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GENETIC AND ENVIRONMENTAL EFFECTS ON GRAIN SIZE UNIFORMITY IN WINTER WHEAT

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ABSTRACT

Grain size is an important yield component and also influences flour yield, seed vigor and early plantlet development. It is usually described by the weight (or mass) of thousand grains or average of dimensions of a sample, without paying attention to the variability of individual grains. However, variation in individual grain size is economically important for milling industry, because many small grains are lost during the cleaning process prior to milling and a high proportion of small grains is indicative of a poor flour yield. Also, when grains are sown for a new crop, grain mass variability increases the heterogeneity of germination and leads to non-uniform seedling emergence and crop establishment, often leading to decreased yield.

We analyzed using the software *ImageJ* (http://imagej.nih.gov/ij/) individual grain size distributions of samples 80 to 120 individual grains each, harvested from yield trials at NARDI Fundulea Romania with 13 cultivars grown on four management variants, during two years. Individual grain projected area, as a proxy of grain size, ranged from 8.18 to 29.06 mm^2, and for most cultivars minimum values of individual grain projected area were less than half of maximum values. On average over all management variants and years, coefficients of variation (s%) varied from less than 14% in cultivars Bezostaya 1, Glosa and Pajura to more than 15.5% in cultivars Adelina and Litera. ANOVA for the coefficients of variation of grain projection areas showed that the main source of variation was the effect of cultivars, which was significant when tested both against the Error and the interaction Cultivars*Years. The effect of the four studied managements was significant when tested against the Error, but not when tested against the interaction with Years, suggesting that the managements did not have the same effect in the two years of study.

The large differences between cultivars found in our study provide opportunities in breeding for higher grain uniformity.

Keywords: grain homogeneity, variation, grain projection area, cultivars, management.

INTRODUCTION

Wheat grain size is an important determinant of yield, being the most stable yield component. Grain size has also other economic implications, as it has been shown to be correlated with flour yield, seed vigor and early plantlet development.

Grain size is usually described by the weight (or mass) of thousand grains as a bulk mean of individual grain mass at plot or sample scale. Grain dimensions are also generally described as average length, width, projection area of a sample, without paying attention to the variability of individual grains in the analyzed sample.

However, it is well known that grains in the spike are not uniform in size, as grains formed at the top of the spike or in the central florets of the spikelets are smaller. Similarly, grains formed in the late tillers are generally smaller. As Beral et al. (2020) pointed out, this lack of grain size uniformity is economically important, as a high proportion of small grains is penalized commercially by the milling industry, because many small grains are lost during the cleaning process prior to milling. Also, a high proportion of small grains is indicative of a poor flour yield (Sharma and Anderson, 2004; Nuttall et al. 2017). Increased grain homogeneity can also be justified on grounds of nutritional quality

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(Beral et al., 2020). For example, dry gluten content is variable between grains in bulk, mainly because of variation linked to their location in the spike (Boz et al., 2012). Micro- and macro-nutrient content also varies between grains (Calderini and Ortiz-Monasterio, 2003), concentrations generally decreasing from basal to apical spikelets.

As Beral et al. (2020) underlined, in crops such as wheat, when grains are sown for a new crop, grain mass variability increases the heterogeneity of germination and leads to non-uniform seedling emergence and crop establishment, often leading to decreased yield (Finch-Savage and Bassel, 2016). For this reason, greater homogeneity in individual mature grain mass in seed lots has become an important breeding objective (Bradshaw, 2006). If better seed uniformity is achieved by sieving, this is accompanied by a loss in usable seed quantity, with economic consequences.

In contrast, in wild species, the variability of individual mature grain mass is often considered a trait that contributes to wider adaptation. Indeed, a large variability in mature seed mass, which is associated with variability of seed performance - e.g. seed vigor (Matilla et al., 2005), seedling growth (Aparicio et al., 2002; Lafond and Baker, 1986) - allows species to adjust to unpredictable environmental conditions, so enhancing survival.

Based on these observations, Beral et al. (2020) speculated that, in the context of climate change and increasingly unpredictable weather, “a greater variability in individual grain size and thus in seed performance, may favor irregular seedling emergence, which may help buffer the impacts of environmental stress events during establishment”, in agricultural crops too. However, a correlation between higher individual seed size variation and yield stability in agricultural crops remains to be demonstrated.

MATERIAL AND METHODS

The field experiments were conducted at the National Research and Development Institute (NARDI) Fundulea - Romania (44º27'45" N latitude and 26º31'35” E longitude, 68 m above sea level) in 2016/2017 and 2017/2018 seasons. Weather conditions during the experiments are summarized in Table 1. A detailed comparison of the weather conditions in the two years of study with the average of many years is beyond the aims of this paper. Nevertheless, overall the conditions of the 2016/2017 and 2017/2018 seasons were considered representative for the region in the last years.

Table 1. Rainfall and average temperatures during wheat vegetation period

Month	2017		2018	
	average t°C	rainfall	average t°C	rainfall
October	10.3	74.4	11.7	111.6
November	5.7	48.8	6.9	49.2
December	-0.3	0	3.6	27.8
January	-5.5	35.4	0.8	36
February	0	50.5	2.2	58.6
March	8.6	47.6	3.3	40.6
April	10.6	73.6	15.8	2.4
May	16.8	65.8	19.4	34
June	22.2	96.4	22.6	120.6
Total rainfall		492.5		480.8

The experiments were carried out was a cambic chernozem soil, formed on loessoide deposits.

Thirteen winter wheat cultivars were tested with four crop management variants, which included:

- usual recommended practices without and with fungicide foliar treatments;
- no supplementary N fertilization;
- late sowing (about 30 days later than the recommended date).

The analyses of individual grain size distributions were based on measurements on two replications of 80 to 120 individual grains each. Two main metrics were calculated for each cultivar from the data provided by the software *ImageJ* (http://imagej.nih.gov/ij/), namely average of individual grain projected area and grain projected area variance. Coefficients of variation of the grain projected area were calculated as a ratio between the standard deviation and the mean, for each cultivar and each environment.

ANOVA was used to estimate significance of cultivar and environment (years*crop management) effects on the variance of coefficients of variation.

RESULTS AND DISCUSSION

Individual grain projected area ranged from 8.18 to 29.06 mm^2, while cultivar average grain projected area varied from minimums of 10.42-12.41 to maximums of 23.81-26.51 (Table 2). For most cultivars minimum values of individual grain projected area were less than half of maximum values. This illustrates the wide range of variation of grain size.

It is worth noting that differences between cultivars varied more for minimum values (19%) than for maximum values (11%), suggesting that cultivars differed more in the share of small grains than of large grains.

Table 2. Maximum and minimum individual grain projected area of 13 cultivars grown with different crop managements

		2017				2018				
Cultivar	Grain size	Treated	Not treated	N0	Late sowing	Treated	Not treated	N0	Late sowing	Average
Bezostaya 1	max	23.25	26.17	24.85	25.05	24.78	25.17	23.53	23.55	**24.54**
	min	*10.92*	*11.24*	*10.71*	*12.59*	*12.39*	*12.38*	*11.41*	*11.32*	***11.62***
Glosa	max	24.50	23.85	26.15	23.80	23.80	26.49	25.23	24.09	**24.74**
	min	*10.42*	*13.36*	*12.72*	*12.34*	*11.50*	*14.30*	*13.01*	*11.64*	***12.41***
Pajura	max	23.98	25.05	21.64	23.83	23.85	27.15	24.23	20.77	**23.81**
	min	*11.92*	*10.33*	*10.19*	*13.58*	*11.14*	*13.18*	*10.11*	*9.42*	***11.23***
Miranda	max	26.64	25.00	26.75	24.73	24.30	26.96	24.09	23.18	**25.20**
	min	*14.76*	*9.55*	*13.20*	*14.28*	*9.50*	*13.05*	*12.50*	*8.46*	***11.91***
Amurg	max	28.33	25.52	26.99	26.61	26.91	27.32	25.14	25.26	**26.51**
	min	*12.31*	*11.26*	*10.77*	*13.48*	*10.17*	*12.64*	*14.17*	*10.23*	***11.88***
Voinic	max	23.09	27.52	25.30	24.96	25.89	26.27	25.98	21.22	**25.03**
	min	*11.25*	*10.00*	*11.09*	*10.90*	*11.40*	*10.21*	*10.10*	*10.20*	***10.64***
Pitar	max	24.98	26.67	24.22	26.95	23.20	26.76	24.17	22.41	**24.92**
	min	*12.83*	*12.60*	*10.06*	*11.66*	*10.24*	*11.95*	*10.53*	*9.59*	***11.18***
Boema	max	24.93	23.46	24.94	25.81	24.20	25.62	23.32	22.09	**24.29**
	min	*10.81*	*10.67*	*12.42*	*11.75*	*11.50*	*12.51*	*8.63*	*8.50*	***10.85***
Izvor	max	25.98	23.33	27.55	27.90	25.40	26.94	22.90	21.86	**25.23**
	min	*12.39*	*10.17*	*11.05*	*11.70*	*12.10*	*12.19*	*10.03*	*9.49*	***11.12***
Otilia	max	25.37	23.14	23.59	23.47	24.50	27.60	23.66	22.09	**24.18**
	min	*12.94*	*10.22*	*11.61*	*11.72*	*12.10*	*13.80*	*9.17*	*8.18*	***11.22***
Ursita	max	25.06	24.94	23.55	25.58	24.02	25.48	23.74	21.14	**24.19**
	min	*11.81*	*11.73*	*10.51*	*11.62*	*11.61*	*12.13*	*9.66*	*8.85*	***10.99***
Adelina	max	26.12	27.17	28.53	24.06	23.89	28.37	24.16	20.66	**25.37**
	min	*10.55*	*10.09*	*11.96*	*10.97*	*9.75*	*12.31*	*9.65*	*10.29*	***10.70***
Litera	max	25.87	24.44	25.84	23.70	25.10	29.06	25.20	23.18	**25.30**
	min	*12.16*	*9.96*	*11.57*	*9.93*	*10.30*	*9.94*	*10.91*	*8.63*	***10.42***
Crop managements	max	**28.33**	**27.52**	**28.53**	**27.90**	**26.91**	**29.06**	**25.98**	**25.26**	**26.51**
	min	***10.42***	***9.55***	***10.06***	***9.93***	***9.50***	***9.94***	***8.63***	***8.18***	***10.42***

Smaller differences in size were observed between managements for minimum grain size, ranging from 8.18 mm^2 to 10.42 mm^2, while the maximum grain sizes varied less (25.26 to 29.06)

The large variability of grain size for each analyzed sample from a cultivar grown with a crop management variant can be also observed from frequency distributions. In Figure 1 we present four examples (two cultivars - Litera and Bezostaya 1, with two variants of crop management - usual recommended practices without fungicide foliar treatment and late sowing). The histograms illustrate the difference between the two cultivars and to less extent between the two crop managements, showing that grains of Bezostaya 1 are clearly more uniform.

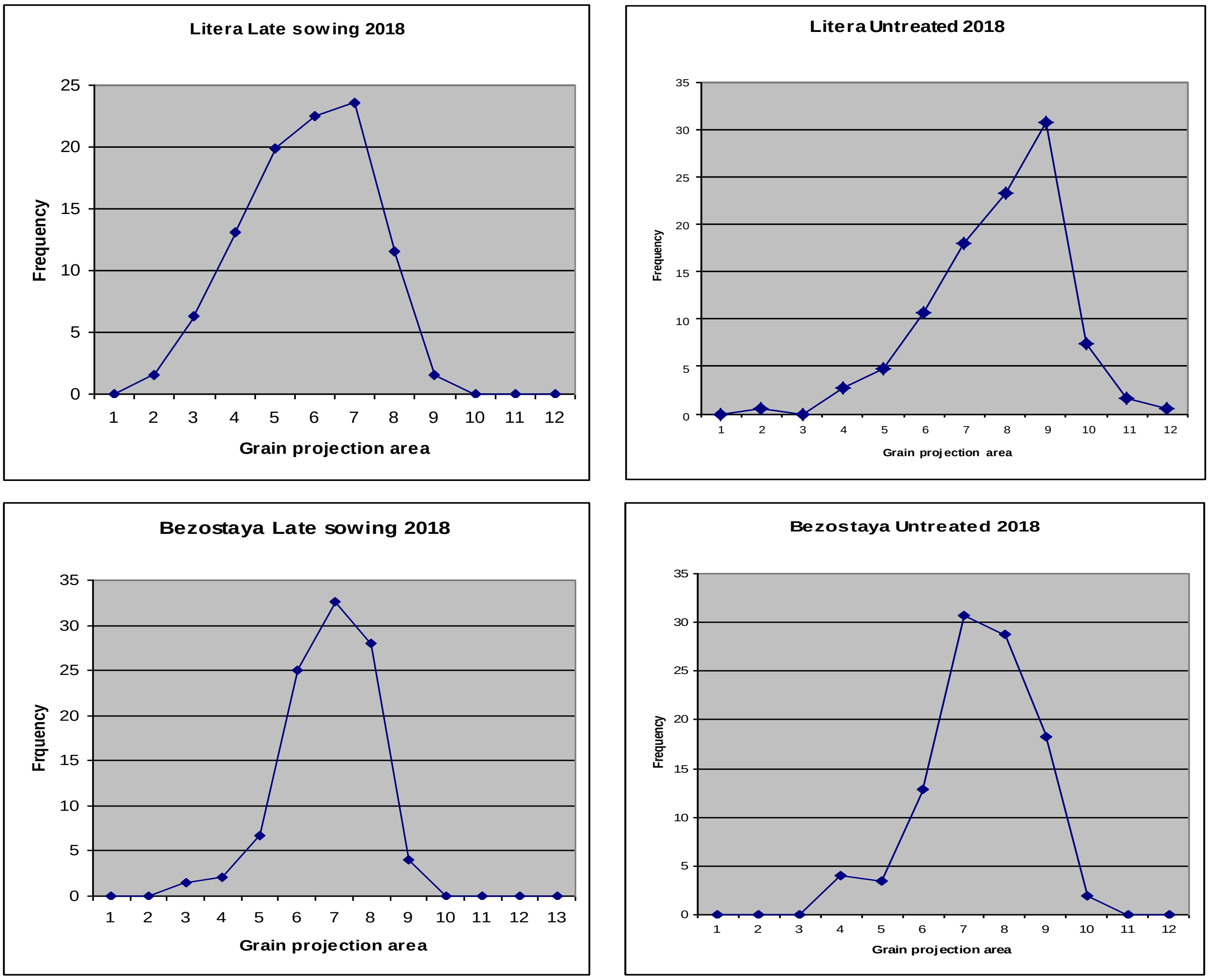

Figure 1. Frequency distribution of grain projection area in two cultivars grown under two variants of crop management

Better description of grain projection area variation, respectively uniformity, is provided by the coefficients of variation (Table 3). On average over all management variants and years, coefficients of variation varied from less than 14% in cultivars Bezostaya 1, Glosa and Pajura to more than 15.5% in cultivars Adelina and Litera. Average differences between coefficients of variation among the four studied crop management variants were less obvious, from 13.85% for the usual management without fungicide treatment in 2018, to 15.5% for late sowing also in 2018.

Table 3. Coefficients of variation for grain projection areas in 13 winter wheat cultivars grown with four managements during two years

	2017				2018				
Cultivar	Treated	Not treated	N0	Late sowing	Treated	Not treated	N0	Late sowing	Average
Bezostaya 1	14.29	13.53	11.87	13.87	11.74	12.96	14.29	12.19	**13.09**
Glosa	14.41	14.82	12.60	12.79	13.96	11.29	12.13	14.14	**13.27**
Pajura	12.91	13.98	13.83	11.85	13.48	12.55	13.97	14.76	**13.42**
Miranda	13.67	14.32	14.57	13.12	15.38	11.57	12.69	17.62	**14.12**
Amurg	14.52	13.55	16.61	13.93	14.25	13.66	13.16	14.38	**14.26**
Voinic	14.35	14.84	14.31	13.47	15.67	13.93	13.47	14.72	**14.35**
Pitar	13.07	14.99	16.08	14.73	14.39	14.54	14.49	14.77	**14.63**
Boema	14.22	12.96	14.09	15.78	13.73	14.70	16.10	16.79	**14.80**
Izvor	13.71	14.31	16.14	15.77	15.45	16.04	14.81	14.52	**15.09**
Otilia	14.09	14.37	15.13	14.62	15.09	15.33	15.59	17.65	**15.23**
Ursita	15.69	13.77	17.10	16.79	14.49	14.17	14.16	16.16	**15.29**
Adelina	14.15	16.85	14.91	14.33	16.10	14.63	17.37	16.24	**15.57**
Litera	14.91	14.93	17.89	14.51	15.86	14.65	15.37	18.23	**15.79**
Average	14.15	14.40	15.01	14.27	14.58	13.85	14.43	15.55	**14.53**

ANOVA for the coefficients of variation (s%) of grain projection areas shows that the main source of variation was the effect of cultivars, which was significant when tested both against the Error and the interaction Cultivars*Years (Table 4).

The effect of the four managements studied was significant when tested against the Error, but not when tested against the interaction with Years. This suggests that the managements did not have the same effect in the two years of study.

The only significant interaction, both when tested against Error and against the triple interaction, was the one involving Management*Years, which suggests that the effect of the tested managements were much influenced by the weather conditions of the two testing years.

Table 4. ANOVA for the coefficients of variation of grain projection areas

Source of variation	SS	df	MS	F tested against Error	F tested against IA with Years
Cultivars	149.37	12	12.44	7.39***	5.08**
Management	19.38	3	6.46	3.83*	0.62^{ns}
M*Y	30.96	3	10.32	6.12**	3,23*
C*M	61.12	36	1.69	1.00^{ns}	$0,52^{ns}$
C*Y	29.39	12	2.45	1.45^{ns}	$0,76^{ns}$
C*M*Y	114.84	36	3.19	1.89^{ns}	1.00
Error	175.16	104	1.68	1.00	
Total	581.39	207			

Beral et al. (2020) demonstrated that individual grain size variation is subjected to both genetic and environmental control. In our study the effect of genetic differences between cultivars was more important, but this could be partly due to relatively limited diversity of the tested environments.

Highest average grain size uniformity was found in cultivar Bezostaya 1. This was not unexpected, since this cultivar was the only one among the cultivars included in this study, which did not carry the GA insensitive dwarfing alleles *Rht-B1,* known for its pleiotropic effect on increasing tillering and spikelet fertility (Allan, 1986; Börner et al., 1993). However, we also found important differences in grain size uniformity among the semidwarf cultivars, suggesting that large possibilities of breeding for grain uniformity also exist in modern short-stem cultivars. Beral et al. (2020) found that Grain Size Variance heritability ranged between 0.52 and 0.85, making selection for grain uniformity a relatively efficient task.

Tashiro and Wardlaw (1990) observed that single grains exhibit different responses to post-anthesis temperature stress, depending on their position on the spike or within a spikelet. Therefore, higher grain size uniformity under post-flowering stress might be associated with increased tolerance to abiotic stress (Beral et al., 2020).

CONCLUSIONS

Often neglected, grain size uniformity within samples deserves attention for reducing losses during the cleaning process prior to milling or during the seed processing, for increasing flour yield and for ensuring more uniform seedling emergence and crop establishment,

Although, the smallest average coefficient of variation of the grain area projection was found in a taller non semidwarf cultivar, differences detected among the studied modern semidwarf cultivars offer interesting possibilities of genetic progress in wheat breeding programs.

REFERENCES

Allan, R.E., 1986. *Agronomic comparisons among wheat lines nearly isogenic for three reduced - height genes*. Crop Sci., 26(4): 707-710.

Aparicio, N., Villegas, D., Araus, J.L., Blanco, R., Royo, C., 2002. *Seedling development and biomass as affected by seed size and morphology in durum wheat*. J. Agric. Sci., 139: 143-150. https://doi.org/10.1017/ S0021859602002411

Beral, A., Rincent, R., Le Gouis, J., Girousse, C., Allard, V., 2020. *Wheat individual grain-size variance originates from crop development and from specific genetic determinism*. PLoSONE 15(3): e0230689. https://doi.org/10.1371/journal. pone.0230689

Börner, A., Worland, A.J., Plaschke, J., Schumann, E., Law, C.N., 1993. *Pleiotropic effects of genes for reduced height (Rht) and day-length insensitivity (Ppd) on yield and its components for wheat grown in Middle Europe*. Plant Breeding, 11(3): 204-216.

Boz, H., Gerçekaslan, K.E., Karaoğlu, M.M., Kotancilar, H.G., 2012. *Differences in some physical and chemical properties of wheat grains from different parts within the spike*. Turk. J. Agric. For., 36: 309-316. doi:10.3906/tar-1102-41

Bradshaw, A.D., 2006. *Unravelling phenotypic plasticity - why should we bother?* New Phytologist, 170: 644-648. https://doi.org/10.1111/j.1469-8137.2006.01761.x

Calderini, D.F., and Ortiz-Monasterio, I., 2003. *Grain position affects grain macronutrient and micronutrient concentrations in wheat*. Crop Science, 43: 141-151. https://doi.org/10.2135/cropsci2003.1410

Finch-Savage, W.E., and Bassel, G.W., 2016. *Seed vigour and crop establishment: extending performance beyond adaptation*. EXBOTJ, 67: 567-591. https://doi.org/10.1093/jxb/erv490

Lafond, G.P., and Baker, R.J., 1986. *Effects of genotype and seed size on speed of emergence and seedling vigor in nine spring wheat cultivars*. Crop Science, 26: 341-346. https://doi.org/10.2135/ cropsci1986.0011183X002600020027x

Matilla, A., Gallardo, M., Puga-Hermida, M.I., 2005. *Structural physiological and molecular aspects of heterogeneity in seeds: a review*. Seed Science Research, 15: 63-76. https://doi.org/10.1079/SSR2005203

Nuttall, J.G., O'Leary, G.J., Panozzo, J.F., Walker, C.K., Barlow, KM., Fitzgerald, G.J., 2017. *Models of grain quality in wheat - A review*. Field Crops Research, 202: 136-145. https://doi.org/10.1016/j.fcr.2015.12.011

Sharma, D.L., and Anderson, W.K., 2004. *Small grain screenings in wheat: interactions of cultivars with season. Site and management practices*. Aust. J. Agric. Res., 55: 797. https://doi.org/10.1071/AR03265

Tashiro, T., and Wardlaw, I.F., 1990. *The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat*. Australian Journal of Plant Physiology, 17(5): 551-561.

***http://imagej.nih.gov/ij/. Java image processing program, National Institute of Mental Health, Bethesda, Maryland, USA (last accessed 14.04.2016).