

GENETIC DIVERSITY REGARDING GRAIN SIZE AND SHAPE OF COMMON WINTER WHEAT

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Abstract

Grain characteristics regarding grain size and shape, such as: thousand kernel weigh, grain area, length, width, circularity, and test weight are important components of grain yield and quality in wheat. A set of 22 winter wheat varieties, which included old and new varieties and advanced breeding lines were tested across 2018-2019 and 2019-2020, at NARDI Fundulea, to estimate the historic evolution of these features and the correlation of them with grain yield. Shape grain characteristics were analysed with Marvin seed analyser. ANOVA showed very significant differences both between varieties and between years. The thousand kernel weigh was positively correlated with other parameters of grain shape, such as: grain area, width, length and circularity. The year of release was negatively correlated with some grain parameters, such as length, circularity and positively correlated with yield. It is necessary to pay attention in breeding to increase grain size and weight, but only if this will not be associated with the correlated negative changes in the other components of yield, which might lead to a decrease in grain yield.

Key words: grain size and shape, genetic diversity, wheat, yield.

INTRODUCTION

Grain shape and size are important components of grain yield and quality in wheat. Grain size is an important physical indicator of seed quality that affects early vegetative growth and is frequently related to yield, market grade factors and harvest efficiency.

Wheat kernel size and shape influence flour yield and market price. Seed size has significant impact on seedling emergence percentage.

Grain morphology analysis can play an important role in determining quality of wheat grain especially regarding market value (Kumari et al., 2015).

With increasing world population, it has been estimated that the global demand for wheat will increase by a further 60% by 2050 (Licker et al., 2010). It is a huge challenge to ensure global food security through sustainable wheat production for the projected population, in the context of the increasing adverse impact of climate change (Palm et al., 2010).

In the past four decades, improvement of grain yield has come from increased grains per square meter or larger grain sizes, due to the utilization of *Rht* genes in wheat breeding (Calderini & Reynolds, 2000).

However, TGW is a complex trait, and is largely controlled by several grain traits, including grain size and shape (Zhang et al., 2014). TGW is characterized by a higher heritability than grain yield itself (Deng et al., 2011), and it is positively correlated with agronomic yield (Maccaferri et al., 2011) and flour yield (Williams et al., 2013). In a previous study, the correlation coefficient of grain yield with TKW ranged from -0.4 to +0.6, with most trials showing practically no correlation, and only one trial having significant positive correlation (Mandea et al., 2019). The high heritability values (59% to 96%) in most of the cultivars studied so far proved that this character is phenotypically the most-stable yield component (Giura & Săulescu, 1996; Huang et al., 2006; Sun et al., 2009; Patil et al., 2013). Grain size is mainly characterized by grain weight and area, whereas shape means a relative proportion of the main growth axes of the grain (Gegas et al., 2010). Grain shape is generally estimated by length, width, vertical perimeter, sphericity and horizontal axes proportion (Bresghello & Sorrells, 2007). Many studies have shown that wheat grain size and shape are positively correlated with TGW and they affect flour yield, end-use quality and market price (Evers et al.,

1990; Breseghello and Sorrells, 2006; Williams et al., 2013; Rasheed et al., 2014). Theoretical models predict that milling yield could be increased by optimizing grain size and shape with large and spherical grains being the optimum grain morphology (Evers et al., 1990). To gain deeper insights into the genetic basis of grain size and shape variation, Gegas et al. (2010), studied several different populations of recombinant doubled haploids (DH) that capture a broad spectrum of the phenotypic variation present in the elite winter wheat germplasm pool. Grain material from accessions of primitive wheat species and modern elite varieties were measured to determine the phenotypic structure of the traits and assess the extent of variation retained in domesticated wheat. They showed that grain size is largely independent of grain shape both in the DH populations and in the primitive wheat species and that there is a significant reduction of phenotypic variation in grain shape in the breeding germplasm pool probably as a result of relatively recent bottleneck. This phenotypic structure is attributed to a distinct genetic architecture where common genetic components are involved in the control of those traits in different wheat varieties (Gegas et al., 2010). Moreover, the emergence of hexaploid, common or bread wheat, followed by further selection and extensive breeding, led to a crop species of significant financial and nutritional importance since it provides one-fifth of the calories consumed by humans today (Dubcovsky & Dvorak, 2007). Archaeobotanical evidence from around the Fertile Crescent region indicates that the transition from the diploid wild einkorn (*Triticum monococcum* ssp. *aegilopoides*; AmAm) and tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccoides*; BBAA) to the domesticated forms (*T. monococcum* ssp. *monococcum* and *T. turgidum* ssp. *dicoccum*, respectively) was associated with a trend toward larger grains (Fuller, 2007). Several other quality criteria used by the industry are influenced by grain morphology. Grain size was also found to be associated with various characteristics of flour, such as protein content and hydrolytic enzymes activity, which in turn determine baking quality and end-use suitability (Evers, 2000). Abdipour et al. (2016), analysed a set of 98 bread wheat landraces from

different geographic regions of Iran, across 2013-2014 and 2014-2015 to determine the phenotypic diversity and relations between thousand grain weight (TGW), grain morphology and grain quality. They found that the genotypes were significantly different for all traits, which reflects the high levels of diversity. Significant positive correlations were observed between TGW and grain size (or shape), except for the aspect ratio (AR) and roundness. However, grain quality traits, especially GPC, had significant negative correlation with TGW. The present study was conducted to estimate the historical changes regarding grain characteristics and the association between different grain morphological traits with grain yield.

MATERIALS AND METHODS

A set of 22 winter wheat varieties, which included old and new varieties and advanced breeding lines was tested across 2018-2019 and 2019-2020, at National Agricultural Research and Development Fundulea (44°30' N, 24°10' E, 68 m above sea level), as material in this study. The tested cultivars are shown in table below (Table 1):

Table 1. The tested cultivars, the provenience and the year of release

No. crt.	Variety	Provenience	Year of release
1	A15	Romanian cultivar	1939
2	Bezostaia 1	Russian cultivar	1961
3	Dacia	Romanian cultivar	1971
4	Iulia	Romanian cultivar	1974
5	Fundulea 29	Romanian cultivar	1979
6	Arieșan	Romanian cultivar	1985
7	Fundulea 4	Romanian cultivar	1987
8	Drobia	Romanian cultivar	1993
9	Alex	Romanian cultivar	1994
10	Boema 1	Romanian cultivar	2000
11	Glosa	Romanian cultivar	2005
12	Apache	French cultivar	2005
13	Izvor	Romanian cultivar	2008
14	FDL Miranda	Romanian cultivar	2011
15	Otilia	Romanian cultivar	2013
16	Avenue	French cultivar	2013
17	Pitar	Romanian cultivar	2015
18	Voinic	Romanian cultivar	2020
19	Ursita	Romanian cultivar	2021
20	FDL Amurg	Romanian cultivar	advanced breeding line
21	FDL Armura	Romanian cultivar	advanced breeding line
22	FDL Abundant	Romanian cultivar	advanced breeding line

The genotype panel was planted under the open-field conditions, on chernozem soil (pH: 6.3-6.8; humus: 3%), in plots of 6 m², using recommended crop management.

Weather conditions during the experiments are summarized in table 2. From the point of view of the grain characteristics, the temperatures during the grain filling are of interest.

Table 2. Weather conditions during the two seasons of research

Season	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Average temperature (°C)											
2018	19	11.7	6.7	3.6	0.8	1.6	3.3	15.7	19.4	22.6	22.8
2019	19.1	13.4	5.2	-0.1	-1.2	3.8	9.3	11.2	17.2	23.6	23
2020	19.3	12.8	10.3	4	0.9	5.2	8.3	12.2	17	21.7	25.1
Rainfall (mm)											
2018	12.2	111.6	49.2	27.8	36	58.6	40.6	2.4	34	120.6	83
2019	28.6	10.8	23	43	53.8	21.4	21.6	51.4	124	74.6	87.4
2020	6.2	38	33.2	16.2	2	16.6	27.8	14	58	68.4	34.2

The characteristics regarding grain size and shape were analysed with a Marvin seed analyser (high-throughput method).

The analysed parameters were: grain area, length, width, circularity; in addition to these characteristics, we also analysed the test weight and the thousand kernel weight; all of these traits were analysed in three replicates.

ANOVA was used to estimate the significance of differences between wheat genotypes regarding grain characteristics.

RESULTS AND DISCUSSIONS

ANOVA with two factors (genotype and season) calculated for grain width showed very significant differences for both, genotype and year, and also for interaction (Table 3).

Table 3. ANOVA for grain width, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	SS	df	MS	F	F crit
Cultivars	0.57	21	0.027	24.438***	1.677
Seasons	0.10	1	0.104	93.015***	3.949
Interaction	0.17	21	0.008	7.140***	1.677
Within	0.10	88	0.001		
Total	0.94	131			

*** = very significant at P<0.1%.

ANOVA with two factors (genotype and season) calculated for grain length showed very significant differences for both, genotype and year, and also for interaction (Table 4).

The differences between the genotypes, regarding grain width and length, are showed in table below (Table 5).

Table 4. ANOVA for grain length, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	SS	df	MS	F	F crit
Cultivars	12.96	21	0.617	285.50***	1.68
Seasons	2.28	1	2.283	1056.18***	3.95
Interaction	0.27	21	0.013	5.98***	1.68
Within	0.19	88	0.002		
Total	15.70	131			

*** = very significant at P<0.1%.

Table 5. The differences between genotypes, regarding grain width and length and their significance

No. crt.	Variety	Average values and significance	Average values and significance
		Width (mm)	Length (mm)
1	A15	2.90	6.008
2	Bezostaia 1	2.91	6.260***
3	Dacia	3.06***	5.813 ^{oo}
4	Iulia	2.92	6.113**
5	Fundulea 29	2.79 ^{ooo}	5.810 ^{oo}
6	Arieşan	2.96	6.989***
7	Fundulea 4	2.89	6.263***
8	Drophia	2.95	6.048*
9	Alex	2.91	6.211***
10	Boema 1	2.91	5.956
11	Glosa	2.95	6.089**
12	Apache	2.95	5.835 ^{oo}
13	Izvor	2.84 ^{oo}	5.664 ^{ooo}
14	FDL Miranda	2.85 ^{oo}	6.094**
15	Otilia	2.90	5.608 ^{ooo}
16	Avenue	2.93	5.726 ^{ooo}
17	Pitar	2.99*	5.745 ^{ooo}
18	Voinic	2.91	5.671 ^{ooo}
19	Ursita	2.90	5.833 ^{oo}
20	FDL Amurga	3.11***	6.046*
21	FDL Armura	2.88	5.469 ^{ooo}
22	FDL Abundent	2.95	5.700 ^{ooo}

DL 5%= 0.054 DL 5%= 0.075

*** = very significant positive at P<0.1%; * = significant positive at P<5%; ^{oo} = very significant negative at P<0.1%; ^{ooo} = distinct significant negative at P<1%.

The genotypes with higher grain width were: the old cultivar Dacia, the relatively new cultivar Pitar and the breeding line FDL Amurg. The genotypes with smaller grain width were: the old cultivar Fundulea 29, the relatively new cultivars Izvor and FDL Miranda.

Related to grain length, many varieties had a positive significance, namely: the old varieties Bezostaia 1, Iulia, Arieşan, Fundulea 4, Drophia, Alex, the relatively new varieties Glosa and FDL Miranda and the breeding line FDL Amurg. However, many varieties had a negative significance for the grain length, namely: the old varieties Dacia and Fundulea 29, the relatively new varieties Apache, Izvor, Otilia, Avenue, Pitar, Voinic, Ursita and the breeding line FDL Armura and FDL Abundent.

ANOVA with two factors (genotype and season) calculated for grain area showed very significant

differences for both, genotype and year, and for interaction too (Table 6).

Table 6. ANOVA for grain area, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	SS	df	MS	F	F crit
Cultivars	81.53	21	3.88	80.99***	1.68
Seasons	25.71	1	25.71	536.35***	3.95
Interaction	5.29	21	0.25	5.25***	1.68
Within	4.22	88	0.05		
Total	116.75	131			

*** = very significant at P<0.1%.

ANOVA with two factors (genotype and season) calculated for grain circularity showed very significant differences for both, genotype and year, and also for interaction (Table 7).

Table 7. ANOVA for grain circularity, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	SS	df	MS	F	F crit
Cultivars	0.22	21	0.010	104.056***	1.677
Seasons	0.01	1	0.010	98.997***	3.949
Interaction	0.02	21	0.001	7.253***	1.677
Within	0.01	88	0.000		
Total	0.25	131			

*** = very significant at P<0.1%.

The differences between the genotypes, regarding grain area and circularity, are showed in table below (Table 8).

Table 8. The differences between genotypes, regarding grain area and circularity and their significance

No. crt.	Variety	Average values and significance	Average values and significance
		Area (mm ²)	Circularity (mm)
1	A15	12.94	1.42
2	Bezostaia 1	13.52***	1.44**
3	Dacia	13.31*	1.35 ^{oo}
4	Iulia	13.18	1.42*
5	Fundulea 29	11.98 ^{ooo}	1.42 ^o
6	Arieşan	15.26***	1.53***
7	Fundulea 4	13.44**	1.45***
8	Dropia	13.36*	1.41
9	Alex	13.54***	1.43**
10	Boema 1	12.95	1.41
11	Glosa	13.30*	1.41
12	Apache	12.64	1.39
13	Izvor	11.83 ^{ooo}	1.40
14	FDL Miranda	12.90	1.44**
15	Otilia	12.07 ^{ooo}	1.36 ^{ooo}
16	Avenue	12.40 ^{oo}	1.38 ^{oo}
17	Pitar	12.90	1.36 ^{ooo}
18	Voinic	12.25 ^{oo}	1.37 ^{oo}
19	Ursita	12.57	1.39
20	FDL Amurg	13.93***	1.37 ^{oo}
21	FDL Armura	11.65 ^{ooo}	1.36 ^{ooo}
22	FDL Abundent	12.44 ^{oo}	1.37 ^{oo}

DL5% = 0.37 DL 5% = 0.016

*** = very significant positive at P<0.1%; ** = distinct significant positive at P<1%; * = significant positive at P<5%; ^{ooo} = very significant negative at P<0.1%; ^{oo} = distinct significant negative at P<1%.

The genotypes with higher grain area were: the old genotypes like Bezostaia 1, Dacia, Arieşan, Fundulea 4, Dropia, Alex and two relatively new genotypes also, like Glosa (released in the last two decades) and the advanced breeding line FDL Amurg. The genotypes with smaller grain area were: the old genotype Fundulea 29, the relatively new genotypes (released in the last two decades), Izvor, Otilia, Avenue, Voinic and the breeding lines FDL Armura and FDL Abundent. The varieties with higher values for the index of circularity were: the old varieties Bezostaia 1, Iulia, Arieşan, Fundulea 4, Alex and the relatively new variety FDL Miranda. The varieties with smaller grain circularity were: the old varieties Dacia, Fundulea 29, the relatively new varieties: Otilia, Avenue, Pitar, Voinic, the breeding lines FDL Amurg, FDL Armura, FDL Abundent. The smaller values of the circularity index show a rounder grain, a desirable character in milling industry.

ANOVA with two factors (genotype and season) calculated for thousand kernel weight (TKW) showed very significant differences for both, genotype and year, and also for interaction (Table 9).

Table 9. ANOVA for TKW, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	SS	df	MS	F	F crit
Cultivars	1349.83	21	64.28	71.44***	1.68
Seasons	38.09	1	38.09	42.34***	3.95
Interaction	123.77	21	5.89	6.55***	1.68
Within	79.17	88	0.90		
Total	1590.86	131			

*** = very significant at P<0.1%.

ANOVA with two factors (genotype and season) calculated for test weight (TW) showed very significant differences for both, genotype and year, and for interaction also (Table 10).

Table 10. ANOVA for TKW, for 22 genotypes, tested in two seasons (2018-2019; 2019-2020)

Source of Variation	SS	df	MS	F	F crit
Cultivars	290.93	21	13.85	19.67***	1.68
Seasons	1186.80	1	1186.80	1684.85***	3.95
Interaction	48.39	21	2.30	3.27*	1.68
Within	61.99	88	0.70		
Total	1588.11	131			

*** = very significant at P<0.1%; * = significant at P<5%.

The differences between the genotypes, regarding TKW and TW are shown in table below (Table 11).

Table 11. The differences between genotypes, regarding TKW and TW and their significance

No. crt.	Variety	Average values and significance	Average values and significance
		TGW(g)	TW (Kg/hl)
1	A15	39.65	75.62
2	Bezostaia 1	42.94***	77.17*
3	Dacia	43.44***	73.80°°
4	Iulia	41.63*	76.87
5	Fundulea 29	35.98°°°	77.08*
6	Ariesan	46.91***	75.10
7	Fundulea 4	40.30	76.77
8	Dropia	41.95**	76.67
9	Alex	40.08	75.02
10	Boema 1	40.25	76.73
11	Glosa	40.82	77.47
12	Apache	38.21	74.32
13	Izvor	36.27°°°	76.48
14	FDL Miranda	38.32	74.75
15	Otilia	36.37°°°	77.17*
16	Avenue	36.60°°°	72.75°°°
17	Pitar	40.48	77.77***
18	Voinic	37.78°	78.22***
19	Ursita	38.68	77.52**
20	FDL Amurg	45.61***	78.08***
21	FDL Armura	33.12°	74.07°
22	FDL Abundent	37.78°	75.60

DL5%= 1.54 DL 5%= 1.36

*** = very significant positive at P<0.1%; ** = distinct significant positive at P<1%; * = significant positive at P<5%; °°° = very significant negative at P<0.1%; ° = significant negative at P<0.1%.

The varieties with higher TKW were: the old varieties, Bezostaia 1, Dacia, Iulia, Ariesan, Dropia and the breeding line FDL Amurg; the varieties with a smaller TKW were: the old variety Fundulea 29, the new varieties Izvor, Otilia, Avenue, Voinic and the breeding lines FDL Armura and FDL Abundent.

The varieties with higher TW were: the old varieties Bezostaia 1 and Fundulea 29, the new varieties Otilia, Pitar, Voinic, Ursita, the breeding line FDL Amurg; the varieties with smaller TW were: the old variety Dacia, the relatively new variety Avenue and the breeding line FDL Armura.

We analysed the correlations between all these parameters with the year of release and yield (Table 12). We obtained significant positive correlation coefficients between TKW and grain area, width, length and circularity.

The grain area was positively correlated with grain width, length, and circularity. The grain length was positively correlated with grain circularity. The test weight was not correlated with any of the other parameters. The year of release was negatively correlated with some grain parameters, such as length, circularity and positively correlated with yield.

Table 12. The correlation coefficients between analysed parameters

	Year of release	area	width	length	circularity	TW	TKW	yield
Year of release	1							
area	-0,35	1						
width	0,11	0,52	1					
length	-0,45	0,92	0,14	1				
circularity	-0,44	0,67	-0,27	0,91	1			
TW	0,09	0,04	0,01	0,02	-0,002	1		
TKW	-0,37	0,93	0,67	0,77	0,460	0,20	1	
yield	0,75	-0,19	-0,09	-0,18	-0,111	0,33	-0,27	1

P 5%=0.42.

The bold correlation coefficients are positive significant at P<5%.

The italic correlation coefficients are negative significant at P<5%.

The relation between year of release and some grain characteristics (including TKW and grain length) was negative, but positive with yield (Figures 1, 2 and 3).

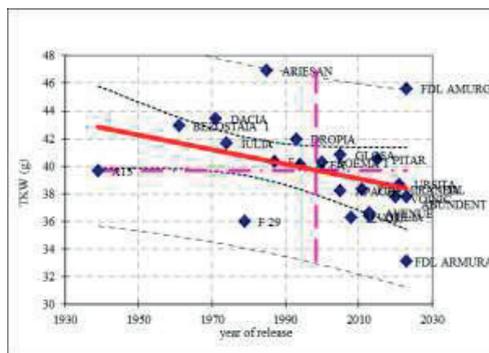


Figure 1. The relationship between year of release and TKW

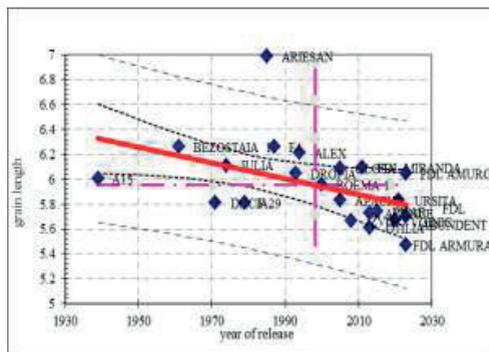


Figure 2. The relationship between year of release and grain length

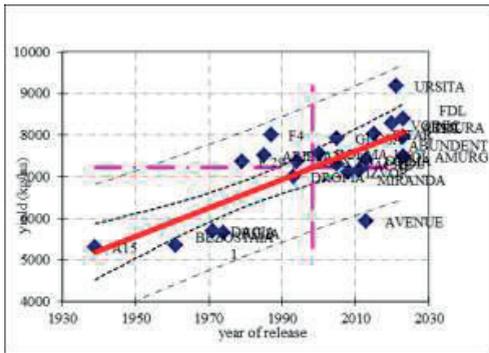


Figure 3. The relationship between year of release and yield

Yield of flour per grain is dependent on grain size and also shape, as this determines the proportion of the grain that is taken up by the endosperm relative to other grain parts. Breeders routinely select new varieties for improvements in grain yield, a component of which is grain size, but have not previously selected specifically for grain shape (<https://europepmc.org>).

Moreover, the knowledge of morphology of wheat grains is important for designing machines for sowing, handling, milling, cleaning, storing, and conveying purposes. This strategy might be helpful for wheat breeders to develop new varieties with better grain features to improve the milling and baking quality of wheat (<https://europepmc.org>).

Grain shape (and size), density, and uniformity are important attributes for determining the market value of wheat grain since they influence the milling performance (i.e., flour quality and yield) (Evers et al., 1990). Theoretical models predict that milling yield could be increased by optimizing grain shape and size with large and spherical grains being the optimum grain morphology (Evers et al., 1990). However, accurate characterization of grain size and shape remains a big challenge due to complex nature of wheat grain shape. (Houle et al., 2010; Patil et al., 2013).

Gegas et al. (2010), revealed that grain size and shape are largely independent traits in both primitive wheat and in modern varieties. Moreover, their results showed a significant reduction of phenotypic variation in grain shape in the modern germplasm pool compared with the ancestral wheat species, probably as a result

of a relatively recent bottleneck. In our study, the varieties (some old, new and breeding lines) were significantly different for all analysed parameters. We noticed that, over time, some grain characteristics decreased, while grain yield increased.

CONCLUSIONS

The selection for grain yield led, over time, to a decrease (reduction) of grain size and weight. However differences between varieties were noted and the ones that have positive deviations from the regression on year of release deserve attention.

To counteract this trend, it is necessary to pay attention in breeding to increasing the grain size and weight (especially due to the positive effect on milling properties), but only if this will not be associated with the correlated negative changes in the other components of yield, leading to a decrease in grain yield.

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