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GRAIN MORPHOMETRY ANALYSIS OF ROMANIAN WINTER BARLEY CULTIVARS REGISTERED DURING 1959-2019 PERIOD

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ABSTRACT

Grain morphometry is presently among the main targets of modern barley breeding. Three yield trials with winter barley cultivars were performed in the 2018-2020 period at the National Agricultural Research and Development Institute Fundulea in order to analyze the weight of thousand grains and grain morphometry (grain length, grain width, grain area, grain circularity, grain length-width ratio, and factor form density) of six and two-row registered Romanian winter barley cultivars.

Acquired image analysis of grains produced by 25 winter barley cultivars in three years with different grain filling conditions, showed that variation of climatic conditions significantly influenced the thousand grain weight (TGW), grain length (Gl), grain width (Gw), grain area (Ga), grain circularity (Gc), grain length-width ratio (L/W ratio) and factor form density (FFD). The barley cultivars were significantly different for all studied traits, which underlined a high level of diversity.

All traits were on average different between six-row and two barley cultivars. TGW was highly correlated to Ga, Gw, and FFD. The PCA analysis revealed that PC1, PC2, and PC3 explain 99.1% of the amount of variation. Our paper provides extended information and the newest grain morphometry insight of an old, oldest, and modern barley germplasm released in three different breeding periods.

Keywords: winter barley, cultivar, grain morphometry, factor form density.

INTRODUCTION

In 2013, Kesavan et al., studied the seed size as a priority trait in cereal crops, having in view that yield depends on seed size and this character is affected by abiotic and biotic factors. Barley grain weight is mainly determined by grain length and width, which are quantitative traits, controlled by polygenes or QTLs (Sun et al., 2013). Grain length, grain width, and grain weight of barley contribute to the grain quality (Lai et al., 2017).

The weight of thousand grains (TGW) analysis has been made by many researchers. So, Ayoub et al. (2002) studied a cross between two-row with six-row barley; Sameri and Komatsuda (2007) investigated a cross oriental × occidental barley genotypes; Comadran et al., (2011) located loci controlling yield-related traits in a multi-environmental trial data; Walker et al. (2013) dissected OTLs associated with endosperm hardness, grain density, grain size, and malting quality; Wang et al. (2019) detected barley agronomic traits by SNP markers; Xu et al. (2018) applied a genome wide association analysis (GWAS) of grain yield-associated traits in a large barley collection (379 different growth habit and row type cultivars).

In some studies done overtime, QTLs for grain length (Gl) (Ayoub et al., 2002; Walker et al., 2013; Watt et al., 2018), grain width (Gw) (Cu et al., 2016 in a doubled haploid population derived from two Australian malting barley varieties), grain length-width ratio (L/W ratio) (Sameri and Komatsuda, 2007; Kalladan et al., 2013), grain area (Ga) (Xu et al., 2018), grain diameter (Gd) (Cu et al., 2016) were detected and mapped on all seven barley chromosomes.

Wang et al. (2019), after studying a DH population, identified 45 barley genes for grain size and weight and evaluated nine traits determining grain size and weight [TGW, Gl, Gw, L/W ratio, Ga, grain perimeter - Gp (mm),

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grain diameter - Gd (mm), grain roundness - Gr (mm) and factor form density - FFD (g/mm^2)].

An alternative to collecting much more phenotypic data about biological material (plant genetic resources from ex-situ collections) and to enhance the use of genetic resources is the analysis of historical data collected, for instance, during their multiplication (González et al., 2018; Philip et al., 2019). The obtained data can serve both to validate the analysis of historical data and to create a bio-digital resource centers useful for researchers and breeders (González et al., 2018; Philip et al., 2018).

This study was performed to assess and analyze for the first time the grain morphometry of six and two-row Romanian winter barley cultivars (from ex-situ collection), registered during the 1959-2019 period.

A comparison between grain traits of six-row versus two-row winter barley and between oldest cultivars (registered between 1959 and 1986 year) versus old cultivars (registered between 1992 and 2000 year) and modern varieties (registered since 2003 year till 2019) was made.

MATERIAL AND METHODS

The grain morphometry of twenty-five Romanian winter barley cultivars, from yield trials performed at NARDI Fundulea (44°26`N latitude and 26°31`E longitude) during the 2018-2020 period were analyzed (Table 1). This set comprised the barley varieties released from 1959 to 2019 (nineteen six-row and six two-row barley varieties), obtained from different germplasm combinations (Table 1) by pedigree method, except two cultivars created by *bulbosum* method (six-row barley Smarald and two-row barley Gabriela cultivars).

For a period of 60 years, the barley breeding process was divided into three periods: first breeding period with six cultivars released (1959-1986), second breeding period with nine cultivars released (1992-2000), and third breeding period with 10 cultivars released (2003-2019).

The trials were conducted according to the local practices for winter barley production (peas as preceding crop, 100 kg/ha P_2O_5 applied in autumn, sown in October, 100 kg/ha urea applied in spring, one treatment with herbicide and a fungicide were applied).

In the experimental field, a randomized block design experiment with three replications was performed. Each plot has a 5 m length, consisted of eight rows and the number of seeds was calculated on 350 seeds/m^2, according to the evaluated germination of seeds and thousand weight grains. Seeds were sown by a Wintersteiger seed drill, at 3 cm deep between October 9-12 each year and at the end of June, the cultivars were harvested. The obtained quantity was weighted, all the samples were cleaned in triplicate and 100 grains from each were counted with Contador instrument (Pfeiffer, Germany) and with an electronic balance with 2 decimals (Précisa, Switzerland) the thousand weight grains (TGW) were determined.

By an optical grain analysis instrument (Marvin seed analyser, Germany) the grain morphometry was acquired: grain length (Gl), grain width (Gw), grain area (Ga), grain circularity (Gc), grain length/width ratio (L/W ratio).

In order to assess the difference in barley grain density of cultivars, the factor form density (FFD) was calculated according to the formula adapted after Giura and Săulescu (1996), in Eq. (1):

$$FFD = \frac{TGW}{(1000 \text{ x } Gl \text{ x } Gw)} \qquad (1)$$

where: TGW - thousand grain weight; Gl - grain length; Gw - grain width.

Table 1. Year of registration, row type and genealogy of 25 winter barley cultivars released in three different breeding period

No.	Name of cultivar	Year of registration	Row type	Breeding method	Genealogy
First breeding period					
1	Cenad 345	1959	6	pedigree	Unknown
2	Intensiv 2	1968	6	pedigree	(Cenad 396 x Rekord)
3	Miraj	1974	6	pedigree	{Fundulea 296/64 x [(B Ky x 259.711) x 124]}
4	Productiv	1981	6	pedigree	(Ager x Mo B 475)
5	Grivița	1981	2	pedigree	(Azuga x Fundulea 1328-69)
6	Precoce	1986	6	pedigree	(3B504 x Miraj)
Second breeding period					
7	Laura	1992	2	pedigree	(Grivița individual reselection)
8	Amical	1993	6	pedigree	(Cernomoreţ x Rachel)
9	Andreea	1994	2	pedigree	(Egmond x Grivița)
10	Dana	1993	6	pedigree	(Dilana x Fundulea 38-81)
11	Mădălin	1994	6	pedigree	[(M 251-500Y-500B-500Y) x Miraj]
12	Sistem	1996	6	pedigree	(Fundulea 1594-78 x Fundulea 123-79)
13	Andrei	1998	6	pedigree	(Productiv x H.V.W. 2247)
14	Compact	1998	6	pedigree	(No.77/74 x Fundulea 298-72)
15	Mareșal	2000	6	pedigree	(Fundulea 630-90 x Productiv)
Third breeding period					
16	Cardinal FD	2003	6	pedigree	(H 259 x Fundulea 3-83)
17	Univers	2004	6	pedigree	[Atlas 68 x (Fundulea 38-81 x Fundulea 30-81)]
18	Ametist	2012	6	pedigree	(Rany x Samaki) x 3F
19	Artemis	2012	2	pedigree	(Obruk 86 x Andra)
20	Smarald	2013	6	*bulbosum*	(Miraj x Activ) x H 251
21	Simbol	2015	6	pedigree	(35 GI/98 x Fundulea 8-81-97)
22	Onix	2017	6	pedigree	(Carola x Fundulea 8-31-2001)
23	Gabriela	2017	2	*bulbosum*	[NS 525 x (Azuga x Fundulea 1328-69)]
24	Lucian	2018	6	pedigree	Balkan x (H 251 x Fundulea 3-83)
25	Diana	2019	2	pedigree	(Anita x Fundulea 8-110-2000)

Meteorological station from NARDI Fundulea provided data regarding the rainfall and average temperature during the barley grain filling stage, by each month decades.

The barley grain filling season (April - June) from the studied periods was different regarding average temperature and rainfall (Table 2). A comparison between experimental years showed large differences between them, both the climatic presented elements. In 2018, each average decade temperature was higher comparing each decade from 2019 with 3.2-6.0°C in April, 0.7-4.6°C in May and almost similar in June for both years (except the third decade of June from 2019, when the average temperature was increased with 4.3°C comparing with the same decade from 2018). As average recorded temperature, the 2020 year was a little bit warmed in the second and third decade of April and in the first and second decade of May comparing with 2019. Regarding the first two decades of June, in this year the temperature was lower than the previous years in the same decades.

As quantity and distribution, the average rainfall was also contrasting. In 2019, the total fallen amount from April to June was 250.2 mm, higher than the same period from 2018 with 107.2 mm and not equally distributed. If we compare the experimental years on decades, the rainfall from the beginning of May to the end of June second decade, the differences oscillated from 4.0 mm to 61.8 mm so, the studied years registered different conditions for grain filling, 2018 with a higher average of temperature and 2019 with a high quantity of rainfall, provided different testing conditions. Under 2020-year conditions, the amount of average rainfall was much lower compared to the first and second year both in decadal and monthly distribution.

Table 2. Total rainfall (mm) and average temperature (°C) recorded during the grain barley filling stages (April-June), NARDI Fundulea, 2018-2020

Year		2018	2019	2020	2018	2019	2020
Month	Decade	Average t (ºC)	Average t (ºC)	Average t (ºC)	Average rainfall (mm)	Average rainfall (mm)	Average rainfall (mm)
April	1-10	**13.4**	10.2	9.49	2.0	5.6	**0.0**
	11-20	**15.5**	9.5	12.8	**0.2**	**41.6**	5.6
	21-30	**18.5**	13.9	14.4	0.2	4.2	8.4
May	1-10	**19.3**	14.7	15.8	**16.0**	**20.0**	32.0
	11-20	17.8	17.1	20.2	**14.8**	**39.2**	**0.4**
	21-31	21.1	19.8	15.2	**3.2**	**65.0**	25.6
June	1-10	22.8	21.6	20.2	**16.6**	**50.4**	15.6
	11-20	24.8	24.6	21.3	**10.6**	**19.8**	38.4
	21-30	**20.3**	**24.6**	23.7	93.4	4.4	14.4
Total rainfall April - June (mm)					**157.0**	**250.2**	**140.4**

To determine the effects of cultivar (C), environment (E), and cultivar x environment (V x E interaction), two-way analysis of variance with MSTAT statistical software (taking into consideration the cultivar, environment, and the cultivar x environment interaction) for all the characters were performed. The results were presented as means and analysed according to the variation scale (Ceapoiu, 1968) described by the coefficient of variation (CV%). Significant differences between barley cultivars means were assessed by the Fisher´s least significant differences (LSD) test.

Principal component analysis (PCA) based on correlation matrix and Pearson correlation between the analyzed traits was performed using OPSTAT software (Statistical Software Package for Agricultural Research Workers, Sheoran et al., 1998).

RESULTS AND DISCUSSION

In this study, acquired digital seeds imaging provided a complete description of grain size and shape by a modern instrument, namely the Marvin seed analyzer. The obtained results indicate that trait varieties size differences depend on environmental conditions (temperature and rainfall quantity and distribution), as evidenced by the significant effects of the Y, C, and Y x C interactions of the analysed winter barley traits.

Analysis of main effects (Table 2) of year (Y), cultivar (C), and two-way interactions of year x cultivar (Y x C) showed significant differences ($P<0.01$). These results revealed that grain of varieties differed in grain weight, length, width, area, circularity, L/W ratio, FFD and were influenced by year, cultivar and their interactions.

Table 3. Analysis of variance (probability value in italics), standard deviation (SD), standard error (SE) and limit significance differences (LSD) for winter barley cultivars analysed

Source of variation	df	TGW (g)	Ga (mm^2)	Gl (mm)	Gw (mm)	Gc (mm)	L/W ratio	FFD (g/mm^2)
Year	2	*0.0000*	*0.0008*	*0.0009*	*0.0008*	*0.0000*	*0.0000*	*0.0014*
Cultivar	24	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
Y x C	48	*0.0000*	*0.0000*	*0.0242*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
SD		2.72	0.61	0.25	0.10	0.08	0.13	0.10
SE		0.41	0.14	0.09	0.06	0.06	0.08	0.09
LSD (5%)		0.9	0.30	0.35	0.04	0.04	0.05	0.03

Averaged obtained traits values over all cultivars were as follows: TGW - 43.24 g, Ga - 17.52 mm^2, Gl - 8.22 mm, Gw - 3.20 mm, Gc - 1.77 mm, L/W ratio - 2.59 mm and FFD - 1.14 g/mm^2 (Table 4). Analysis of the average grain traits, revealed an ascending trend regarding TGW among different breeding period, from 42.01 g (first breeding period) to 44.14 g (third breeding period) with the highest coefficient of variation in the third breeding period (8.65%) due to a decreasing of minim value (from 40.85 g to 39.69 g) and an increase of maximum value with 5.32 g (from 44.82 g - second breeding period to 50.14 g - third breeding period).

Ga, Gw, L/W ratio, and FFD registered maximum values in the third breeding period (18.45 mm, 3.44 mm, 2.78, and 1.36 g/mm^2, respectively). Gl and Gc had maximum values in the second breeding period (old cultivars).

The modern varieties grain traits coefficient of variation is higher than old cultivars with 6.37% for TGW (from 42.22 g to 44.13 g). The differences regarding the CV for the other traits between old cultivars and modern cultivars are moderate: 2.25% for GA, 1.67 for Gw, and 7.97% for FFD which showed a tendency to increase compared with the second breeding period while the other traits (Gl, Gc, and L/W ratio) registered a slight decrease.

Table 4. Traits mean values, range and coefficient of variation in three different breeding period (25 winter barley cultivars)

Traits	TGW (g)	Ga (mm^2)	Gl (mm)	Gw (mm)	Gc (mm)	L/W ratio	FFD (g/mm^2)
First breeding period (oldest cultivars)							
Range	40.85-46.25	16.76-18.16	8.06-8.41	3.11-3.33	1.72-1.85	2.47-2.73	1.03-1.26
Mean	42.01	17.16	8.21	3.16	1.80	2.62	1.09
CV (%)	5.00	3.08	1.71	2.63	2.98	3.39	7.83
Second breeding period (old cultivars)							
Range	41.87-44.82	17.35-18.41	7.65-8.61	3.12-3.33	1.61-1.87	2.31-2.75	1.11-1.23
Mean	43.05	17.68	8.33	3.21	1.79	2.62	1.15
CV (%)	2.28	2.13	4.00	2.36	5.11	6.00	3.87
Third breeding period (modern cultivars)							
Range	39.69-50.14	16.54-18.45	7.89-8.40	3.04-3.44	1.60-1.84	2.30-2.78	1.01-1.36
Mean	44.14	17.60	8.12	3.23	1.73	2.54	1.15
CV (%)	8.65	4.38	2.28	4.03	3.90	5.04	11.84
Breeding period (1959-2019)							
Average	43.24	17.52	8.22	3.20	1.77	2.59	1.14
CV (%)	2.24	1.83	4.54	1.21	2.29	1.96	3.28

In terms of row number and the entire barley breeding period (1959-2019), the mean of TGW, Ga, Gl, Gw, Gc, L/W ratio, and FFD is presented in Table 5. For these traits, the differences had maintained between six-row and two-row cultivars, larger for TGW (from 42.10 g for six-row barley to 46.85 g for two-row barley), moderate for Ga, Gw, and FFD but smaller for six-row barley, and higher for two-row barley. Gl, Gc, and L/W ratio was larger for six-row barley and smaller for two-row barley. The differences between barley cultivars grains having the same grain length and grain weight assessed with the factor form density (FFD) trait, showed a slight increase comparing six-row barley with two-row barley (only 0,15 g/mm^2).

For both six-row and two-row barley and all seven traits, the coefficient of variations varied between 1.52% and 7.31%, the values did not exceed the 10% threshold, which underlined the high stability of these characters for both six-row and two-row winter barley varieties registered since 1959 till 2019.

Table 5. Comparison of grain traits six-row versus two-row winter barley cultivars (mean and coefficient of variation), the 1959-2019 period

Type row cultivars average and coefficient of variation	TGW (g)	Ga (mm^2)	Gl (mm)	Gw (mm)	Gc (mm)	L/W ratio	FFD (g/mm^2)
Six-row cultivars average (1959-2019 period)	42.10	17.35	8.29	3.16	1.80	2.65	1.10
Coefficient of variation (CV%)	3.55	3.02	2.64	1.78	2.59	2.89	6.82
Two-row cultivars average (1959-2019 period)	46.85	18.08	7.99	3.36	1.66	2.39	1.25
Coefficient of variation (CV%)	5.67	3.09	2.70	1.52	2.80	2.96	7.31

To quantify the contribution of the studied traits (TGW, Ga, Gl, Gw, Gc, L/W ratio, and FFD), principal component analysis (PCA) was carried out (according to the methodology of Sheoran et al., 2020) and the number of principal components that accounts for the variance was determined (Table 6). The principal component with an eigenvalue greater than 1 was PC1 (4.64768) and PC2 (2.2018) and also the level of variance is explained by these principal components. The first principal component (PC1) explains 66.4% of the amount of variation and the second (PC2) explains 31.45%. The first three components explain 99.1% of the trait variation and therefore these components have to be used as selection criteria for barley germplasm utilized as genitors in the barley breeding.

Table 6. Eigenvalues of the correlation matrix for the seven principal components (PC) and the percentage of variance explained by the successive principal components

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	4.64768	2.20180	0.08578	0.05066	0.01236	0.00097	0.00075
% variance	66.395	31.454	1.2254	0.72371	0.17653	0.013926	0.010726
Cumulative %	0.664	0.978	0.991	0.998	1.000	1.000	1.000

For relevance, the magnitudes and direction of the correlation eigenvectors matrix were examined. Coefficients taken into account when grain traits were associated with principal components are bolded values (Table 7). The relationship between the correlation coefficients of grain traits with the components' axes is described by the association of the principal components with each trait. The results showed that most associations are presented by PC1, PC2, PC7, and the traits (five or six). The first principal component (PC1), the second (PC2) and the seventh (PC7) have a large positive association with Gw (0.456, 0.491, and 0.477, respective), PC2 and PC7 with Gl (0.611 and 0.480), PC5 with FFD (0.681) and PC6 show the highest positive association with L/W ratio (0.710). The PC3, PC4, PC5, and PC6 present the largest negative association with TGW, Ga, and Gl (-0.687, -0.520, -0.542, and -0.567).

Table 7. Eigenvectors of the correlation matrix (grain component weights to principal main components)

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
TGW	**0.436**	0.18	**-0.687**	-0.048	**-0.520**	-0.136	0.118
Ga	**0.335**	**0.452**	**0.387**	**-0.542**	-0.146	0.066	**-0.463**
Gl	-0.195	**0.611**	0.128	-0.035	0.138	**-0.567**	**0.480**
Gw	**0.456**	0.054	**0.491**	**0.381**	-0.262	0.328	**0.477**
Gc	**-0.376**	**0.382**	0.034	**0.599**	**-0.395**	0.041	**-0.442**
L/W	**-0.403**	**0.328**	-0.217	-0.294	-0.027	**0.710**	**0.303**
FFD	**0.385**	**0.365**	-0.268	**0.336**	**0.681**	0.204	-0.168

Table 8. Pearson correlation matrix of phenotypic winter barley analysed traits

Trait	TGW	Ga	Gl	Gw	Gc	L/W	FFD
TGW	1						
Ga	0.838^{***}	1					
Gl	-0.162^{ns}	0.310^{ns}	1				
Gw	0.917^{***}	0.769^{***}	-0.336^{ns}	1			
Gc	-0.613^{**}	-0.220^{ns}	0.853^{***}	-0.738^{***}	1		
L/W	-0.673^{***}	-0.299^{ns}	0.804^{***}	-0.828^{***}	0.971^{***}	1	
FFD	0.936^{***}	0.944^{***}	0.140^{ns}	0.851^{***}	-0.360^{ns}	-0.456^{*}	1

In order to obtain the correlation coefficient among the seven grain traits based on the mean value of the three-year data, Pearson correlation matrix of phenotypic winter barley analysed traits (correlation table - www.real-statistics.com) were performed (Table 8).

The results showed that TGW had a significantly positive correlation with Gw (0.917^{***}), which is according also with results of Walker et al. (2013), Ga (0.838^{***}), and FFD (0.936^{***}), showing a very strong positive relationship and the fact that this parameter is influenced by all of them. The correlation between Gc, grain L/W ratio and TGW was negatively significant (r = -0.62 and r = -0.67) while Gl, Gc, and grain L/W ratio were uncorrelated with Ga. There was a significant negative correlation between Gc and grain L/W ratio and Gw (r = - 0.74 and r = - 0.83).

Furthermore, the FFD showed strong positive correlations with TGW, Ga, and Gw ($r = 0.94^{**}$, $r = 0.95^{**}$, and $r = 0.85^{**}$), and it was uncorrelated with Gl and Gc, and negatively slighter correlated with grain L/W ratio.

CONCLUSIONS

Analysis of barley grain cultivars released in the 1959-2019 period by image method, showed that traits determining morphometry of Romanian winter barley grain cultivars were dependent on the environment, cultivar as well as the interaction of the environment with cultivar. This method can help to evaluate and describe barley germplasm originated from different countries, too.

We found high diversity within barley cultivars for the analysed traits, especially for Ga, Gw, and FFD. A high correlation of Ga, Gw, and FFD with TGW explained most of TGW variation.

The dissected components of barley grain have evolved on average from the first to the second breeding period through increases of Ga, Gl, and Gw followed in the third breeding period by slight modifications in Gw and a constant FFD.

Our results show a slight reduction of phenotypic trait variation in barley grain morphometry in the modern germplasm in comparison with the oldest and old barley cultivars, as a result of large differences (10.45 g) between the minimum and maximum value of cultivars TGW, registered between the 2003-2019 years.

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