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A breeding concept to improve the performance of locally cultivated bread wheat (*Triticum aestivum* L.) cultivars

Vasileios Greveniotis[1], †Stylianos Zotis[2], Evangelia Sioki[3], Constantinos Ipsilandis[2]*

[1]Department of Agricultural Technology, Technological and Educational Institution of Thessaly, Larissa, Greece
[2]Department of Agricultural Technology, Technological and Educational Institution of Western Macedonia, Florina, Greece
[3]Cotton Centre, Hellenic Agricultural Organization – DEMETER, Karditsa, Greece
**Corresponding author: vgreveni@mail.com, vgreveni@teilar.gr*

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Abstract: The objective of this study was to explore the internal variability in six established F7 commercial wheat cultivars for breeding purposes. They are sown traditionally in the region of Western Macedonia, Greece. Spikes of the six cultivars were sown in 2008 in separate rows. A head to row selection scheme was applied for two growing periods in order to select lines within the cultivars, based on various traits such as: the total spike number, the spike weight per row, the 1000-kernel weight and the specific weight . The final selection was based on the specific weight and the four best rows from each cultivar were selected. All selected lines were tested in a field trial with a randomised complete block design (RCB). The original seed of the cultivars were used as controls. Statistically significant differences were found for all the studied traits. The line selections differed from the original cultivars, sometimes highly significantly. In conclusion, commercial cultivars that are sown traditionally for many years may contain exploitable variability, which reveals, that the continuous selection within cultivars is necessary to avoid cultivar deterioration and to improve the yield and other traits. The results indicate a degeneration of grain yield from 8% to 20%. Although eye-selection restricts off-types, our results mainly indicate new variability and cultivar performance deterioration under extreme biotic and abiotic stress.

Keywords: continuous selection; inbred lines; internal variability; stress tolerance

Commercial wheat cultivars must incorporate a high yield potential and stability across various environments in order to be successful (Stratilakis & Goulas 2003). Monogenotypic cultivars are considered homogeneous without any noticeable variation (Yates *et al*. 2012). Thus, research on a continuous cultivar selection is limited under the belief of cultivar uniformity. For inbreeders like wheat (*Triticum aestivum* L.), the exploitation of an additive genetic variation together with the removal of deleterious genes, is the predominant step for the commercial cultivar development in a breeding programme (Fasoulas 1988; Rasmusson & Philips 1997; Duvick *et al*. 2004). Also, inbred cultivars are easily maintained by farmers, by keeping part of the harvest seed for the next season (Friis-Hansen 1996), but this may result in off-types and a reduced field yield (Khan *et al.* 2007; El-Kalla *et al.* 2010). Searching for such a newly developed variation, Fasoula and Boerma (2007) showed that intracultivar variation was present in soybeans. McClintock (1984) stated that the plant genome is dynamic and can be self-modified under different environmental conditions exhibiting adaptability under extreme conditions. In order to

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face environmental challenges, plants employ various mechanisms of genome reconstruction like mutations of certain loci, crossing over, inversions, silencing of genes, etc, thus, developing new genetic combinations (Cullis 1990). Parlevliet (2007) summarised the contaminating and degrading forces that act within cultivars. The continuous rearrangement of the plant genome indirectly imposes restrictions, or even principles on the natural selection expression. As a consequence, Fasoula and Fasoula (2000) proposed the non-stop selection of genetic materials, as a constant improvement of a crop's yield and the quality of the released cultivars. A continuous selection seems to be necessary for eliminating random and deleterious mutations and exploiting new favourable variations (genetic combinations), either genetic or epigenetic (Fasoulas 1993). Epigenetic variation is heritable (through meiosis or mitosis) and results as a response to the environment pressure (Riggs & Porter 1996).

Selection under very low density was used to reveal the within the cultivar variation in several crops, such as the grain yield of bread wheat (*Triticum aestivum* L.) under varying competitive conditions (Fasoula 1990), in snap beans (*Phaseolus vulgaris* L.) (Traka-Mavrona *et al.* 2000) and cotton (*Gossypium hirsutum* L.) for the yield and tolerance to wilt caused by *Verticillium dahliae* Kleb. (Fasoulas 1988). In bread wheat and barley (*Hordeum vulgare* L.) under salinity stress, Ipsilandis *et al.* (2011) found internal variability in the commercial cultivars for drought tolerance as a result of the flexibility of the genomes of the two species, but the genetic mechanism for such a behaviour was undefined.

Useful gene pools and an effective breeding methodology are the main factors for successful wheat cultivar development programmes. Local landraces and mixed cultivars and/or segregating populations following hybridisation are the main gene pools (Agorastos & Goulas 2005; Poehlman & Sleper 2006). Modern breeding programmes use different gene pools to exploit genetic variation. This may ensure adaptation and stability under various environmental conditions (Greveniotis & Fasoula 2016). Local traditional farmers exploit the possible variability and adaptation of old cultivars and local landraces because of their good potential to cope with biotic and abiotic stresses (Belluci *et al.* 2013; Lopes *et al.* 2015; Dwivedi *et al.* 2016). After all, the incorporation of tolerance to biotic and abiotic stresses is a primary target of modern breeders (Duvick 2005).

The objective of this study was to explore the internal variability for breeding purposes in six established F7 commercial wheat cultivars sown traditionally in the region of Western Macedonia, Florina, Greece. Florina has extreme environmental conditions and biotic and abiotic pressures negatively affect the yield. These six cultivars have been cultivated in the same remote and isolated fields for almost 20 years and our effort was to improve their performance locally, in the specific environmental conditions, by establishing a specific pure-line breeding programme based on a head to row evaluation.

MATERIAL AND METHODS

As the initial material, 200 individual plants of each of the six different F7 commercial cultivars (A: Generoso, B: Vergina, C: Vitsi, D: Irnerio, E: Yecora, F: Nestos) were selected by eye-judgment (visual selection) in each of the six remote and isolated bread wheat farms, in the year 2008. These cultivars have been sown traditionally for almost 20 years in those fields, without buying any more certified seed (it was purchased once 20 years ago), but by only keeping the harvest seed, in the region of Western Macedonia, Greece.

During the period 2008–2009, the spikes of these 200 × 6 plants were sown in separate rows. These lines entered a pure-line selection programme conducted in the farm of the Technological Education Institute of Western Macedonia (in Florina, Greece, 40°46'N, 21°22'E, 705 m a.s.l., soil type SL, Sandy Loam: sand 61.2%, silt 27.6%, clay 11.2%, pH 6.25), based on a head to row evaluation (Poehlman & Sleper 2006). The total spike number per row, spike weight per row, number of kernels and the 1000-kernel weight (TKW in g) were measured. Selection was based on the mean spike weight (in g), calculated by the division of the spike weight by the total spike number, for each row and, thus, the 50 best rows of each cultivar were selected.

During the period 2009–2010, the seeds from the 50 best rows for each cultivar were sown in separate rows (300 rows in total). The spike weight per row, the 1000-kernel weight (in g) and the specific weight (bulk density in g/l) were measured. The selection was based on the specific weight (bulk density) and the four best rows from each cultivar were selected (based on Yabwalo *et al.* 2018; Greveniotis *et al.* 2019).

The final evaluation was conducted in two periods: 2010–2011 and 2011–2012. The four best rows (1 to 4)

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from each cultivar together with the original cultivar as a check (30 genetic materials in total), were sown in a randomised complete block design (RCB), with three replications. Each plot consisted of 7 rows, 6 m long and with 25 cm line interval (350 plants/m^2). The grain yield (GY in kg/ha), the 1000-kernel weight (TKW in g) and the specific weight (bulk density in g/l) of each plot were measured. An ANOVA was performed for each year separately and in total as well (year as additional factor). The genetic materials were considered as a fixed factor. The analyses were based on Steel and Torrie (1980) and the means were separated according to Duncan's method. The total sum of squares was used to estimate the contribution of the two factors (the genetic materials and year) based on the expected mean squares of the model (McIntosh 1983). Finally, the genotypic variability coefficient (GCV), phenotypic variability coefficient (PCV), repeatability (R^2) and broad sense heritability (H^2) were calculated according to Johnson *et al.* (1955), Akcura (2009) and Kaya and Akcura (2014). The coefficient of variation (CV%) was also computed.

RESULTS AND DISCUSSION

The farmers' experience in many Greek areas, lead to the choice of certain wheat cultivars and to the subsequent cultivation of this small group of cultivars for many years by seed keeping. Although commercially released cultivars are considered homogeneous, exploitable genetic variation among the single plants within each cultivar exists and the mechanisms that generate variation are present (Fasoula 1990; Haun *et al.* 2011), especially under the biotic and abiotic pressures of extreme environments (Dwivedi *et al.* 2016). Fasoula (1990) applied a divergent selection for the yield in the bread wheat cultivar Siete Cerros, based on the honeycomb designs (Fasoulas 1988) and, as a result, the lines developed from the selected plants showed 8% higher and 9% lower yield in the RCB trials. Also, Gonzalez *et al.* (2011) concluded that within elite wheat germplasm, which could be used directly in breeding programmes, there is variation in the developmental dynamics of florets with a possible impact on the yield. Furthermore, degradation of the cultivars is usually due to the increased off-types reaching 4% after two cultivating periods, as reported by El-Kalla *et al.* (2010), and 7% from the early stage outcrossing as reported by Gains *et al.* (2007).

Table 1. The factor analyses (ANOVA) for each trait measurement: the grain yield (GY), specific weight (bulk density) and the 1000-kernel weight (TKW) including the coefficient of variation (CV)

Effects	GY	Bulk density	TKW
Environment (E)	ns	ns	ns
Genotypes (G)	***	***	***
G × E	ns	ns	ns
CV (%)	11.94	2.83	2.27

***Significant at a 0.001 probability level; ns – not significant

From our dataset, it is apparent that the variation present is due to the genetic differences and the statistical level of confidence is very high (Table 1, ANOVA for each trait measurement). A combination of data in Tables 1 and 2 (that presents the averages of the sum of squares treatment partitioning (%) for the genotype (G), the environment (E) and the G × E interaction for each trait measurement) and Table 3 (that presents the means for each trait measurement of the selected genetic materials across the two years) revealed that the genetic differences are the main source of variation even within each cultivar. In Table 2, the genotypes contribute 41–86% of the total variability and in Table 3, many selected lines yielded more than the original cultivar by 12% for the GY in cultivar A, 9% in cultivar B, 18% in cultivar C, 8% in cultivar D, 17% in cultivar E and 20% in cultivar F. Also, under the bulk density, the selected lines yielded more than the original cultivar by 0% in cultivar A, 4% in cultivar B, 9% in cultivar C, 2% in cultivar D, 3% in cultivar E and 5% in cultivar F. Finally, for the TKW, the selected lines yielded more than the original cultivar by 2% in cultivar A, 6% in cultivar C, 1% in cultivar D, 5% in cultivar E and 4% in cultivar F, with the exception being cultivar B. Cultivars B and D seems to be more stable and without significant differences with their selections,

Table 2. The averages of the sum of the squares treatment partitioning (%) for the genotype (G), the environment (E) and the interaction (G × E) for each trait measurement: the grain yield (GY), the 1000-kernel weight (TKW) and the specific weight (bulk density)

Factors/traits	GY	Bulk density	TKW
Environment (E)	1	1	3
Genotypes (G)	41	86	52
G × E	1	1	7

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while C and F exhibited the greatest differences, indicating the differences in the breeding methods, the quality of the seed production (Fehr 1987) and the farmers' practices (in sowing). Xiao and He (2003) reported that most of the genes affecting the TKW have additive effects and, thus, selection for TKW in the early generations of breeding is highly effective, but this was not reflected in our findings where the differences between the selected lines were relatively low, maybe because of the stability of these commercial cultivars. Also, for the TKW trait in the wheat breeding, the numbers of favourable alleles in the modern cultivars indicate that there is still considerable genetic potential (variability) for use in genome selection (Wang *et al.* 2012) and, thus, this trait needs more investigation. These positive results were realised although the presence of various insects was apparent and the possible damages were described previously in the same region (Deligeorgidis *et al.* 2012). They also referred to the fact that the yield losses are usually about 5% or more.

Table 3. The means for each trait measurement: the grain yield (GY), the 1000-kernel weight (TKW) and the specific weight (bulk density), of the six cultivars (A, B, C, D, E, F) and their four selections (1, 2, 3, 4) forming 30 genotypes, across the two experimental years

Genotypes	GY (kg/ha)	Bulk density (g/l)	TKW (g)
A1	4200^{cde}	741.8^{bcde}	39.77^{ghi}
A2	5130^{abc}	771.2^{abc}	42.18^{abc}
A3	4670^{abcde}	774.2^{ab}	42.15^{abcd}
A4	4660^{abcde}	776.3^{ab}	41.72^{abcdef}
Original A (check)	4600^{abcde}	775.7^{ab}	$41.33^{abcdefgh}$
B1	4710^{abcde}	646.5^{fg}	$40.43^{bcdefghi}$
B2	4990^{abcd}	638.2^{fg}	39.65^{hi}
B3	3700^{e}	630.2^{g}	39.43^{i}
B4	4550^{abcde}	674.7^{f}	$40.33^{cdefghi}$
Original B (check)	4570^{abcde}	649.5^{fg}	$41.48^{abcdefg}$
C1	4360^{bcde}	774.2^{ab}	$40.67^{abcdefghi}$
C2	4390^{bcde}	784.5^{a}	41.88^{abcde}
C3	4560^{abcde}	767.3^{abcd}	40.00^{fghi}
C4	4610^{abcde}	791.2^{a}	$40.90^{abcdefghi}$
Original C (check)	3920^{de}	728.0^{de}	39.65^{hi}
D1	4550^{abcde}	776.8^{ab}	42.50^{a}
D2	4110^{cde}	760.3^{abcd}	$40.92^{abcdefghi}$
D3	4850^{abcd}	784.0^{a}	41.80^{abcdef}
D4	4760^{abcde}	780.5^{ab}	42.30^{ab}
Original D (check)	4510^{abcde}	764.8^{abcd}	42.08^{abcd}
E1	4710^{abcde}	781.3^{ab}	$41.02^{abcdefghi}$
E2	4740^{abcde}	785.8^{a}	42.30^{ab}
E3	4640^{abcde}	784.5^{a}	41.70^{abcdef}
E4	4710^{abcde}	778.7^{ab}	$41.53^{abcdefg}$
Original E (check)	4070^{cde}	761.7^{abcd}	40.28^{defghi}
F1	4850^{abcd}	731.5^{cde}	41.63^{abcdef}
F2	5540^{a}	751.5^{abcde}	41.65^{abcdef}
F3	5350^{ab}	733.0^{cde}	41.85^{abcdef}
F4	5090^{abc}	752.3^{abcde}	41.82^{abcdef}
Original F (check)	4610^{abcde}	713.5^{e}	40.18^{efghi}

The means followed by different letter(s) within the same column are significantly different according to Duncan's test ($P \leq 0.05$)

Regarding the CV (%) in Table 1, it was found to be high for the grain yield, but relative very low for the bulk density and the 1000-kernel weight, indicating the relative genetic heterogeneity of the cultivars for this trait (Fasoulas 1988), since the environmental effects are very low. Also, Francis and Kenneberg (1978) consider that low CV % values indicate the stability of the cultivars. Under these considerations, Fasoula and Fasoula (2000) stated that the cultivar uniformity seems to be indispensable for high yields because of the lack of the unfavourable effects of genetic heterogeneity and the unequal sharing of the resources. Uniform cultivars exhibit reduced competition resulting in maximum plant yield per area. In our paper, cultivars B and D showed two of the greatest grain yield values, together with cultivar A which a exhibited similar behaviour and cultivar F which exhibited a rather unstable performance. The latest may indicate cultivar heterogeneity on one hand and a kind of narrow population buffering that boosts the yield components on the other hand (Fasoula & Fasoula 1997). This narrow population formation may be a result of the farmers' practices that keep part of the wheat seed after harvesting to be used in sowing for the next year's cultivation.

Table 4 presents the means of the selected genetic materials for the two years separately. For the bulk density, the differences between the selections were greater for the year 2012. The A1 progenies exhibited lower performance for the bulk density in both years (749 and 734.7 g/l). D2 showed also a similar behaviour, but, in 2011, the difference was not statistically significant. In the F materials, there were the greatest statistical differences. For the TKW, the differences were slightly greater in the year 2011. It seems that the year effect may reveal cultivar het-

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erogeneity for the different traits and the multiyear evaluation is a more reliable procedure for detecting cultivar uniformity.

Table 5 presents the genotypic variability coefficient (GCV), the phenotypic variability coefficient (PCV), the repeatability (R^2), the broad sense heritability (H^2) and the experimental CV (%) for the two years of the experimentation. The bulk density showed a PCV of 6.23 to 6.89, which is lower than the grain yields (from 8.48 to 8.62) and higher than the TKW (from 1.96 to 2.75). The GCV for the bulk density is the greatest portion of the total PCV and, as a result, the broad sense heritability is very high (around 94%) and the repeatability is also high (near 90%). For the grain yield in the bread wheat, Fellahi *et al*. (2013) found a high PCV of 28.45 and a

Table 4. The means for each trait measurement: the 1000-kernel weight (TKW) and the specific weight (bulk density), of the six cultivars (A, B, C, D, E, F) and their four selections (1, 2, 3, 4), for the two years separately (2011, 2012)

Genotypes	Bulk density (g/l)		TKW (g)	
	2011	2012	2011	2012
A1	749.0^{abcde}	734.7^{cdef}	39.83^{fghi}	39.70^{ef}
A2	773.7^{ab}	768.7^{abc}	42.70^{ab}	41.67^{abcd}
A3	771.3^{abc}	777.0^{ab}	42.57^{ab}	41.73^{abc}
A4	784.3^{a}	768.3^{abc}	42.20^{abcd}	41.23^{abcde}
Original A (check)	781.3^{a}	770.0^{abc}	41.63^{abcdef}	41.03^{abcdef}
B1	645.3^{fg}	647.7^{gh}	39.70^{ghi}	41.17^{abcde}
B2	634.7^{fg}	641.7^{h}	39.33^{hi}	39.97^{cdef}
B3	628.7^{g}	631.7^{h}	39.03^{i}	39.83^{def}
B4	671.7^{f}	677.7^{g}	39.87^{fghi}	40.80^{abcdef}
Original B (check)	648.0^{fg}	651.0^{gh}	40.95^{bcdefgh}	42.00^{ab}
C1	780.3^{a}	768.0^{abcd}	41.00^{bcdefgh}	40.33^{bcdef}
C2	788.3^{a}	780.7^{ab}	42.17^{abcd}	41.60^{abcd}
C3	774.0^{a}	760.7^{abcde}	40.20^{efghi}	39.80^{def}
C4	793.7^{a}	788.7^{a}	41.17^{abcdefg}	40.63^{abcdef}
Original C (check)	727.0^{de}	729.0^{ef}	40.03^{fghi}	39.27^{f}
D1	780.7^{a}	773.0^{ab}	42.77^{ab}	42.23^{a}
D2	776.3^{ab}	744.3^{bcdef}	41.33^{abcdefg}	40.50^{abcdef}
D3	793.0^{a}	775.0^{ab}	42.03^{abcde}	41.57^{abcde}
D4	783. ^{a}	777.7^{ab}	42.83^{ab}	41.77^{abc}
Original D (check)	776.7^{ab}	752.8^{abcde}	42.17^{abcd}	42.00^{ab}
E1	790.3^{a}	772.3^{ab}	41.47^{abcdefg}	40.57^{abcdef}
E2	791.0^{a}	780.7^{ab}	42.97^{a}	41.63^{abcd}
E3	787.0^{a}	782.0^{ab}	42.17^{abcd}	41.23^{abcde}
E4	782.3^{a}	775.0^{ab}	41.97^{abcde}	41.10^{abcdef}
Original E (check)	769.7^{abcd}	753.7^{abcde}	40.67^{cdefghi}	39.90^{cdef}
F1	728.7^{cde}	734.3^{cdef}	41.93^{abcde}	41.33^{abcde}
F2	756.0^{abcde}	747.0^{bcde}	42.00^{abcde}	41.30^{abcde}
F3	734.7^{bcde}	731.3^{def}	42.37^{abc}	41.33^{abcde}
F4	752.7^{abcde}	752.0^{abcde}	42.17^{abcd}	41.47^{abcde}
Original F (check)	717.0^{e}	710.0^{f}	40.37^{defghi}	40.00^{cdef}
Column *F*-test	***	***	***	**
CV (%)	3.07	2.57	2.26	2.28

**, ***significant at a 0.01 and 0.001 probability level; the means followed by the different letter(s) within the same column are significantly different according to Duncan's test ($P \leq 0.05$)

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Table 5. The genotypic variability coefficient (GCV), the phenotypic variability coefficient (PCV), the repeatability (R^2) and the broad sense heritability (H^2) for the two years of the experimentation, for each trait measurement: the grain yield (GY), the 1000-kernel weight (TKW) and the specific weight (bulk density), of the six cultivars, for the two years separately (2011, 2012)

	GCV		PCV		H^2		R^2	
	2011	2012	2011	2012	2011	2012	2011	2012
Grain yield	3.94	5.99	8.48	8.62	21.55	48.48	41	51
Bulk denstity	6.66	6.05	6.89	6.23	93.39	94.34	89	90
TKW	2.42	1.45	2.75	1.96	22.50	45.33	70	53

GCV of 11.97% and an H^2 of 17.69. Also, Singh and Upadhyay (2013) reported a high PCV of 27.2 and a GCV of 26.54% for the grain yield, with high heritability because of the large proportion of GCV. The highest values in the bread wheat have been reported by Degewione *et al*. (2013) and the yield PCV was found to be 41.57, while the GCV was 38.25% with high heritability also. In the durum wheat *T. durum* L., Akcura (2009) reported a GCV of 7.27 and a PCV of 13.3% with a relative low heritability of 31.1. In the bread wheat, Kaya and Akcura (2014) found the heritability to be 33% for the grain yield and 32% for the TKW. For the TKW in the bread wheat, Moghaddam *et al*. (1997) reported a PCV of 18.7 and a GCV of 17.7% and, thus, the heritability reached 90%. Singh and Ceccareli (1996) depicted that very high heritability (over 80%), indicates the easier and effective selection for the specific character. A high PCV accompanied by a high GCV and, thus, the heritability of a certain trait, indicates that the selection for this trait may be effective since the genotype is better expressed through the phenotype (Singh *et al*. 1994).

Various studies have shown the plasticity (flexibility) of the genome (Cullis 1990; Rasmusson & Philips 1997; Brunner *et al*. 2005; Lolle *et al*. 2005, Fasoula & Boerma 2007; Ipsilandis *et al*. 2011). Under this consideration, Fasoula and Fasoula (2000) analysed the concept of nonstop selection as a very important procedure of extensive testing, concluding that the breeder will gain fundamental knowledge on some important issues: whether the selection for agronomic traits within advanced generations of selfing is feasible, whether a heritable variation is constantly being created and also, whether cultivars deteriorate with time because they are not constantly being improved. Trait flexibility and plasticity in the phenotypic expression, such as the grain number in the wheat, leads to greater heritability and subsequently to the greater relation with the final goal which is the total yield (Sandras & Slafer 2012).

The results indicate a degeneration in the grain yield from 8 to 20% according to the cultivar. This is a record of almost 20 years, while El-Kalla *et al*. (2010) reported 4% after two cultivating periods. Although the eye-selection restricted off-types, these results mainly indicate the newly-developed variability and less, off-type deterioration.

Concluding, commercial cultivars that are sown traditionally for many years may contain (or developed) exploitable internal variability which depict that a selection within the cultivars may be effective for improving the total yield and other quantitative or qualitative traits. Many of these cultivars may not be protected any more. The concept of continuous selection seems to be a tool for improving the cultivars and overcoming problems of deterioration, useful for the official seed foundations that may need to preserve the productivity. Also, incorporation of tolerance to the biotic and abiotic stresses may be valuable for the preservation of the yield's performance, especially under common insect infections that may reduce productivity. Cultivar uniformity seems to be indispensable for high yields because of the lack of the unfavourable effects of the genetic heterogeneity and the unequal sharing of the resources. Uniform cultivars exhibit reduced competition resulting in the maximum plant yield per area. In our paper, cultivars B and D showed two of the greatest grain yield values, together with cultivar A. Cultivar F exhibited a rather unstable performance. The latest may indicate cultivar heterogeneity on one hand and buffering that boosts yield components on the other hand. This behaviour may be a result of the farmers' practices that keep part of the wheat seed after harvesting to be used in sowing for the next period of cultivation.

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