# The impact of crop management regime on oil content and fatty acid composition in hulless and covered spring barley

L. Legzdina<sup>1,\*</sup>, E. Ivdre<sup>2</sup>, D. Piliksere<sup>1</sup>, A. Vaivode<sup>1</sup>, I. Mierina<sup>2</sup> and M. Jure<sup>2</sup>

<sup>1</sup>Institute of Agricultural Resources and Economics, Priekuli Research Centre, Zinātnes iela 2, LV-4126 Priekuļi, Latvia

<sup>2</sup>Institute of Technology of Organic Chemistry, Faculty of Materials Science and Applied Chemistry, Riga Technical University, P.Valdena iela 3/7, LV-1048 Rīga, Latvia <sup>\*</sup>Correspondence: linda.legzdina@arei.lv

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Abstract. Lipids are a minor nutritional component of barley (*Hordeum vulgare* L.) grain and have not been as widely explored as the major components. The aim of this study was to investigate the effect of genotype and environment, including conventional farming system with three crop management regimes, differing in agrochemical input, and organic farming system, on oil content and fatty acid composition in grain of two covered and four hulless spring barley genotypes during two growing seasons. Genotype significantly affected oil content and it was on average 4.26% and ranged in individual barley samples from 2.87 to 5.53%. We found linoleic, oleic, palmitic,  $\alpha$ -linolenic, stearic and capric fatty acids in average proportions of 55.6; 21.3; 18.6; 3.7; 0.6 and 0.4%, respectively. Higher average oil content and proportion of  $\alpha$ -linolenic acid was found in covered barley. Crop management regime did not significantly affect oil content but had some effect on the proportion of linoleic,  $\alpha$ -linolenic, oleic and stearic acid. Decrease of chemical inputs was in favour of oil content and proportion of  $\alpha$ -linolenic, oleic and stearic acid. Waxy hulless barley line with high oil content and a very high proportion of linoleic acid was identified.

**Key words:** conventional farming, different input of agrochemicals, free lipids, organic farming, genotype and environment effects.

# **INTRODUCTION**

Vegetable oils mainly contain fatty acid triglycerides. Fatty acids (FA) form various lipids which are involved e. g. in cell membranes. All FA are classified as saturated and unsaturated, according to the amount of the double bonds into their structure. A high intake of saturated fats is linked with a negative effect on health, especially, on the blood lipid profile, which may be attributed to cardiovascular disease (Mensink, 2016). A part of unsaturated FA is known as essential, e. g. linoleic and  $\alpha$ -linolenic acids which are not synthesized into human body due to the lack of certain desaturases and should be ingested with diet (Tvrzicka et al., 2011). Functional lipids, including polyunsaturated FA, demonstrate a beneficial effect on human health.  $\omega$ -3 and  $\omega$ -6 FA and conjugated

linoleic acid may be useful for the reduction of heart disease, obesity, depression, Alzheimer's disease, Parkinson's disease and atopic dermatitis (Alabdulkarim et al., 2012). The essential FA have a significant role in modulation of gene transcription, function as cytokine precursors, serving as energy sources in complex, and interconnected systems (Glick & Fischer, 2013).  $\alpha$ -Linolenic acid has demonstrated an anti-inflammatory effect, as well as reduces stroke risk, size, and/or consequences (Blondeau et al., 2015).

## Oil content and FA composition in barley

Lipids are a minor nutritional component of barley (*Hordeum vulgare* L.) grain and have not been as widely investigated as other components. Most of the authors have reported barley lipid content in a range of 1.9–3.7% and up to 7.3% in specific high-sugar genotypes, however, it is affected by the choice of analytical procedures, differing between the studies (Osman et al., 2000; Newman & Newman, 2008). Seefeldt et al. (2011) found that lipid biosynthesis in barley mutants with increased lipid content happens ten days earlier than in normal barley. Barley was found to have a little higher oil content than that of wheat, similar to that of rice and lower than in sorghum and oat, the latest being the cereal with superior oil content (Liu, 2011).

Similar to other cereals, oilseeds and legumes, also in barley five major FA are palmitic, stearic, oleic, linoleic and  $\alpha$ -linolenic acid. Several other FA in much lower concentrations are reported as well (Liu, 2011). Linoleic acid is the dominant one found in a range from 50 to 60%, followed by palmitic acid from 18 to 25%, oleic acid from 11 to 22%,  $\alpha$ -linolenic acid from 2 to 8% and stearic acid from 0.3 to 4.6% (De Man, 1985; Osman et al., 2000; Newman & Newman, 2008; Liu, 2011; Ciołek et al., 2012; Gangopadhyay et al., 2017). Qian et al. (2009) found ten FA in hulless barley (HB) bran oil with a 75% proportion for linoleic acid but no  $\alpha$ -linolenic acid. Barley oil was found to have higher relative content of linoleic and  $\alpha$ -linolenic acids and lower content of oleic acid than that of oats, rice or sorghum (Osman et al., 2000; Liu, 2011).

## Oil and FA distribution in barley grain

About 18% of total lipids are concentrated in embryo, 77% in endosperm and 5% in the hulls of barley grain (Newman & Newman, 2008). The hull has a much lower oil content than the following grain outer part fractions and also the completely dehulled grain. Oil concentration in grain is decreases in the direction from the outer parts to inner core; the decrease is very rapid for the first few outer layers followed by gradual reduction. The increase of palmitic and stearic acids and the decrease of oleic and  $\alpha$ -linolenic acids towards the grain central part are shown proving a higher amount of unsaturated FA in the whole grain products (Liu, 2011). Barley hull fraction is relatively rich in palmitic and stearic acids but low in  $\alpha$ -linolenic acid (De Man, 1985). Qian et al. (2009) reported 8.1% crude oil content in HB bran.

#### Effects of genotype and environment

Significant differences between barley varieties in respect to the content of linoleic, palmitic, oleic and stearic acids have been reported (Gangopadhyay et al., 2017). De Man (1985) found that genotype has a larger effect on barley total FA (TFA), linoleic, oleic and stearic acid contents than growing location and the effect of both factors is similar for palmitic and  $\alpha$ -linolenic acids. Significant differences in TFA content are found between two-rowed and six-rowed barley but not between winter and spring type barley.

The effect of *meteorological and soil conditions* on oil and FA content has been comparatively widely studied for various field crops. Analysis of large dataset on many species in China indicated an increase in total unsaturated FA content with increasing latitude and a negative correlations between mean annual temperature and precipitation and the ratio of unsaturated FA (Zhang et al., 2015). Wheat experiments in controlled environments showed, that a rise of growing temperature causes increase of palmitic and oleic acids and a decrease of linoleic and  $\alpha$ -linolenic acids (Shamloo et al., 2017). Similar results are reported for oilseed crops (Schulte et al., 2013; Gauthier et al., 2017). De Man (1985) found that palmitic acid content in barley correlates positively with the temperature before flowering, oleic acid has a positive connection to the precipitation before flowering and temperature during ripening, linoleic acid correlates positively to temperature before flowering, and  $\alpha$ -linolenic acid is positively affected by temperature before flowering and precipitation during grain ripening.

Only a few results are published on the *effect of fertilizers and crop management* systems on oil and FA contents in food crops. Lower crude fat content in organically versus conventionally grown cereals including barley was reported (Mikulioniene & Balezentiene, 2009). De Man & Dondeyne (1985) reported that nitrogen fertilizer slightly lowers TFA content and significantly decreases oleic acid proportion and increases palmitic acid proportion in barley. A more recent report on sunflower showed that nitrogen fertilizer affects proportion of oleic and linoleic acids positively, palmitic and stearic acids negatively (Abd EL-Satar et al., 2017). Another study on wheat did not find significant effects of nitrogen fertilizer on FA content (Wojtkowiak et al., 2018). For durum wheat no significant *effect of organic/conventional farming* system on FA was found, however, the interaction of genotype and farming system was significant for palmitic, linoleic and oleic acids (Beleggia et al., 2013). Ciołek et al. (2012) reported more lipids in cereals grown under conventional farming system if compared to organic system with the exception of hulless barley (HB) genotype, and more unsaturated FA under organic farming in most cases.

The aim of our study was to investigate the effect of genotype and environment, including conventional farming system with three crop management regimes, aimed to achieve different yield levels, and organic farming system, on oil content and FA composition in hulless and covered spring barley grain.

## MATERIALS AND METHODS

## Field trials

Field trials with two covered and four hulless spring barley genotypes were established under four crop management regimes: conventional crop management (C) system with three agrochemical input levels (C1, C2 and C3) and organic crop management (O) system, in years 2011 and 2012 as described in Legzdiņa et al. (2018). The three agrochemical input levels were aimed to obtain potential yield of 4 t ha<sup>-1</sup> (C1) and 6 t ha<sup>-1</sup> (C2 and C3). The amount of nitrogen supplied was 90 and 140 kg ha<sup>-1</sup>, respectively. For C3 foliar fertilizer Kristalon white, containing several mineral elements, was applied in addition to the same input of agrochemicals as for C2. Weather conditions were comparatively warmer and drier (especially in June and the beginning of July) in 2011 and a bit cooler with precipitation above the long-term average in 2012.

## **Barley genotypes**

The covered barley (CB) genotypes were varieties currently under production 'Jumara' and 'Rubiola', one hulless barley (HB) variety 'Irbe' and three advanced HB breeding lines. The lines were chosen considering their potentially elevated content of health promoting biologically active compounds. Lines PR-4651 and PR-5099 are waxy barley with comparatively high concentrations of  $\beta$ -D-glucans; PR-5099 is derived from cross with waxy variety 'Washonubet' providing high concentration of tocols as reported by Ehrenbergerová et al. (2006). Line PR-3808 contains a source with a very high content of protein and lysine in its pedigree.

#### Grain chemical composition analysis

The grain yield of the complete plots was harvested, dried and cleaned with a 1.7 mm sieve. One representative grain sample was assembled from all replications for analysis. The grain moisture content ranged from 10.2 to 12.5%. For all analysis barley grain was ground to pass through a sieve (d < 0.065 mm).

**Oil content.** Standard method ISO 734–1:2001 was applied to determine the free lipid content. Each sample was analysed in three to five replicates. Briefly, the grains were ground, dried at 105-110 °C and extracted with petroleum ether (fraction 40–70 °C) in a Soxhlet apparatus.

**Derivatization of barley oil triglycerides for gas chromatography (GC) analysis.** Barley oil (80 mg) and potassium hydroxide (0.9 mg) was refluxed in methanol (2.8 mL) for 20 min. After cooling the mixture, the solution with petroleum ether was transferred to a test tube, washed with brine (2×2 mL) and water (3×2 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The sample was purified prior to GC analysis on silica column (eluent petroleum ether: chloroform, 2 : 1).

**FA composition.** Gas chromatograph (Agilent Technologies 6890N Network GC system, USA) with flame ionization detector was used. The sample of fatty acid methyl esters (FAME) of barley oil was dissolved in hexane (concentration of the sample  $10-20 \text{ mg mL}^{-1}$ ) and analysed on DB-Wax capillary column (0.32 mm ×30 m, 0.25 µm). The temperature conditions were as follows: the initial oven temperature was 140 °C, then the temperature was increased to 230 °C with a rate of 5 °C min<sup>-1</sup>, followed by holding the temperature for 15 minutes, increasing the temperature to 250 °C with rate 25 °C min<sup>-1</sup> and holding the same temperature for an additional 5 minutes. FAME were identified by comparing the retention times with standard compounds (Supelco mixture of 14 FAME from C8 to C24). Helium was used as a carrier gas (flow rate 25 mL min<sup>-1</sup>). Each FAME was quantified by calibration curves with acceptable correlation coefficients.

**Other compounds.** Analysis of  $\alpha$ -tocopherol and total polyphenol content was performed as described by Legzdiņa et al. (2018). Content of crude protein, starch and (1 $\rightarrow$ 3) and (1 $\rightarrow$ 4)  $\beta$ -D-glucans in the dry matter were determined using a NIT analyzer, Infratec 1241 (Foss, Denmark). Lysine content in grain was measured using InfraXact NIR Spectroscopy analyser (Foss, Denmark).

#### Statistical analysis

Data were tested for normality by the *Shapiro-Wilk test* and the homogeneity of variances by the *Levene's test*. The means and significant differences by *T-test*, *one-way* 

ANOVA and Tukey's HSD post-hoc tests (for normally distributed data) or Welch-t test and Games-Howell post-hoc tests (for non-normally distributed data) and Kendall's correlation coefficients of the data were evaluated using the IBM SPSS Statistics 23 software. To estimate the stability of oil concentration and FA distribution ecovalence (Wi) was computed as described by Becker & Leon (1988) and expressed in percentage of the total interaction sum of squares. Values close to zero indicate high stability.

# RESULTS

## **Oil content**

Oil content was on average 4.26%, and it ranged in individual barley samples from 2.87% in high lysine HB line PR-3808 to 5.53% in CB variety 'Rubiola' (Fig. 1. Both samples grown under C1 management regime in 2011). The *effect of genotype* on oil content was highly significant (Table 1). In 2011 and over both years the mean oil content of CB group significantly surpassed that of HB. CB variety 'Jumara' provided the highest mean in 2011, however, oil content of waxy HB line PR-5099 did not significantly surpassed CB varieties and the mean value of them over both years did not significantly differ from CB varieties. Commercial HB variety 'Irbe' and high lysine line PR-3808 were the poorest in oil, but line PR-4651 had contradictory results in each year. Oil content was the most stable according to *ecovalence* for HB 'Irbe' with low values and for PR-5099 with high values, for the other genotypes it was similarly unstable (Table 2).



**Figure 1.** Oil content (%) in covered ('Jumara', 'Rubiola') and hulless ('Irbe', PR-4651, PR-3808, PR-5099) barley grain grown under organic (O) and conventional management regimes (C1, C2, C3), 2011–2012 (mean  $\pm$  SE). Bars with different letters show significant differences within management regimes (p < 0.05).

No significant *effect of crop management regime or year* on oil content was found. A trend to increase the average oil content over the genotypes with the decrease of mineral fertilizer amount applied  $(C3\rightarrow C2\rightarrow C1\rightarrow O)$  can be seen with a 0.3% difference between O and C3 for average data over both years. The difference between O and C systems was significant (Table 1). However, this trend was not always the case, whilst

looking at the data of individual genotypes (Fig. 1). Mean oil content over the years under O system was always higher than in C system for HB genotypes but not for CB varieties.

Genotype/ crop management	Oil, %			Palmitic C16:0, %			Stearic C18:0, %			Capric C10:0, %
	2011	2012	average	2011	2012	average	2011	2012	average	2011
Jumara	4.90a	4.31c	4.59ab	23.1	21.0	22.0a	0.8	0.6	0.7	0.0
Rubiola	4.48a	4.10cd	4.30abc	18.7	20.6	19.6a	0.6	0.8	0.7	1.3
Irbe	3.90b	3.54d	3.71d	20.9	19.3	20.1a	0.3	0.4	0.4	2.2
PR-4651	3.65b	4.71ab	4.20bc	19.4	18.7	19.0a	0.4	0.4	0.4	0.9
PR-3808	3.63b	4.47bc	4.06cd	19.2	20.8	20.0a	1.5	0.7	1.1	0.0
PR-5099	4.53a	4.93a	4.69a	11.0	9.9	10.5b	0.0	0.6	0.3	0.0
Genotype, p	***	***	***	ns	ns	***	ns	ns	ns	ns
0	4.40	4.46	4.43	18.5	16.5	17.5	0.6	1.4a	1.0	0.0
C1	4.26	4.32	4.29	19.5	20.7	20.1	0.9	0.6ab	0.7	0.7
C2	4.01	4.39	4.18	20.1	17.2	18.6	0.6	0.2b	0.4	1.2
C3	4.18	4.08	4.13	16.7	19.1	17.9	0.3	0.2b	0.2	1.0
Management, p	ns	ns	ns	ns	ns	ns	ns	***	ns	ns
Average	4.20	4.30	4.25	18.7	18.4	18.6	0.6	0.6	0.6	0.7
Year, p	ns			ns			ns			-
HB/CB, O/C*	CB>HB ***		CB>HB ***; O>C **		CB>H B **			O>C ***	O>C **	C>O ***

**Table 1.** Oil content and saturated fatty acid proportion in hulless and covered barley grain under organic (O) and conventional management regimes (C1, C2, C3), 2011–2012

\*\* -p < 0.05; \*\*\* -p < 0.01; ns - p > 0.05; p - p-value; a, b, c - different letter indicate significant differences (p < 0.05) between genotypes or management regimes. \*HB/CB – significant difference between means of hulless and covered barley groups. O/C – significant difference between means in organic and conventional farming systems.

*Oil content correlated* significantly negative with the content of protein, lysine and  $\alpha$ -tocopherol in grain (Table 3). There were no significant correlations between oil content and grain yield, 1,000-grain weight and volume weight.

## Fatty acid compositions

We found in barley grain samples linoleic (C18:2), oleic (C18:1), palmitic (C16:0),  $\alpha$ -linolenic (C18:3), stearic (C18:0) and capric (C10:0) acids in average proportions of 55.6, 21.3, 18.6, 3.7, 0.6 and 0.4%, respectively (Tables 1 and 4). Capric acid was found in only seven samples of three genotypes grown in 2011 under C regimes with a proportion 1.3–3.6%. The effect of genotype was significant for linoleic and palmitic acid proportion over both years and for  $\alpha$ -linolenic acid proportion in 2012. Significant differences between CB and HB genotype groups were found for palmitic acid proportion in 2012 and for  $\alpha$ -linolenic acid proportion in 2012 and over both years with higher means for CB. However, only one HB line was significantly lower than the rest of the genotypes for palmitic acid and two lines showed a trend to lower  $\alpha$ -linolenic acid proportion but the rest were close to CB genotypes.

Waxy HB line PR-5099 was different from the other genotypes for its FA composition, especially under higher input C2 and C3 regimes: it had very high proportion of linoleic acid (up to 88.4% under C3 in 2011) and low proportion of palmitic acid (down to 2.3% under C3 in 2012) if compared to the other genotypes (Fig. 2). PR-5099 also was low in  $\alpha$ -linolenic and oleic acid in mo st of the cases. Despite the highest proportion of linoleic acid among the genotypes in all but one environment (C1 in 2011) this line was the most unstable for this FA (Table 2).



**Figure 2.** Fatty acid composition of covered barley 'Jumara' (A), hulles barley 'Irbe' (B) and hulless waxy barley PR-5099 (C) grown under organic (O) and conventional (C1, C2, C3) management regimes in 2011 and 2012.

CB variety 'Rubiola' was the most unstable for proportions of  $\alpha$ -linolenic and palmitic acids. Exceptionally high proportion of  $\alpha$ -linolenic acid (14.6%) and the only case with 0.0% palmitic acid was found for the sample of 'Rubiola' with the maximal

oil content over the experiment grown under C1 in 2011. In the same year under C2 management regime the proportions were with opposite trends:  $0.0\% \alpha$ -linolenic and maximum value for palmitic acid proportion (30.7%) accompanied with low linoleic acid (36.2%).

Linoleic acid proportion correlated significantly negative with oleic, stearic, and palmitic acids

**Table 2.** Stability of oil content and fatty acidproportion in barley genotypes over four cropmanagement regimes in 2011 and 2012

Genotype	Ecovalence (W <sub>i</sub> ), %								
	oil	C 18:1	C 18:2	C 18:3	C 16:0	C 18:0			
Jumara	23.4	35.5	6.8	12.2	2.1	22.2			
Rubiola	24.8	19.5	28.7	43.2	50.2	15.7			
Irbe	5.4	3.1	2.7	19.5	6.2	11.0			
PR-4651	19.0	10.6	11.1	3.4	7.1	6.6			
PR-3808	21.8	15.0	10.3	15.6	7.3	38.4			
PR-5099	5.6	16.3	40.4	6.1	27.0	6.0			

and positively with the content of polyphenols,  $\alpha$ -tocopherol and starch in grain (Table 3). Proportion of  $\alpha$ -linolenic acid was negatively related to protein, lysine and  $\beta$ -glucan content in grain.

**Table 3.** Significant coefficients of Kendall's correlation between barley oil content, FA proportions and some other grain compound content

Com-	Protein	Lucine	Polyphen	α-Toco-	Starch	β-	$C_{16.0}$	C 18.1	C 18.0
pounds	Tiotem	Lysnie	ols	pherol	Startin	Glucans	C 10.0	C 10.1	C 18.0
oil	-0.233*	-0.214*		-0.204*					
C 18:2			$0.297^{**}$	$0.215^{*}$	0.264**		-0.291**	-0.638**	-0.327**
C 18:3	-0.262*	-0.285**				-0.256*		$-0.208^{*}$	
C 18:1			-0.365**	-0.273**	-0.202*				
C 16:0					-0.268**				
* <i>p</i> < 0.0	5, ** <i>p</i> <	0.01.							

Crop management regimes significantly influenced the proportion of linoleic, oleic and stearic acid in 2012 and  $\alpha$ -linolenic acid over both years (Tables 1 and 4). Significantly higher linoleic acid proportion was in samples grown under conventional farming regimes if compared to organic farming and the average over genotypes increased with mineral fertilizer amount applied in 2012. In 2011 average linoleic acid proportion increased according to management regime sequence  $C1 \rightarrow C2 \rightarrow C3$ , however, the mean linoleic acid proportion of organically grown samples surpassed those of C1 and C2. The opposite situation in both years was observed for oleic acid proportion: it was significantly higher under O system in comparison to the C system in 2012 and with a trend to increase with the reduction of mineral fertilization  $C3 \rightarrow C2 \rightarrow C1$ and similar values for O and C3 regimes in 2011. For  $\alpha$ -linolenic acid proportion the mean genotype values in both years had a slight trend to increase with the decrease of agrochemical input with the highest means under O system. It is proved also by negative correlation between the FA proportion and management regimes in order  $O \rightarrow C1 \rightarrow C2 \rightarrow C3$  (r = -0.227, p < 0.05). Mean stearic acid proportion was significantly higher under O system and also had a trend to increase with decreasing mineral fertilization (correlation with management regimes r = -0.343, p < 0.01).

The conditions of *growing year* affected significantly and conversely the proportions of linoleic and oleic acids (Table 4).

Genotype/	Linoleic C18:2, %			α-Linolenic C18:3, %			Oleic C18:1, %		
crop management	2011	2012	average	2011	2012	average	2011	2012	average
Jumara	60.3	50.2	55.2b	4.4	4.6a	4.5	11.5	23.7	17.6
Rubiola	51.9	49.4	50.6b	5.6	4.7a	5.2	22.0	24.6	23.3
Irbe	55.6	49.4	52.5b	2.8	4.8a	3.8	18.3	25.9	22.1
PR-4651	56.4	49.5	52.9b	4.0	4.0ab	4.0	19.1	27.5	23.3
PR-3808	53.8	48.8	51.3b	3.3	1.2b	2.2	22.6	28.7	25.6
PR-5099	74.8	67.6	71.2a	2.9	1.6ab	2.2	11.3	20.3	15.8
Genotype, p	ns	ns	***	ns	***	ns	ns	ns	ns
0	59.9	41.7b	50.8	5.2	4.3	4.7a	15.9	36.1a	26.0
C1	54.4	53.7ab	54.0	4.5	3.7	4.1ab	20.1	21.4b	20.7
C2	57.1	55.9a	56.5	2.8	3.3	3.0ab	18.4	23.4b	20.9
C3	63.8	58.6a	61.2	2.8	2.7	2.7b	15.5	19.5b	17.5
Management, p	ns	**	ns	ns	ns	**	ns	***	ns
Average	58.8	52.5	55.6	3.8	3.5	3.7	17.5	25.1	21.3
Year, p	**			ns			***		
		C>O			CB>HB	CB>HB **	;	O>C	
HB/CB, O/C		***			***	O>C **	•	***	

**Table 4.** Unsaturated fatty acid proportion in hulless and covered barley grain under organic (O) and conventional management regimes (C1, C2, C3), 2011–2012

\*\* -p < 0.05; \*\*\* -p < 0.01; ns - p > 0.05; p - p-value; a, b, c - different letter indicate significant differences (p < 0.05) between genotypes or management regimes. \*HB/CB - significant difference between means of hulless and covered barley groups. O/C - significant difference between means in organic and conventional farming systems.

# DISCUSSION

## Range of oil content and FA distribution

The barley grain *oil content* found by us with an average of 4.25% and maximum 5.65% was relatively high if compared to that reported in other studies (Osman et al., 2000; Newman & Newman, 2008; Liu, 2011; Ciołek et al., 2012). Some reports suggest that genetic factors controlling waxy starch in barley may be linked to genes that increase total lipids (Newman & Newman, 2008); in our study waxy line PR-5099 was superior according to average data and also showed high stability for oil content, whereas the oil content of the other waxy line PR-4651 appeared to be more affected by the environment.

The maximum linoleic acid proportion detected in waxy HB line PR-5099 grown under C crop management regimes with highest agrochemical inputs (71.5–88.4%) noticeably surpassed that reported in barley grain in other studies (De Man, 1985; Osman et al., 2000; Newman & Newman, 2008; Liu, 2011; Ciołek et al., 2012; Gangopadhyay et al., 2017), and this line can be considered to be used for the specific purpose to produce linoleic acid rich oil. Qian et al. (2009) found linoleic acid proportion 75% in HB bran oil, but it cannot be comparable to our results in whole grain because the proportion of unsaturated FA is higher in outer parts of grain (Liu, 2011).

A very high proportion of  $\alpha$ -linolenic acid (25%) in combination with low linoleic acid (39%) was found in a HB genotype by Osman et al. (2000). Among our samples, exceptionally high  $\alpha$ -linolenic acid proportion (14.6%) was identified in one sample of CB 'Rubiola' also possessing the maximum oil content and complete lack of palmitic acid. We do not have an explanation why this particular sample was so different.  $\alpha$ -Linolenic acid was not detected in several HB and CB samples. The range of this FA, found in the majority of samples (0.4–7.0%), is comparable to that reported in other studies (De Man, 1985; Osman et al., 2000; Newman & Newman, 2008; Liu, 2011; Ciołek et al., 2012; Gangopadhyay et al., 2017).

We only found a few published precedents on identification of capric acid in barley grain. Youssef et al. (2012) reported it in comparable amounts to our results (1.5-2.0%).

# Hulless/covered grain type

The average oil content in CB significantly surpassed that of HB in 2011 and also over both years. However, this difference cannot be explained by covered/hulless grain type, because hulls contain a relatively small amount of oil in accordance with the results of Liu (2011). In addition, higher fat content in naked oat genotypes if compared to husked varieties are reported in oat (Sterna et al., 2014), leading us to think that the most likely differences between genotypes were caused by some other genetic factors, independent from the presence or absence of the hulls. Comparable and even higher oil content to CB varieties in HB PR-5099 also supports this statement. To prove this hypothesis, we would need to repeat the experiment with dehulled CB grain. It must also be taken into account that CB cannot be used for food purposes directly because the hulls are inedible and the grain must be dehulled during production of food.

Our results, combined with those reported by other authors, suggest that differences in *FA composition* are also mostly due to genotype and not due to the grain type. Liu (2011) comparing one HB and one CB variety showed a trend to a higher proportion of oleic acid and a lower proportion of linoleic and  $\alpha$ -linolenic acids in HB. According to our results we can point on HB PR-3808 being slightly superior for oleic acid and CB surpassing some but not all HB genotypes for  $\alpha$ -linolenic acid, however, HB PR-5099 was exclusively superior for linoleic acid proportion. High palmitic acid proportion in barley hulls if compared to other grain parts reported by De Man (1985) and higher proportion of palmitic acid in CB compared to HB demonstrated by Youssef et al. (2012) are generally in agreement with our results. However, only the HB line PR-5099 had significantly lower palmitic acid proportion than the other CB and HB genotypes.

# **Environment and fertilization**

Our data do not support the findings of De Man (1985) that palmitic and  $\alpha$ -linolenic acids are affected more by environmental conditions than the other FA. Environmental factors of the particular year were not significant for both previously mentioned FA, but there was some environmental effect due to crop management regime or farming system for the five main barley grain FA.

The negative effect of nitrogen fertilizer on oleic acid proportion reported by De Man & Dondeyne (1985) and Stepien et al. (2019) is in accordance with our work, however, we did not find any increase in palmitic and  $\alpha$ -linolenic acid proportions by increasing the fertilizer amount, but a slight opposite trend. There are also some parallels between our data and those presented by Abd EL-Satar et al. (2017) on sunflower in

respect of a positive effect of nitrogen fertilizer on linoleic and negative effect on stearic acid proportions. According to our data, a decrease of chemical input was in favour of oil content and proportions of unsaturated oleic and  $\alpha$ -linolenic acids and also saturated stearic acid, but did not favour unsaturated linoleic acid proportion. Particularly important this finding might be in respect of  $\omega$ -3  $\alpha$ -linolenic acid in relation to health promoting organically grown food products. A significant positive correlation between the amount of Mn fertilizer applied and linoleic acid content found by Stepien et al. (2019) is in agreement with our data, showing the highest average proportion of this FA in C3 management regime, were a number of mineral elements including Mn was applied in form of foliar fertilizer.

In respect to meteorological conditions of the *growing year*, linoleic acid proportion was significantly higher in 2011 with higher mean air temperature, especially before flowering time. It partially agrees with the finding of De Man (1985) in barley but contradicts with more recent results in other field crops, proving negative correlation between unsaturated FA and temperature increase (Schulte et al., 2013; Zhang et al., 2015; Shamloo et al., 2017). We found a significantly higher proportion of oleic acid in comparatively cooler and wetter year 2012 which agrees with positive correlation for this FA with precipitation showed by De Man (1985), but disagrees with the results on the positive effect of temperature on oleic acid in several other crops (Schulte et al., 2013; Gauthier et al., 2017; Shamloo et al., 2017). The strong negative correlation between linoleic and oleic acids can be explained by oleic acid, being a precursor of unsaturated C:18 FA (De Man & Dondeyne, 1985).

## **Organic/conventional farming system**

We did not find clear support to the finding of Ciołek et al. (2012) that lipid content tends to be higher in conventionally grown CB grain in comparison to organically grown grain. It was true for variety 'Jumara' in both years, but not for 'Rubiola', which had the lowest oil content under C3 management with the highest application of agrochemicals in both trial years. All HB genotypes had highest mean oil content values under O system if compared to C system, which agrees with the trend in previously mentioned study. The same authors reported a higher proportion of unsaturated FA (especially linoleic acid in wheat and  $\alpha$ -linolenic acid in barley) in the oil of organic cereal grain than in conventional grain in most cases. Our results partially support these findings by showing significantly higher mean  $\alpha$ -linolenic acid proportion over both years and also oleic acid proportion in one trial year under O system, but contradict by significantly lower linoleic acid proportion under O system (2012).

## Perspective genotypes for oil with health related compounds

In a study on the content of polyphenols and  $\alpha$ -tocopherol in the same material, we found HB genotypes PR-4651 and 'Irbe' being relatively superior (Legzdiņa et al., 2018). In the current study both of them had a moderate oil content with a particularly good stability for 'Irbe'. In addition, PR-4651 provided moderately high proportion and excellent stability of the valuable  $\alpha$ -linolenic acid giving additional affirmation for the usefulness for healthy food products. The outstanding line PR-5099 for oil and linoleic acid proportion had a moderately low  $\alpha$ -tocopherol content in grain and grain oil, comparatively high polyphenol content in grain and low polyphenol content in grain oil, not contributing to the high antioxidative ability of the oil produced from this genotype.

Barley oil extracted from high oil waxy HB was shown to reduce plasma cholesterol concentration and promote weight gain of chicks if compared to margarine suggesting, that tocotrienol and polyunsaturated FA in barley oil has a hypocholesterolemic effect (Wang et al., 1993). Barley oil cannot be recommended for food directly because the ratio of  $\omega$ -3 and  $\omega$ -6 FA does not match up to the optimum, but it can be used as a good source of linoleic acid due to its anticarcinogenic, antiobese, antidiabetic and antihypertensive properties (Qian et al., 2009; Koba & Yanagita, 2014).

The negative relationships between oil content and several compounds (protein, lysine and  $\alpha$ -tocopherol) and also between  $\alpha$ -linolenic acid proportion and protein, lysine and  $\beta$ -glucan content may indicate difficulties to combine high contents of the respective compounds in the potential varieties during further breeding process. Unlike to our results, a positive relationship between oil and  $\alpha$ -tocopherol content was reported in oat (Sterna et al., 2014).

# CONCLUSIONS

The effect of barley genotype was highly significant for the oil content but only in some cases for proportions of linoleic,  $\alpha$ -linolenic and palmitic acid. We found higher average oil content and proportion of palmitic and  $\alpha$ -linolenic acid in CB than in HB. However, HB line with a high oil content leads to the conclusion, that differences in oil content and FA composition should be explained by other genetic factors and not by hulless and covered grain type.

Crop management regime did not significantly affect oil content in barley grain but had some significant effects on proportions of linoleic,  $\alpha$ -linolenic, oleic and stearic acid. A decrease of chemical input was in favour of oil content and proportion of unsaturated  $\alpha$ -linolenic and oleic acid and also saturated stearic acid, but did not favour linoleic acid proportion. Organic farming system provided higher oil content and proportions of  $\alpha$ linolenic, oleic and stearic acid.

We identified waxy HB line PR-5099 different from other genotypes with a high oil content, a very high proportion of linoleic acid but low proportion of palmitic acid. We suggest this line for linoleic acid-rich oil production, especially when grown under higher mineral fertilizer input.

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