	0.39	0.40	0.36	0.34	0.37
11	0.45	0.41	0.42	0.37	0.37	0.39
12	0.46	0.41	0.43	0.38	0.36	0.39
13	0.39	0.40	0.41	0.35	0.36	0.36
14	0.43	0.40	0.42	0.38	0.37	0.37
15	0.46	0.41	0.42	0.39	0.37	0.38
16	0.50	0.43	0.43	0.39	0.35	0.37
17	0.55	0.44	0.45	0.42	0.38	0.39
18	0.56	0.44	0.45	0.42	0.39	0.39
19	0.51	0.42	0.42	0.39	0.35	0.36
20	0.55	0.42	0.43	0.40	0.37	0.38
21	0.56	0.42	0.44	0.41	0.37	0.40
22	0.56	0.49	0.45	0.45	0.42	0.43
23	0.61	0.52	0.48	0.47	0.45	0.45
24	0.64	0.52	0.49	0.49	0.44	0.45
$LSD_{0.05}$	0.03	0.03	0.04	0.03	0.02	0.03

In lentil, similar to pea, on average over the research years, the lowest ratio of Fv/Fm was observed in the control treatment, which means high exposure to drought at the flowering stage (BBCH 61). The use of hydrogel significantly improved plant condition. In the treatment with hydrogel, the ratio Fv/Fm was 0.67, while in the control it was 0.45. The most effective treatment that contributed to the maximum Fv/Fm values (0.81−0.82) was the cone with the application of hydrogel and mycorrhizal bio formulation, seed treatment and foliar application of micronutrients Reakom-SR-Legumes or Quantum Legumes (BBCH 14). Similar to pea, there was a strong correlation between yield and photosystem indicators at the flowering stage (BBCH 61), $r = 0.97$. The regression dependence is shown in Fig. 3. It should be noted that the

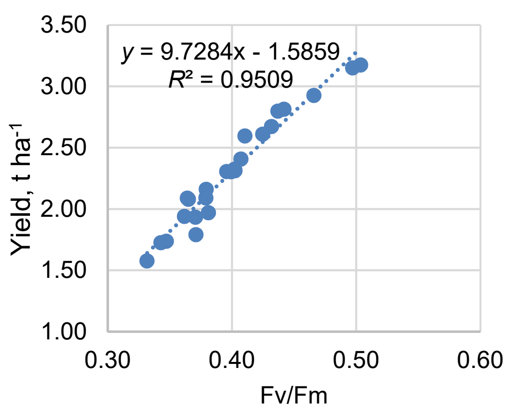


Figure 3. Regression between lentil yield and F_v/F_m.

efficiency of all agronomic practices increases with the optimization of plant provision with water (Larouk et al., 2021; Valcke, 2021).

When analysing the experimental data, we found species-specific values of the content of free proline in the photosynthetic plant organs. On average, the content of (crude) proline was 0.86 µmol g^{-1} in pea and 0.78 µmol g^{-1} in lentil (Table 6). In the control treatments, at the critical stages of growth and development, the maximum concentration of proline was determined as the plants were affected by drought. On the contrary, the application of hydrogel and additional agronomic practices contributed to reducing drought stress in plants as evidenced by the content of free proline in the photosynthetic plant organs.

In the condition of osmotic stress, the content of free proline in pea can increase 100 times (Dar et al., 2016). Other scientists (Lahuta et al., 2022) reported that a 5-day drought led to a fivefold increase in the free proline content in pea and after another 5 days, the content of proline increased 50 times. In our research, we recorded two times higher (in pea) and four times higher (in lentil) free proline content in the control plots.

A study of the content of proline in wheat (Song et al., 2005) showed that a drought-tolerant genotype demonstrated a higher accumulation of proline compared to a susceptible one, with the proline content increasing along with the increasing intensity of drought. Similarly, in maize grown in conditions of water deficit, the content of proline was higher (Anjum et al., 2011; Koskeroglu & Tuna, 2010). The same is true for some other crops (Bartels & Sunkar, 2005; Chaves et al., 2009; Conde et al., 2011; Qin et al., 2011; Roy et al., 2009).

Table 6. The content of free (crude) proline in plant photosynthetic organs (µmol g^{-1}) and yield (t ha^{-1}), average over the years of research

Treatment No	Pea: content of free (crude) proline	Pea: yield	Lentil: content of free (crude) proline	Lentil: yield
1	1.10	2.63	1.40	1.58
2	1.09	2.95	1.38	1.73
3	1.09	2.85	1.36	1.74
4	1.05	3.14	1.29	1.94
5	1.01	3.36	1.23	2.08
6	1.02	3.33	1.22	2.09
7	1.06	2.93	1.11	1.79
8	1.04	3.09	1.00	1.93
9	1.04	3.07	0.98	1.97
10	0.97	3.58	0.78	2.16
11	0.95	3.75	0.71	2.30
12	0.95	3.75	0.72	2.31
13	0.84	3.78	0.65	2.09
14	0.80	4.25	0.56	2.30
15	0.82	4.12	0.55	2.32
16	0.68	4.54	0.48	2.59
17	0.65	4.85	0.46	2.80
18	0.66	4.82	0.46	2.81
19	0.75	4.24	0.45	2.41
20	0.72	4.48	0.44	2.61
21	0.73	4.47	0.43	2.67
22	0.58	5.20	0.39	2.93
23	0.56	5.55	0.36	3.15
24	0.56	5.57	0.36	3.17
$LSD_{0.05}$	0.05	0.21	0.04	0.16

We also determined the type and strength of the correlation between the content of free proline and F_v/F_m in pea and lentil (Figs 4 and 5).

Our results let us assume that there is a strong correlation between the concentration of free proline and Fv/Fm in the studied crops, as correlation coefficients for pea (r = -0.97) and lentil (r = -0.86) corresponds to a very strong level of correlation. Some studies do not fully agree on the effectiveness of using the Fv/Fm indicator for the identification of stress in plants. Thus, when studying the reaction of plants to treatment with cadmium in doses of Cd20 and Cd25, it was found that the proline concentration in bean leaves increased 2.5 and 1.3 times, while Fv/Fm changed differently (Alle et al., 2019).

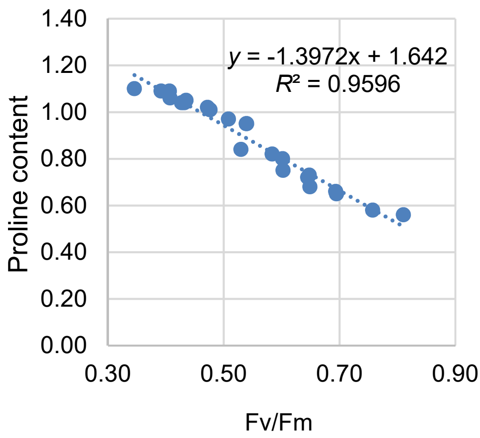


Figure 4. Regression between the content of proline and F_v/F_m in pea.

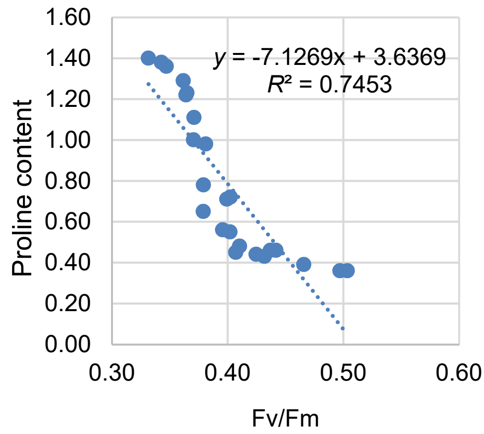
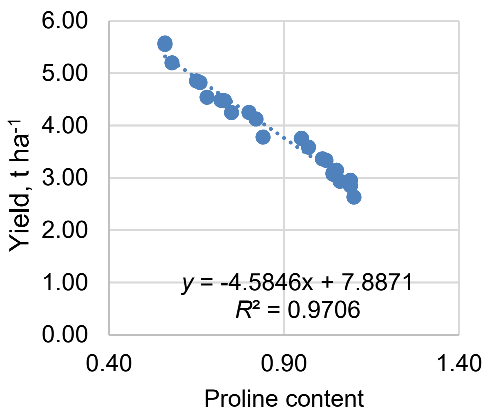


Figure 5. Regression between the content of proline and F_v/F_m in lentil.

Quite interesting is the dynamics of free proline concentration in the photosynthetic organs of lentil (leaves and tendrils) in comparison with the efficiency of the plant photosystem. In our opinion, the low level of correlation of the studied signs is due to the fact that lentil slows down its growth under the influence of stress and recovers in the event of favourable conditions. Consequently, with an increase in water deficit in lentil, other mechanisms for regulating plant stress are most likely involved. Thus, according to other researchers, water deficit during flowering reduces plant height, leaf area and dry matter accumulation, which leads to a decrease in the dry matter content in the biomass and seeds (Shrestha et al., 2005). Foti et al. (2021) showed the general metabolic disturbance in the lentil metabolism in response to drought stress. The metabolic response included the accumulation of D-fructose, a-trehalose, myoinositol and L-tryptophan, which indicates their crucial role in the response to drought and their potential to be used as biomarkers for the effective selection of drought-resistant germplasm. This corresponds to our assumptions about a more complex reaction to water deficit in lentil.

We also identified regression relationships between the content of proline in the studied crops and their yield (Figs 6, 7). The obtained patterns show that a high concentration of free proline might be an indicator of plant stress caused by water deficit, which is associated with crop productivity. Thus, we obtained correlation coefficients for pea $r = -0.98$ and lentil $r = -0.88$, which correspond to a very strong level of correlation. The obtained data positively correlates with recent publications of other authors (Baldocchi et al., 2020; Guo, et al., 2022; Larouk et al., 2021).



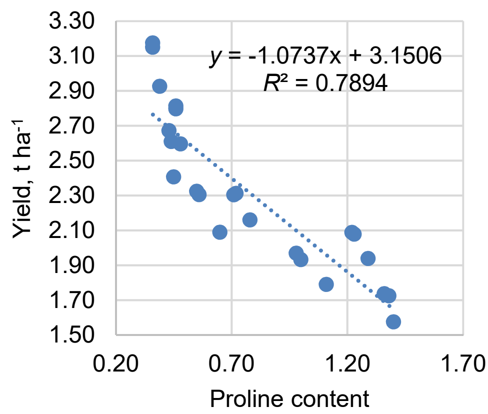


Figure 6. Regression between the content of proline and yield in pea.

Figure 7. Regression between proline content and seed yield in lentil

Regarding the influence of the studied agronomic practices, the application of hydrogel contributed to better conditions for water supply; therefore, pea yield was 1.15 t ha^{-1} higher than in the control. Application of hydrogel in lentil also ensured an increase in yield by 0.51 t ha^{-1} (Table 6) as it provided a smooth course of the critical for lentil stages - BBCH 30−30 and 61−69 (Coyne et al., 2020). In our experiments, the application of hydrogel interacted quite well with other experimental factors, especially with seed treatment and application of mycorrhiza-forming bio formulation ensuring pea yield increase of 2.57 t ha^{-1} compared to the control, while seed treatment and mycorrhiza-forming bio formulation ensured a 1.91 t ha^{-1} yield increase and seed treatment alone increased yield by only 0.51 t ha^{-1}. Lentil demonstrated a similar yield pattern, with a yield increase of 0.36 t ha^{-1}, 1.02 t ha^{-1} and 1.35 t ha^{-1}, respectively.

Foliar application of micro fertilisers in pea had a rather strong effect on the plants in the absence of other studied practices. Whereas in lentil, micro fertilisers showed maximum efficiency under the combination of all studied practices. This is consistent with the findings of other researchers, who found that lentil, compared to pea, had a better-developed root system, and therefore, provided its needs for nutrients through absorption from the soil (Khodanitska, 2019). Under the favourable conditions for biomass formation ensured by the application of other agronomic measures plants respond better to the application of micro fertilisers (Hospodarenko & Musiyenko, 2020).

In the studies of Le et al. (2018) and Akhtar et al. (2020) rhizobacteria formulations applied to soil worked especially effectively in dry periods as they increased the water use efficiency of plants and improved crop productivity (Backer et al., 2018). Growth regulators positively affected plant conditions in the study of Zeroual et al. (2023). However, Lamaoui et al. (2018) noted that the contribution of growth regulators to the alleviation of drought stress in plants is rather supplementary and they work well when combined with other agronomic practices. The contribution of hydrogel is more obvious as it helps plans obtain additional water every day, thereby contributing to better growth and development.

The effectiveness of the chlorophyll fluorescence method as a criterion for assessing the optimality of the agrocenosis of field crops has been proven against the background of various fertilisation options in the studies of Herritt et al. (2021) and Guo et al. (2022). Therefore, the interaction of the experimental factors obtained by us, especially the influence of the foliar application of micro fertilisers, should be further investigated in deep as a separate experimental factor.

CONCLUSIONS

1. Measuring the ratio of the variable to the maximum fluorescence F_v/F_m of the plant photosystem is demonstrated to be an efficient way of early detection of drought stress in pea and lentil. A very strong correlation was found between F_v/F_m and proline content in plant vegetative organs of pea (r = -0.97) and lentil (r = -0.86). A high concentration of proline in the photosynthetic plant organs indicates drought stress in plants leading to lower crop yield. The correlation between yield and proline content was also very strong, with r = -0.98 in pea and r = -0.88 in lentil.

2. All studied practices were effective for the alleviation of drought stress in plants; the most efficient for pea was the application of all studied agronomic practices i.e., incorporation of hydrogel (Aquasorb) and mycorrhizal bio formulation (Mycofriend) to the soil, seed treatment (Kelpak SC), and foliar application of micro fertilisers (Biovit or Freya-Aqua Legumes) at BBCH 14, which resulted in F_v/F_m values of 0.81–0.82 and ensured a yield increase of 2.92 (with Biovit) and 2.62 t ha^{-1} (with Freya-Aqua Legumes) compared to control. Similar to pea, lentil showed the maximum value of Fv/Fm (0.67) under the application of all studied agronomic practices, with the correlation coefficient between yield and Fv/Fm at the flowering stage (BBCH 61) r = 0.97 and yield increase of 1.57 (with Biovit) and 1.60 t ha^{-1} (with Freya-Aqua Legumes) compared to control

3. Further research should clarify the interaction between the proposed agronomic practices. Also, the effect of the foliar application of micro fertilisers should be further investigated in the context of the development of protocols for their application aimed at alleviating drought stress in crops.

REFERENCES

Ain-Lhout, F., Zunzunegui, M., Diaz-Barradas, M.C., Tirado, R., Clavijo, A. & Garcia-Novo, F. 2001. Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. *Plant Soil* **230**, 175–183.

Akhtar, S.S., Amby, D.B., Hegelund, J.N., Fimognari, L., Großkinsky, D.K., Westergaard, J.C., Müller, R., Moelbak, L., Liu, F. & Roitsch, T. 2020. Bacillus licheniformis FMCH001 increases water use efficiency via growth stimulation in both normal and drought conditions. *Front. Plant Sci.* **11**, 297 http://doi.org/10.3389/fpls.2020.00297

Al-Khayri, J.M. 2002. Growth, proline accumulation and ion content in sodium chloride-stressed callus of date palm. In vitro *Cell Dev. Biol. Plant* **38**, 79–82. doi: 10.1079/IVP2001258.

Alle, V., Osvalde, A., Vikmane, M. & Kondratovics, U. 2019. The effect of cadmium and lead pollution on growth and physiological parameters of field beans (*Vicia faba*). *Agronomy Research* **17**(S2), 1261–1272. https://doi.org/10.15159/AR.19.062

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.

Anjum, S.A., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C. & Lei, W. 2011. Morphological, physiological and biochemical responses of plants to drought stress. *Afr. J. Agric. Res.* **6**, 2026−2032. doi: 10.5897/AJAR10027

Arafa, S.A., Attia, K.A., Niedbała, G., Piekutowska, M., Alamery, S., Abdelaal, K., Alateeq, T.K., Ali, M., Elkelish, A. & Attallah, S.Y. 2021. Seed Priming Boost Adaptation in Pea *Plants under Drought Stress. Plants (Basel)*. **10**, 2201. doi: 10.3390/plants10102201

Arrobas, M., Aguiar, P. & Rodrigues, M.A. 2016. A comparison of a pasture ley with a maize monoculture on the soil fertility and nutrient release in the succeeding crop. *Journal Archives of Agronomy and Soil Science* **62**(6).

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S. & Smith, D.L. 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci*. **9**, 1473. http://doi.org/10.3389/fpls.2018.01473

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.

Bartels, D. & Sunkar, R. 2005. Drought and salt tolerance in plants. *Crit. Rev. in Plant Sci*., 23−58. doi: 10.1080/07352680590910410

Brouziyne, Y., Abouabdillah, A., Hirich, A., Bouabid, R., Rashyd, Z. & Benaabidate, L. 2018. Modeling sustainable adaptation strategies toward climate-smart agriculture in a Mediterranean watershed under projected climate change scenarios. *Agricultural Systems* **162**, 154–163.

Buschmann, C. 2007. Variability and application of the chlorophyll fluorescence emission ratio red/far-red leaves. *Photosynthesis Res.* **92**, 261−271.

Buschmann, C. 2008. Recommended taking multiple measurements per leaf to find potential infection locations as a substitute for fluorescence leaf imaging. Botanik 2, Universität Karlsruhe (TH), 76128 Karlsruhe (in Germany).

Carillo, P. & Gibon, Y. 2011. Protocol: Extraction and determination of proline. http://prometheuswiki.publish.csiro.au/tiki-index.php?page=Extraction+and+determination+of+proline

Cavender-Bares, J. & Fakhri, A. 2004. From Leaves to Ecosystem: Using Chlorophyll Fluorescence to Assess Photosynthesis and Plant Function in Ecological Studies. *Chapter 29, Chlorophyll a Fluorescence a Signature of Photosynthesis, edited by George Papaqeorgiou and Govindjee*. Springer, pp. 746−747.

Chaves, M.M., Flexas, J. & Pinheiro, C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* **103**, 551−560. doi: 10.1093/aob/mcn125

Choukri, H., Hejjaoui, K., El-Baouchi, A., El haddad, N., Smouni, A., Maalouf, F., Thavarajah, D. & Kumar, S. 2020. Heat and drought stress impact on phenology, grain yield, and nutritional quality of lentil (*Lens culinaris* Medikus). *Front. Nutr*. **7**, 596307. doi: 10.3389/fnut.2020.596307

Conde, A., Chaves, M.M. & Geros, H. 2011. Membrane Transport, Sensing and Signaling in Plant Adaptation to Environmental Stress. *Plant Cell Physiol*. **52**, 1583−1602. doi: 10.1093/pcp/pcr107

Coyne, C.J., Kumar, S., von Wettberg, E.J.B., Marques, E., Berger, J.D., Redden, R.J., Ellis, T.H.N., Brus, J., Zablatzká, L. & Smýkal, P. 2020. Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. *Legume Science* **2**, e36. https://doi.org/10.1002/leg3.36

Dar, M.I., Naikoo, M.I., Rehman, F., Naushin, F. & Khan, F.A. 2016. Proline accumulation in plants: Roles in stress tolerance and plant development. In Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies; Iqbal, N., Nazar, R., Khan, N.A., Eds., Springer, New Delhi, India. 155–166. http://doi.org/10.1007/978-81-322-2616-1_9

Flexas, J., Briantais, M.J., Cerovic, Z., Medrano, H. & Moya, I. 2000. Steady-State and Maximum Chlorophyll Fluorescence Responses to Water Stress In Grape Vine Leaves: A New Remote Sensing System. *Remote Sensing Environment* **73**, 283–270.

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.

Foti, C., Kalampokis, I.F., Aliferis, K.A. & Pavli, O.I. 2021. Metabolic Responses of Two Contrasting Lentil Genotypes to PEG-Induced Drought Stress. *Agronomy* **11**, 1190. https://doi.org/10.3390/agronomy11061190

Gaur, P.M., Samineni, S., Krishnamurthy, L., Varshney, R.K., Kumar, S., Ghanem, M.E., Beebe, S.E., Rao, I.M., Chaturvedi, S.K., Basu, P.S., Nayyar, H., Jayalakshmi, V. & Babbar, A. 2015. High-temperature tolerance in grain legumes. *Legume Perspect*. **7**, 23–24.

George, C. Papageorgiou & Govindjee (eds). 2006. Chlorophyll a Fluorescence: A Signature of Photosynthesis, pp. 389–400.

Ghanem, M.E., Marrou, H. & Sinclair, T.R. 2015. Physiological phenotyping of plants for crop improvement. *Trends Plant Science* **20**(3), 139–144.

Gitelson, A.A., Buschmann, C. & Lichtenthaler, H.K. 1999. The Chlorophyll Fluorescence Ratio F735/F700 as an Accurate Measure of Chlorophyll Content in Plants. *Remote Sens. Enviro*. **69**, 296–302.

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, 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.

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.

Hoai, N.T.T. & Shim, I.S. 2003. Accumulation of some nitrogen compounds in response to salt stress and their relationships with salt tolerance in rice (*Oryza sativa L.*) seedlings. *Plant Growth Reg*. **41**, 159–164. doi: 10.1023/A:1027305522741

Hospodarenko, H.M. & Musiienko, L.A. 2020. The system of fertilisation of lentil on podzol chernozem. *Proceedings of the 4th International Scientific and Practical Conference "Scientific Principles of Increasing the Efficiency of Agricultural Production"*, November 26–27, Vol. **1**. KhNAU, Kharkiv, p. 150.

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.

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.

Khodanitska, O.O., Khodanitska, E.A., Shevchuk, O.A., Tkachuk, O.O. & Tkachuk, O.A. 2019. The effect of agrostimulin on the processes of lentil seeds germination. Actual issues of geographical and biological sciences: main scientific problems and prospects of research. *Scientific papers of the VSPU* **17**(22), 63−65.

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 needle leaf 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.

Koskeroglu, S. & Tuna, A.L. 2010. The investigation on accumulation levels of proline and stress parameters of the maize (*Zea mays* L.) plants under salt and water stress. *Acta Physiol. Plant* **32**, 541–549. doi: 10.1007/s11738-009-0431-z

Lahuta, L.B., Szabli´nska-Piernik, J. & Horbowicz, M. 2022. Changes in Metabolic Profiles of Pea (Pisum sativum L.) as a Result of Repeated Short-Term Soil Drought and Subsequent ReWatering. *Int. J. Mol. Sci*. **23**, 1704. https://doi.org/10.3390/ijms23031704

Lamaoui, M., Jemo, M., Datla, R. & Bekkaoui, F. 2018. Heat and drought stresses in crops and approaches for their mitigation. *Front. Chem*. **6**, 26. http://doi.org/10.3389/fchem.2018.00026

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.

Le, T.A., Pék, Z., Takács, S., Neményi, A., Daood, H.G. & Helyes, L. 2018. The effect of plant growth promoting rhizobacteria on the water-yield relationship and carotenoid production of processing tomatoes. *HortScience* **53**, 816–822. http://doi.org/10.21273/HORTSCI13048-18

Legendre, R., Basinger, N.T. & van Iersel, M.W. 2021. Low-Cost Chlorophyll Fluorescence Imaging for Stress Detection. *Sensors* **21**(6), 2055.

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.

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, J. & Zhu, J.K. 1997. Proline accumulation and salt-stress-induced gene expression in a salt-hypersensitive mutant of Arabidopsis. *Plant Physiol*. **114**, 591–596.

Marchin, R.M., Ossola, A., Leishman, M.R. & Ellsworth, D.S. 2020. A Simple Method for Simulating Drought Effects on Plants. *Frontiers in Plant Science* **10**, 1715.

Marques de Sá, J.P. 2007. Applied Statistics Using SPSS, STATISTICA, MATLAB and R. Fac. Engenharia, Universidade do Porto, Porto, Portugal, pp. 1–452.

Maxwell, K. & Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* **51**(345), 659–668. https://doi.org/10.1093/jexbot/51.345.659

Meena, M., Divyanshu, K., Kumar, S., Swapnil, P., Zehra, A., Shukla, V., Yadav, M. & Upadhyay, R.S. 2019. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* **5**, e02952 http://doi.org/10.1016/j.heliyon.2019.e02952

Mihaljevic, I., Vuletic, M.V., Šimic, D., Tomaš, V., Horvat, D., Josipovic, M. & Zdunic, Z. 2021. Comparative study of drought stress effects on traditional and modern apple cultivars. *Plants* **10**, 561.

Moisa, C., Copolovici, D., Lupitu, A., Lazar, L. & Copolovici, L. 2019. Drought stress influence on pea plants (*Pisum sativum* L.). *Scien. Tech. Bull-Chem. Food Sci. Eng*. **16**(XVII), 18–22.

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.

Oláh, V., Hepp, A., Irfan, M. & Mészáros, I. 2021. Chlorophyll Fluorescence Imaging-Based Duckweed Phenotyping to Assess Acute Phytotoxic Effects. *Plants* **10**, 2763.

Prysiazhniuk, O., Topchii, O., Slobodianiuk, S., Karpuk, L., Maliarenko, O., Pavlichenko, A. & Svystunova, I. 2020. Lentil: biology and cultivation. Vinnytsia: Tvory, pp. 1–180.

Prysiazhniuk, O.I., Korovko, I.I., Polovinchuk, O.Yu., Shevchenko, O.P., Shklyaruk, S.M., Tanchin, S.M. & Navrotska, E.E. 2017. Determination of fluorescence induction of plant chlorophyll: theoretical and practical basis for the application of the method: methodological recommendations. Kyiv: Nilan-LTD

Qin, F., Shinozaki, K. & Yamaguchi-Shinozaki, K. 2011. Achievements and Challenges in Understanding Plant Abiotic Stress Responses and Tolerance. *Plant Cell Physiol.* **52**, 1569−1582. doi: 10.1093/pcp/pcr106.

Rosa, A.P., Barão, L., Chambel, L., Cruz, C. & Santana, M.M. 2023. Early identification of plant drought stress responses: changes in leaf reflectance and plant growth promoting rhizobacteria selection-the case study of tomato plants. *Agronomy* **13**, 183. https://doi.org/10.3390/agronomy13010183

Roy, R., Mazumder, P.B. & Sharma, G.D. 2009. Proline, catalase and root traits as indices of drought resistance in bold-grained rice (*Oryza sativa*) genotypes. *Afr. J. Biotech*. **8**, 6521−6528.

Rumsey, D.J. 2016. *Statistics For Dummies*. 2nd Ed., John Wiley & Sons Inc., pp. 1–384.

Sen, S., Ghosh, S., Mazumdar, D., Saha, B. & Dolui, S. 2016. Effect of sowing date and variety on phenology and yield of lentil during rabi season. *J. Crop Weed*. **12**, 135–138.

Shrestha, R., Siddique, K.H.M., Turner, N.C., Turner, D.W. & Berger, J. 2005. Growth and seed yield of lentil (*Lens culinaris* Medikus) genotypes of West Asian and South Asian origin and crossbreds between the two under rainfed conditions in Nepal. *Australian Journal of Agricultural Research* **56**, 971–981. doi: 10.1071/AR05050

Simeneh, T.A. 2020. Photosynthesis limiting stresses under climate change scenarios and role of chlorophyll fluorescence. *Cogent Food & Agriculture* **6**, 1–18.

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.

Snowdon, R.J., Wittkop, B., Chen, T.W. & Stahl, A. 2021. Crop adaptation to climate change as a consequence of long-term breeding. *Theoretical and Applied Genetics* **134**, 1613–1623.

Song, S.Q., Lei, Y.B. & Tian, X.R. 2005. Proline metabolism and cross-tolerance to salinity and heat stress in germinating wheat seed. *Russ. J. Plant Physiol*. **52**, 897−904. doi: 10.1007/s11183-005-0117-3

Sperdouli, I. & Moustakas, M. 2014. A better energy allocation of absorbed light in photosystem II and less photooxidative damage contribute to acclimation of Arabidopsis thaliana young leaves to water deficit. *J. Plant Physiol*. **171**, 587–593. http://doi.org/10.1016/j.jplph.2013.11.014

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.

Suprasanna, P., Nikalje, G.C. & Rai, A.N. 2016. Osmolyte accumulation and implications in plant abiotic stress tolerance. *In Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies*; Iqbal, N., Nazar, R., Khan, N.A., Eds., Springer, New Delhi, India. pp. 1–12. http://doi.org/10.1007/978-81-322-2616-1

Szabados, L. & Savoure, A. 2009. Proline: a multifunctional amino acid. *Trends Plant Sci*. **15**, 89−97. doi: 10.1016/j.tplants.2009,11,009

Templer, S.E, Ammon, A., Pscheidt, D., Ciobotea, O., Schuy, C., McCollum, C., Sonnewald, U., Hanemann, A., Förster, J., Ordon, F., von Korff, M. & Voll, L.M. 2017. Metabolite profiling of barley flag leaves under drought and combined heat and drought stress reveals metabolic QTLs for metabolites associated with antioxidant defense. *Journal of Experimental Botany* **68**(7), 1697–1713. doi:10.1093/jxb/erx038

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. *BMC Plant Biology* **19**, 403.

Tsytsiura, Y. 2022. 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. *Agronomy Research* **20**(3), 682–724. https://doi.org/10.15159/ar.22.062

Valcke, R. 2021. Can chlorophyll fluorescence imaging make the invisible visible? *Photosynthetica* **59**(SI), 381–398.

Valliyodan, B. & Nguyen, H. 2006. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Curr. Opin. Plant Biol*. **9**, 189−195. doi: 10.1016/j.pbi.2006,01,019.

Van Zelm, E., Zhang, Y. & Testerink, C. 2020. Salt tolerance mechanisms of plants. *Annual Review of Plant Biology* **71**, 1–31.

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.

Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K. & Yoshiba, Y. 2005. Effects of free proline accumulation in petunias under drought stress. *J. Exp. Bot*. **56**, 1975−1981. doi: 10.1093/jxb/eri195

Zeroual, A., Baidani, A. & Idrissi, O. 2023. Drought stress in lentil (*Lens culinaris*, Medik) and approaches for its management. *Horticulturae*, **9**(1). https://doi.org/10.3390/horticulturae9010001

Zhuang, J., Wang, Y., Chi, Y., Zhou, L., Chen, J., Zhou, W., Song, J., Zhao, N. & Ding, J. 2020. Drought stress strengthens the link between chlorophyll fluorescence parameters and photosynthetic traits. *PeerJ* **8**, e10046. https://doi.org/10.7717/peerj.10046