

# The impact of genotype, environment, and genotype × environment interaction on wheat grain yield and quality

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## Abstract

**Objects and purpose:** In a multifactor field experiment, common winter wheat cultivars developed in Bulgaria were studied. The main factors site (location), season (year) and variety (genotype), the combination of which determines the magnitude and change of the studied characters, have been studied in detail. The aim is to establish and analyze all aspects of the impact of these factors separately, as well as their interaction on yield and several parameters of grain quality. The working hypothesis of the purpose is related to establishing whether there is a sufficiently different magnitude and direction of “genotype x environment” interaction (GE), which can become a prerequisite for a correct assessment of the performance and stability of each variety of the group.

**Methods and approaches:** Wheat varieties were studied during the period (2017-2019) in four locations of the country: Dobrich, Plovdiv, Trastenik, (Russe) and V. Tarnovo. Five grain characteristics were analyzed - Grain Yield (GY), Test Weight of grain (TW), Wet Gluten content of grain (WGC), Grain Protein Content (PC), and Grain Gluten Index (GI). In the statistical analyses, the parameters indicating the quality were taken as quantitative traits. All possible aspects of the „genotype x environment” interaction were investigated using several specialized statistical programs. The changes in the interrelationships between the traits in dynamically changing environmental conditions (location and year) were determined.

**Key results:** Genotype-environment (GE) interactions were found for all traits studied. Traits are differentially influenced by these factors, with GI being the most genetically dependent and GY the least. The full GGE interaction accounts for complex directional and magnitude variation of traits by location and variety. The interactions between the factors show a linear (PC<sub>1</sub>) and non-linear nature (PC<sub>2</sub> - PC<sub>4</sub>), which is the reason for the observed changes in the mean values of each characteristic being statistically reliable in some of the tested varieties. The conditions in the locations differ significantly, which creates prerequisites for the maximum possible expression of GGE in each of the trait, without exception. They cause the established changes in the correlations between the yield and the examined parameters of grain quality, according to the test location.

**Conclusions:** The influence of the “environment” and “genotype” factors for each trait is different as a share of their total variation. The “genotype x environment” interaction is an essential factor that affects all the traits studied. Its full expression designated as GGE is different depending on the genetics of the trait (G) and its tendency to substantial change (GE) in the specific conditions of the locations. Fluctuations in the mean values of the studied varieties, relative to the environmental conditions, allow their correct comparison according to each of the traits.

**Key words:** wheat; genotype x environment; grain yield; grain quality; correlations

**Abbreviations:** (G) – Genotype as a factor; (E) – Environments (conditions), as a factor; (GE) – interaction between the factors genotype x environment; (GGE) – The full interaction between all main factors [(genotype x (genotype x environment)]; PCA(1-4) – Principal component analysis (factors), NIR-near infra-red scanning; SS/MS, (F-ratio) - ratio between the sum of squares and the sum of the mean squares

## INTRODUCTION

Knowledge of the mechanisms of adaptability and plasticity of modern varieties against the background of a huge variety of environmental conditions would be of great benefit to increase the efficiency of breeding in agricultural crops (Schneider, 2022). The relative contribution of genotype (G), environment (E), and genotype-environment (GE) effects on wheat quality is crucial (Williams et al., 2008; Rozbicki et al., 2015).

In the complex interactions between environmental factors and cultivar genetics, it is important to clarify three primary aspects: *First*, what is the role of each of the factors and their interaction on the change of each trait? *Second*, in demonstrating an interaction, what is its magnitude and direction? *Third*, does the interaction “genotype x environment” cause a change in the interrelationships between yield and grain quality, with a view to possible selection by a group of traits? The answers to these fundamental questions would clarify to a great extent the complex interrelationships between the genetic capabilities of the individual variety and, accordingly, the changes in the environment in which it is realized.

Regarding the *first aspect*, there are studies in which these factors have a different manifestation and influence relative to each other. The performance of traits is most significantly influenced by “environments”, as a factor in four of the five traits studied here - for grain yield (Desheva & Deshev, 2021; Dimitrov et al., 2022), for test weight (Panayotova et al., 2021), for wet gluten content (Plavšin et al., 2021) and for grain protein content (Alemu & Gerenfes, 2021; Lemma et al., 2022). In the gluten index, the role of “genotype” is decisive for its performance and changes (Öztürk & Korkut, 2020; Alemu & Gerenfes, 2021; Vida et al., 2022). Studies in cereal crops show the very different role that “genotype” has in the performance of traits. Researchers have diametrically opposed opinions about the effects of “genotype” in the complex picture of its influence on quality-related parameters. For grain yield it can be negligible (Negash & Birr, 2022), moderately high (Dimitrov et al., 2022) or even higher than this of the “environments” as factor (Marcheva, 2021). For the test weight of wheat, the influence of the “genotype” is extremely high (Stoeva, 2012; Desheva, 2016), while in other cereal

crops – very low (Stoyanov, 2020; Panayotova et al., 2021). In the production of wet gluten, a large range is observed in the degree of influence of the “genotype”, which varies from relatively weak (Alemu & Gerenfes, 2021) to relatively strong (Živančev et al., 2021). In the case of grain protein content, the picture is also very different – from a negligibly weak genotype effect (Alemu & Gerenfes, 2021), to a relatively strong direct effect (Dencic et al., 2011; Živančev et al., 2021). In the gluten index (Vida et al. (2014) reported a very strong genotype effect, while in the study of Sekularac et al., 2018), its effect was very weak. Research on the combined effect of “genotype x environment” reveals effects of different severity on the traits studied. For example, in grain yield, the “genotype x environment” interaction can be very weak (Negash & Birr, 2022) or relatively stronger (Marcheva, 2021). The situation for the GE interaction in the other studied indicators is similar and contradictory: for the test weight – from a weak influence (Tsenov et al., 2022c) to a strong influence (Desheva, 2016); for the wet gluten content- from a weak effect (Živančev et al., 2021) to a strong effect (Plavšin et al., 2021); in the grain protein content – from low (Alemu & Gerenfes, 2021), to medium high (Lemma et al., 2022). It is clear that the genotype-environment (GE) interaction picture for individual traits is very different and complex in nature (Quintero et al., 2018). It depends primarily on the specific conditions of the experiment, on the biological and economic differences between the traits of the varieties in the studied group, on their number (Yan, 2021), as well as on the specific period of study.

In connection with the *second aspect*, the analysis of variances is subject to a certain extent to the presence of the so-called “non-linear” change in relation to the change in the conditions of part of the varieties of the studied group, under the influence of the already described factors (G), (E) and (GE). These changes, which occur more as the rule than the exception, are measured through the application of principal component analysis (PCA), which basically divides the direction of change into “linear” and “non-linear”. Linear is the reaction of a variety when the direction of its change is similar to the group and vice versa. This “linear” response of the cultivar group is measured by the parameter  $PC_1$ , which reflects this linear influence of the environment (E) and usually has the highest value (Yan,

2001). All other components after PC<sub>2</sub> show “inadequate” change and the more they are, the stronger this “non-linear” change is. In turn, according to Pacheco et al. (2015), PC<sub>2</sub> expresses the genotype x environment (GE) interaction and the other components its intrinsic non-linearity. This is why PCA was used in conjunction with principal factor analysis of variance (ANOVA). In this regard, the most popular is the statistical model of Gollob (1968), through which even the share (%) of each significant component of PCA is calculated. The published information related to this aspect of the problem appears to be very mixed. First, the number of credible principle components identified is different. It varies from two components (Kyratzis et al., 2022), in the majority of studies they are three to four in number (Alemu & Gerenfes, 2021; Yashavanthakumar et al., 2021), but there are also reports of 10 PC (Tsenov et al., 2022a). Second, the weight of the first relative to all of the other components is also different. According to Tsenov et al. (2022c) for Test Weight, Wet Gluten Content, Protein Content the linear and non-linear nature of change are similar in magnitude, while for Gluten Index the proportion of linear change is twice as high. According to other authors, the share of linear change of the PC trait is lower than 50% (Alemu & Gerenfes, 2021; Plavšin et al., 2021), which means a predominance of the non-linear share of changes in it.

In relation to the *third aspect*, changes in the correlations between the studied traits, according to the conditions of the item, have been reported (Mutwali et al., 2015; Tsenov et al., 2020), which are the result of different combinations of influences of the main environments and the genotype. The latter is very important for wheat breeding, because it largely determines the real possibilities for complex evaluation and parallel selection of difficult-to-match traits and parameters.

The unique combinations between location and season weather conditions and specific cultivar genetics that interact differently in individual traits, indicators or properties is an important part of studying their nature. They are inextricably linked to the evaluation of the genotype under a wide range of environmental conditions in cereals, which are microclimate crops.

The aim of the research is to establish all important aspects of the impact of factors environment

(E), genotype (G) and their interaction (GE) on yield and several parameters of grain quality. The main question is whether there is a sufficiently different magnitude and direction of “genotype x environment” (GE) interaction and whether it creates large enough differences in the performance of the cultivars, with a view to their eventual evaluation for plasticity and stability?

## MATERIAL AND METHODS

### Field experiments

Field trials were organized in four places in the country, which included twenty-four (24) wheat varieties created after 2001 in Bulgaria. The test locations are: the village of Paskalevo (Dobrich), the village of Trastenik (Ruse), Plovdiv (experimental field of the Agrarian University) and Svishtov (Veliko Tarnovo), during the period 2017-2018. The varieties are planted in a “Latin rectangle” scheme, with a plot size of 10 m<sup>2</sup>, in four replications. Sowing was carried out on different dates in individual years, but always in the optimal agronomy period (October 5-20), within two days for all locations. Fertilization throughout the period is N<sub>160</sub>-P<sub>100</sub>-K<sub>100</sub> (in active substance, kg/ha) and is the same for each of the locations of the field experiment. The applied treatments and plant protection for each location is according to the specifics of the season, and at the level of the site it is the same for each of the studied samples (varieties).

### Analysis of traits and patterns

Data were collected for five characteristics: grain yield (GY), ton/hectare; Test Weight, kg (TW); Wet Gluten Content, % (WGC); Grain Protein content, % (PC); grain Gluten Index (GI). The database consists of twenty-two (22) wheat samples (after dropping two for technical reasons). The yield is presented as tons per hectare (t/ha), after recalculation of the harvested amount of grain from each plot at a standard moisture of 14%. According to the studies of Alava et al. (2001) and Zhang et al., (2022) the values of the parameters obtained by the “NIR” method have very high correlations with the classical methods of analysis. The grain quality parameters analysis were made with the help of the INFRAMATIC, 8600 Perten apparatus, according to the mentioned “NIR-method”.

## Statistical analyses

Trait variance by study trait was plotted against each of the main factors in the field experiment: location (A), year (B), and cultivar (C), using the Statgraphics 18 program. The magnitude of their main effects and all combinations of interactions between them were made by applying the “AMMI” model (Gauch, 1988) and the model of Gollob (1968) in the “GEA-R” program (Pacheco et al., 2015). With its help, the method of factorial regression (FR) was applied, through which the complex interactions between the conditions and the traits were established through their covariances. Descriptive statistics and correlations were calculated using modules of the Statgraphics 18 statistical package.

## RESULTS

The traits, according to their variation in the field experiment, could be conditionally divided

into several groups (Table 1). Very highly varying (GY), where the difference between Max. and Min. values is more than 3 times, and the coefficient of variation is very high - 28%; highly variable (WGC, GI), in which the same difference is about 2 times, and the coefficient of variation is 12-13%; slightly varying (TW, PC), the difference in which is about 50%, with a low coefficient of variation of the order of 2-8%. The change of each trait expressed by its total variation (VC) indicates that two of the traits (TW, PC) are probably more affected by the genotype (G) than by the change of conditions (E). For the other two grain quality traits (WGC, GI), the influence of the environment increases, and for (GY) it is the highest.

The independent influence of each of the studied factors on the traits was reliable, except that of the “year” for WGC and GY (Table 2). Location (site) and cultivars (genotype) have a decisive role in the variation of each of the traits studied. For the traits TW, WGC and GY, both factors - “location” and

**Table 1.** Descriptive statistics of quality parameters and grain yield

Statistic	Min	Max	Mean	Variance	Sdev	VC	SE (V)	MAD
TW	75,9	84,5	81,2	3,30	1,82	0,02	0,289	1,530
WGC	17,8	32,5	25,9	8,96	2,99	0,12	0,784	2,426
PC	10,16	15,53	13,07	1,20	1,10	0,08	0,105	0,893
GI	61	100	80	103,50	10,17	0,13	9,060	8,456
GY	3,00	11,92	7,07	3,96	1,99	0,28	0,346	1,499

VAR-Variance (n), Sdev-Standard deviation (n), VC-Variation coefficient, SE (V)-Standard error of the variance, MAD-Mean absolute deviation

**Table 2.** Multifactor ANOVA for all investigated indexes and grain yield (Statgraphics 18)

Traits	Source	A:Location	B:Year	C:Variety	A*B	A*C	B*C
TW	F-ratio	105,61	26,28	6,15	1,45	11,25	1,28
	<i>p-value</i>	<i>0,0000</i>	<i>0,0000</i>	<i>0,0000</i>	<i>0,1996</i>	<i>0,0000</i>	<i>0,1511</i>
WGC	F-ratio	1099,81	1,71	51,88	1,18	33,21	0,93
	<i>p-value</i>	<i>0,0000</i>	<i>0,1842</i>	<i>0,0000</i>	<i>0,3237</i>	<i>0,0000</i>	<i>0,5930</i>
PC	F-ratio	141,66	6,9	22,38	2,3	10,59	1,51
	<i>p-value</i>	<i>0,0000</i>	<i>0,0014</i>	<i>0,0000</i>	<i>0,0387</i>	<i>0,0000</i>	<i>0,0420</i>
GI	F-ratio	15,45	24,45	33,7	10,63	4,06	1,73
	<i>p-value</i>	<i>0,0000</i>	<i>0,0000</i>	<i>0,0000</i>	<i>0,0000</i>	<i>0,0000</i>	<i>0,0109</i>
GY	F-ratio	1371,05	0,17	21,86	0,46	49,39	1,33
	<i>p-value</i>	<i>0,0000</i>	<i>0,8458</i>	<i>0,0000</i>	<i>0,8363</i>	<i>0,0000</i>	<i>0,0147</i>
	Df	3	2	21	6	42	63

All F-ratios are based on the residual mean square error.

“year” determining the “conditions” do not interact. In the first two, there is also no interaction between “year x variety”. For all other traits, the effect of “location” is complicated by its interaction with survey “year”. For all traits, there is a reliably demonstrated “location x variety” interaction. The data show that the variation in the traits studied is the result of the independent influence of “location”, “year” and the interaction “location x variety”, with the exceptions

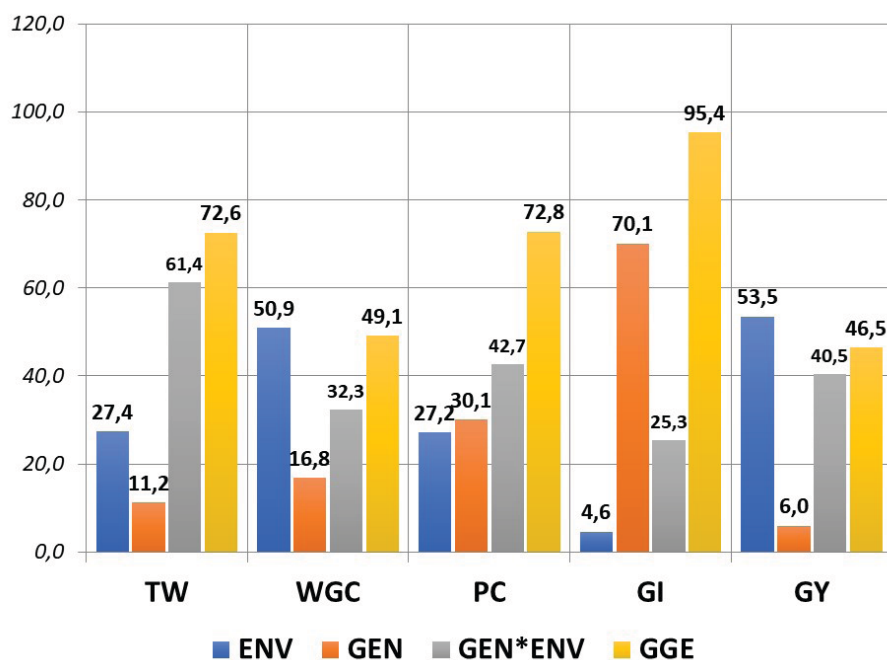
already mentioned. The most resistant to the influence of the factors is the WGC trait, which is not significantly affected by the differences between seasons, (years).

The detailed analysis of variance combined with PCA showed a proven strong influence of each of the factors on the studied traits (Table 3). The established four levels of variation (PC1-PC4) are a signal of a complex multi-layered interaction between

**Table 3.** Analysis of Variances by \*AMMI and \*\*Gollob test for GGE (Genotype Main Effect (G) plus Genotype by Environment (GE)

Source	DF	TW		WGC		PC		GI		GY	
		F-ratio	Pr(>F)	F-ratio	Pr(>F)	F-ratio	Pr(>F)	F-ratio	Pr(>F)	F-ratio	Pr(>F)
ENV	3	77,14	0,0000	1102,18	0,0000	114,87	0,0000	8,73	0,0000	1304,07	0,0000
GEN	21	4,50	0,0000	51,99	0,0000	18,15	0,0000	19,06	0,0000	20,79	0,0000
GEN*ENV	63	8,22	0,0000	33,27	0,0000	8,58	0,0000	2,30	0,0000	46,98	0,0000
PC1	23	268,76	0,0000	445,41	0,0000	87,08	0,0001	13734,54	0,0000	242,70	0,0000
PC2	21	87,84	0,0001	298,26	0,0000	53,34	0,0002	2423,30	0,0000	167,41	0,0000
PC3	19	72,11	0,0001	194,17	0,0000	25,06	0,0010	1114,90	0,0000	15,04	0,0035
PC4	17	63,59	0,0001	141,96	0,0000	27,02	0,0009	624,17	0,0000	5,60	0,0331

\* - AMMI-Additive main effect and multiplicative interaction analysis by PBSTAT, \*\* - Gollob test by GEA-R, F-ratio=SS/MS, Pr(>F) - *p-value*



**Figure 1.** Portion of the effects of environments (ENV), genotypes (GEN) and their interaction (ENV\*GEN) and GGE [(genotype main effect (G) plus genotype-by-environment (GE) interaction)] of the total variation of the field trails



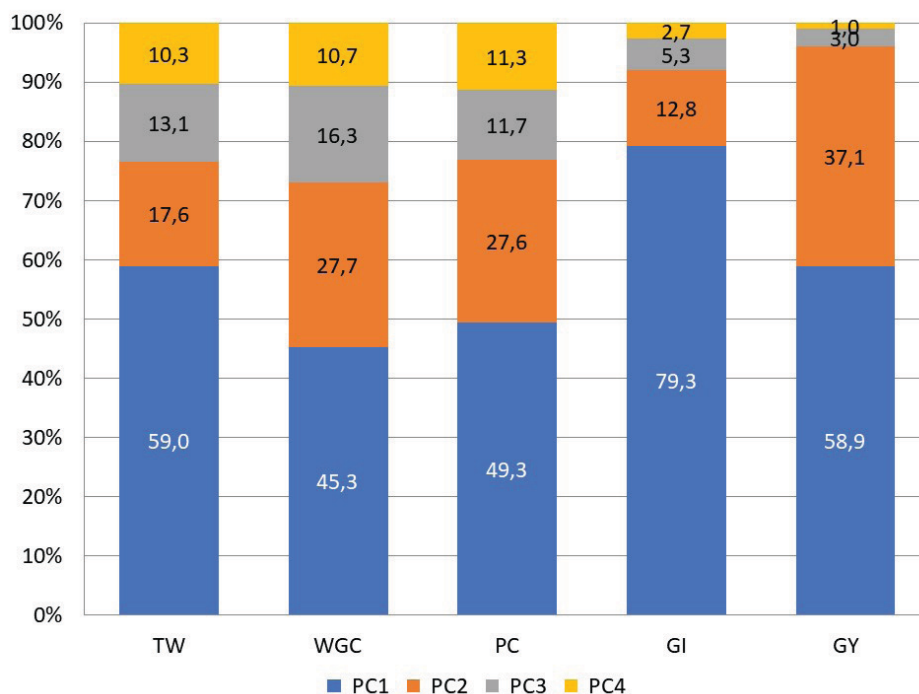
the factors in the study. Without doing additional tests, it is clear that the interaction “genotype x environment” has a cross-over character. The values of the “F-ratio” parameter show different ratios between the individual principle factors in the traits. As a rule, they are highest in the “environment” factor and lowest in the “Genotype” factor, except for the PC and WGC traits. Since the investigated traits are expressed through different measurement units, these values were calculated as a share of its total variation for each trait (Figure 1).

Each of the main factors and the interaction between them has a different share in the total variation of a given trait (Figure 1). The value of each of them is formed as a result of a different combination of effects of the conditions (location by year), genetics (genotype) and the interaction between them. The variation that results from the combination of the genotype (G) and the “genotype-environment” (GE) interaction, denoted GGE (Yan, 2001), is also very different and provides valuable information within each trait and its expression relative to all others.

For the GI trait, for example, there is a very low influence of the environment (4.6%), while for in-

teraction this effect is 25.3%. According to the values of the GGE effects, the traits could be divided into several groups: with a very high value of GGE interaction: 95.4% - for the GI parameter, due to a very low influence of the environment (4.6%); with high values around 73%, for TW and PC traits and relatively low – 46-50%, for WGC and GY. However, significant differences between genotype (G) proportions and the genotype x environment (GE) interaction in these GGE values need to be clarified. For example, in the last mentioned traits WGC and GY, the similar GGE values are a combination of a low genotype effect (6%) in grain yield and 40.5% GE, while in WGC these values are respectively: 16.8% and 32.3%. The high GGE value of GI was overwhelmingly due to the genotype effect (70.1%) in combination with the significantly lower GE effect (25.3%). Between TW and PC traits, whose GGE values are similar, significant differences of G+GE combinations were observed, 11,2% plus 61,4%, in the former and 31.1% plus 42,7%, respectively, in the latter.

The magnitude of the first principle component (PC1) determines the degree of adequate (linear) change of the trait in relation to the change of the

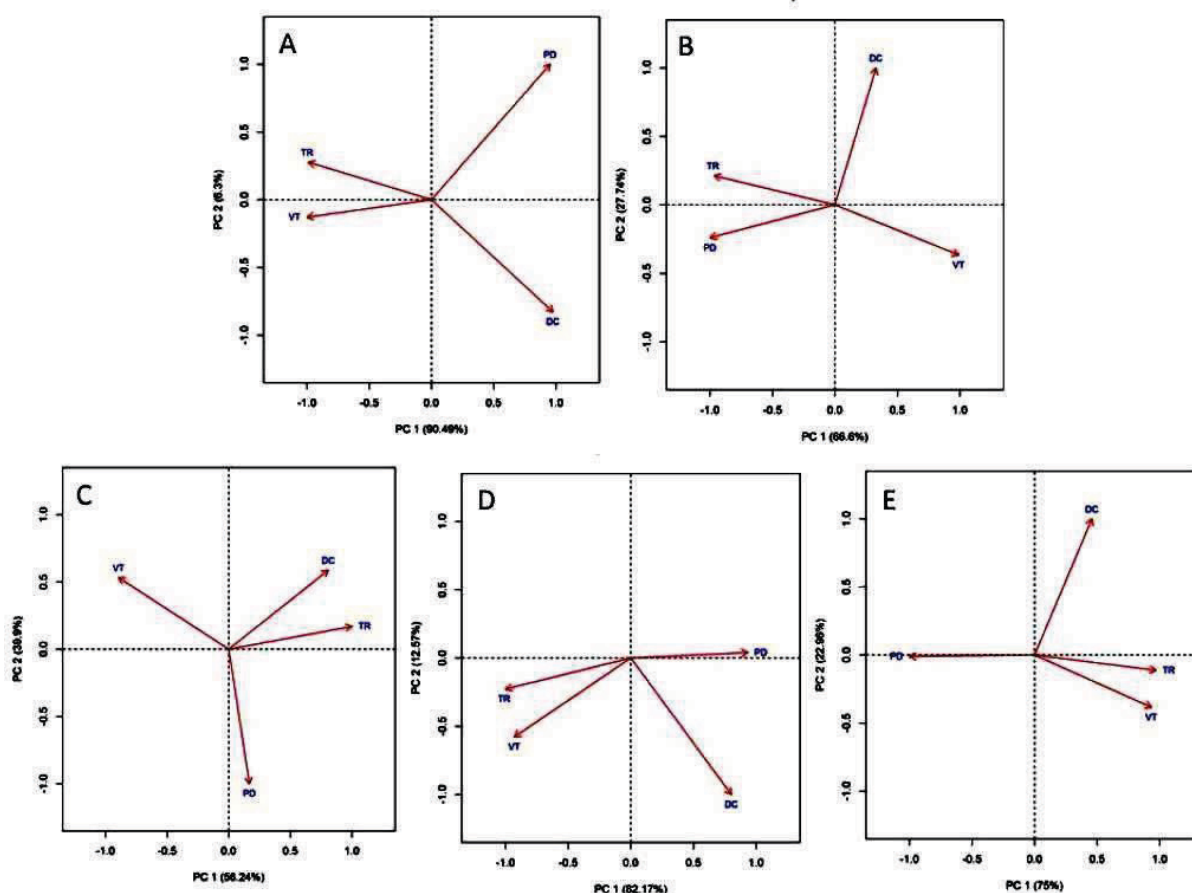


**Figure 2.** Part of total field experiment variability (%) of the significant four principal components for each trait analysed

environmental conditions (Figure 2). According to its values, the GI changes most adequately, and the performance of WGC, is the most unpredictable. The share of the first principle component in the variability of the traits is over 50%, except for the already mentioned GI. This automatically means a high proportion of the sum of the remaining three principle components. Therefore, grain yield and quality (without GI) are traits that vary greatly in degree and direction, primarily by site conditions with some share of variation in season conditions within it. This inherently non-linear change creates major obstacles to an objective assessment of the variety within the group. Difficult to predict variation reduces the degree of predictability of the values of quality parameters of a given variety, under certain conditions of the location. The sites themselves must differ enough (in terms of soil climate) to provoke a different change in a given trait, even

with the anomalies in meteorological conditions of the season. Knowing the magnitude and direction of these changes leads to the correct selection of locations for evaluating each group of varieties. Only in this way could the interactions between factors be evaluated, as well as the adaptability and stability of the variety itself.

Whether there are differences in the conditions of the locations selected here is a question whose answer is found in Figure 3. If the angle between the vectors of the locations is less than  $90^\circ$ , there is a positive correlation, if the angle is more than  $90^\circ$ , it is negative; in the case of  $90^\circ$ , the correlation is 0. With the trait TW, the vectors that are close in terms of conditions are Trastenik and V. Tarnovo; for WGC, the values of Trastenik and Plovdiv are close; for the PC trait, a correlation is established at the location Dobrich and Trastenik; for the gluten index, the Trastenik and V. Tarnovo locations pro-



**Figure 3.** Pearson correlations between the trait means of locations; DC- Dobrich, PD- Plovdiv, TR- Trastenik, VT- V. Tarnovo; A- Test Weight (TW); B- Wet gluten content of grain (WGC); C- Grain protein content (PC); D- Grain gluten index (GI); E- Grain yield (GY)

voke similar values, and for the grain yield, the values are close again at the Trastenik and V. Tarnovo locations. From these results, it is evident that two of the four sites elicit similar values for all of the investigated traits. For three of traits (TW, GI, GY) these are the Trastenik and V. Tarnovo stations. For the other two traits, these are Trastenik locations in combination with Plovdiv, for WGC and in combination with Dobrich, for PC. Trastenik location appears in every pair of similar locations in the different traits. Therefore, it could possibly be removed from the database when evaluating the stability of the varieties later.

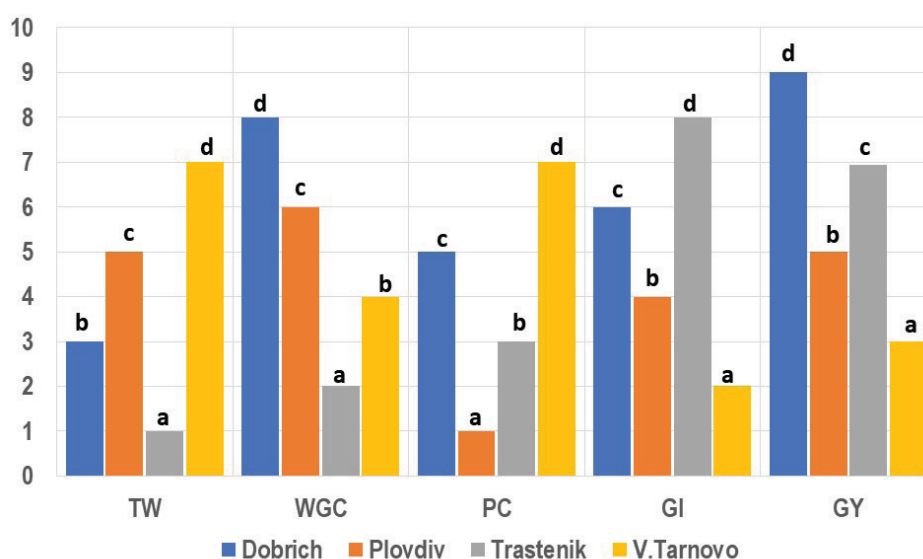
These “similarities” between locations indicate the trends of the direction of variation, but do not directly relate to a change in the mean of the traits in the group of varieties. This can be seen in the summary results in Figure 4. Differences in each trait were statistically significant across all items, without exception. In general, at the Dobrich location, the traits form the highest location means, with the exception of the TW. In the Plovdiv site, four of the traits display the relatively lowest location means, and the other two have the highest location means (TW, PC). In this regard, it can hardly be argued that there are locations with markedly favourable conditions and those with stressful conditions during the growing season. This motley picture of the performance of each trait is an essential prerequisite

for evaluating the studied varieties against the background of locations with significant differences in the environments of each of them.

“Environments”, as a factor, influence each of the traits, through the covariances between them (Table 4). The impact of these covariances was between 18% (TW, GI) and 52% (GY). When each of them is considered “dependent” on the others, then it is found the covariances of the conditions affect it indirectly by changing each of the others, and to a different degree. According to these results, the effects of the interaction E\*TW (26%) and E\*WGC (13%) on grain yield are strong, and the effects of E\*PC (4%) are the weakest. Grain yield as a trait

**Table 4.** Effects of environments (E) on some quality parameters, (% GxE) determined by factorial regression of environment covariance

Interaction E*Trait	As dependent character				
	TW	WGC	PC	GI	GY
E*TW		4,5	7,3	6,4	26,0
E*WGC	3,0		3,6	5,2	13,0
E*PC	3,7	9,5		3,1	4,0
E*GI	7,5	8,4	5,4		9,0
E*GY	2,8	15,6	4,7	4,3	
SUM	17,0	38,0	21,0	19,0	52,0



**Figure 4.** Multivariate comparison of trait means by location (different letters indicate statistical difference at 95% of LSD)



shows, by environments, the strongest effect on WGC 15,6%) and the weakest on TW (2,8%). The effect of E\*PC and E\*GI on WGC is significant (about 10%). It is likely that part of the explanation for the change in correlations across conditions is due to these “masked” indirect effects of covariances between traits whose vector is the environments.

An analogous analysis can be made with respect to the covariances of the interactions of the genotype with the traits (G\*Trait). This would further complicate the picture of the combination of effects on the performance and variation of each trait. Therefore, the data presented here are only to illustrate that the variability of each trait is practically the result, not only of direct interactions between “genotype”, “environment”, “genotype x environment”, but also indirectly through the variances of all others, as a peculiar combination of impacts. All this is direct evidence of the complex nature of the variability of yield and quality in the process of the grain filling. These results suggest the need for tandem (parallel) selection for yield and quality for their possible compromise combination under certain specific conditions.

From this perspective, it is important to understand whether correlations between yield and quality change under these complex combinations of trait interactions. The different conditions of the location and the significant interaction between the “genotype” and the “environment” cause a change in the correlations between grain yield and the quality parameters investigated (Table 5). This change affects all traits as well as research locations. In Dobrich, the correlations between yield and traits do not exist, in Plovdiv they are mostly positive, with the exception of the trait PC, and in V. Tarnovo there are positive (TW), negative (GI) and no correlations (WGC, PC). Against the all-location database, correlations between GY and grain quality traits were negative,

as expected. The only exception is TW, where there is no proven correlation with GY. The relationships between traits at the site level revealed here are useful for their comprehensive evaluation in the breeding process. Trastenik is the location where the negative correlations between yield and quality are most pronounced ( $r=-0.55$  - $0.67$ ). However, the conditions there are very similar to those in V. Tarnovo (Figure 3), in three of the five traits. It was suggested, it could possibly be excluded in a stability assessment. If this is applied, then yield-quality correlations are likely to settle around values that are not statistically significant. This, in turn, could “distort” the assessment of the stability of the quality of a given variety, in the other locations.

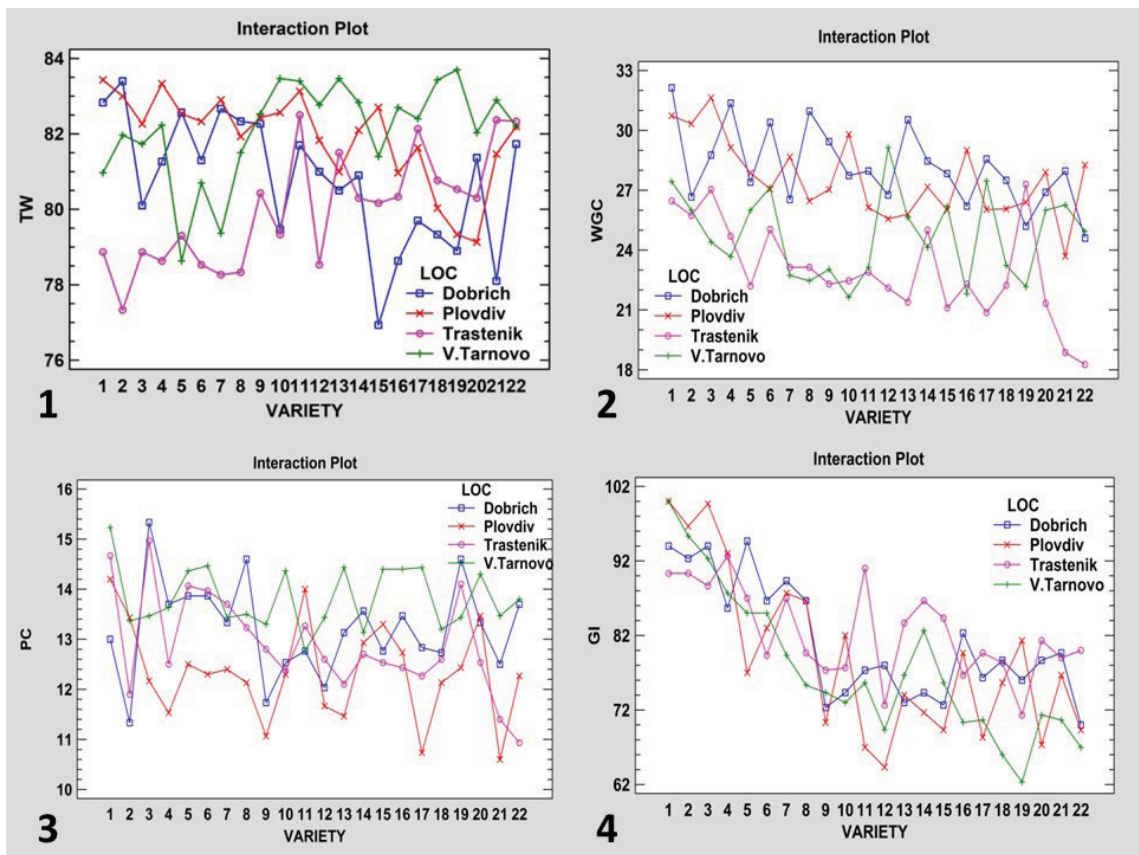
The variation of the genotypes in the conditions of the individual location is so great that the differences between their means could hardly be proved (Figure 5). Each varies differently in magnitude and direction, depending on the location. This complex change in the values of individual varieties is a certain obstacle to their correct and objective assessment. The strong variation of the values of the cultivars in the locations is evidence of the presence of a cross-type of “genotype x environment” interaction. In turn, it is the most important prerequisite for the need to evaluate the performance and change of traits in each variety, compared to the rest of the group.

Despite the effects of a complex GGE interaction, differences between cultivars for each of the traits could be statistically highlighted (Figure 6). The differences between them are the smallest according to TW. There are significant differences between the means of the varieties. The data for grain yield (not presented here) exactly mirror those of the gluten index (Figure 6-4), confirming in principle their strong negative correlation. Each variety ranks differently for each trait. Therefore, on the basis of

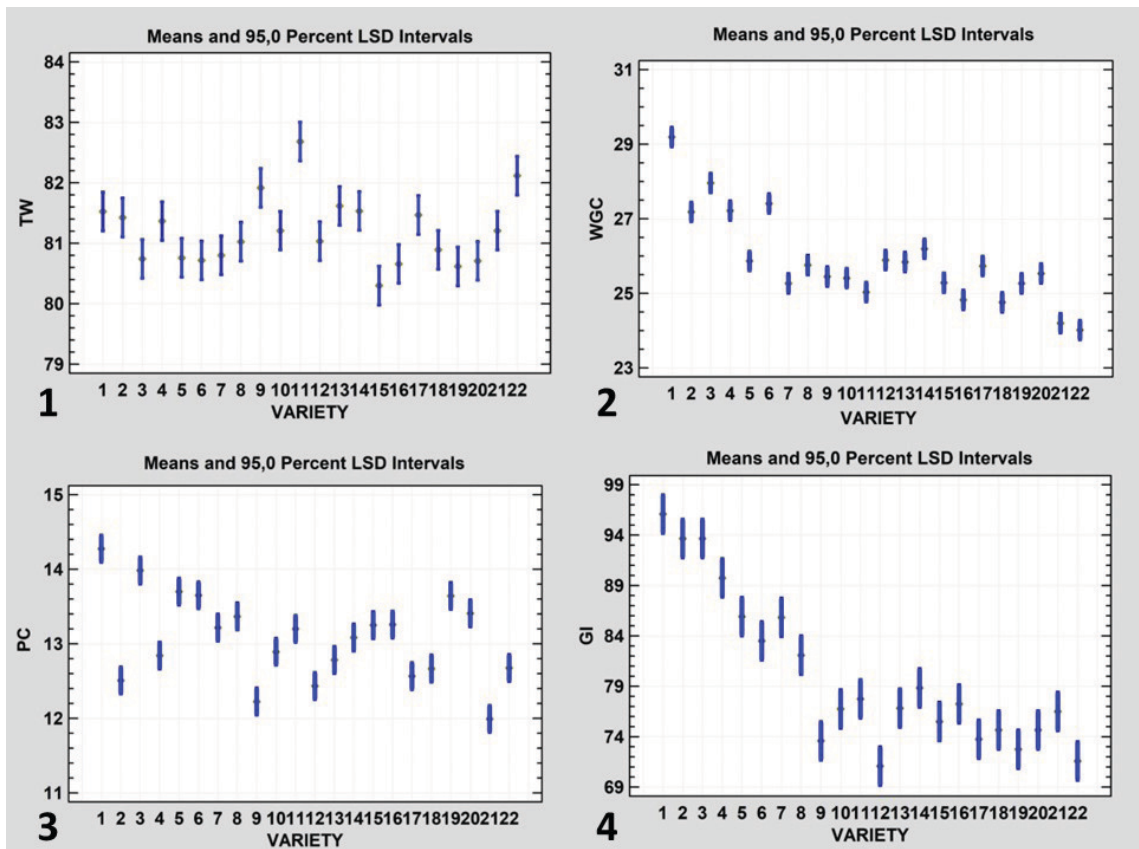
**Table 5.** Pearson correlations between grain yield and quality by locations

Traits	Dobrich		Plovdiv		Trastenik		V. Tarnovo		Overall	
	r	<i>p-value</i>	r	<i>p-value</i>	r	<i>p-value</i>	r	<i>p-value</i>	r	<i>p-value</i>
TW	-0,12	0,583	0,75*	0,000	0,49*	0,022	0,54*	0,009	-0,05	0,824
WGC	-0,32	0,153	0,38*	0,050	-0,67*	0,001	-0,04	0,872	-0,54	0,010
PC	0,07	0,755	0,09	0,700	-0,66*	0,001	0,09	0,702	-0,37*	0,039
GI	0,32	0,153	0,51*	0,015	-0,50*	0,017	-0,85*	0,000	-0,50*	0,019

r- Coefficient of correlation, *p-value*-significance of differences from zero at 95%.



**Figure 5.** Changes in the means of the quality indexes in the tested varieties, caused by the location environments, (1: TW-Test weight; 2: WGC-Wet gluten content; 3: PC-Protein content; 4: GI-Gluten index)



**Figure 6.** Differences between the variety means of the quality parameters (1: TW-Test weight; 2: WGC-Wet gluten content; 3: PC-Protein content; 4: GI-Gluten index)

this difference in ranking, the cultivar can be compared against others on a set of tracked traits. The assessment of the variety after their combination will give full information about its adaptability and variability (stability) compared to other samples in the specific field experience, which will be the subject of a separate study.

## DISCUSSION

*The Test weight (TW)* is directly related to the density of the grain and indirectly to the properties of the flour, and it does not depend on the grain size, but on the conditions of formation and “compaction” during the process of its maturation. (Schuler et al., 1995). The denser the grain, the more strongly this parameter is related to grain yield (Yabwalo et al., 2018). This trait varied least among all studied here (Table 1). The data for this trait in durum wheat are similar (Taneva et al., 2019a). Its performance is influenced by the genotype and the environment, in which the significant role is played by the location and not by the season (Table 2). Nehe et al. (2022) found that it was site conditions in interaction with genotype that most strongly influenced the trait. According to the conclusions in their study, TW is the only trait where breeding has sustained progress, because the role of variety compared to conditions is significantly higher. In common wheat, the genotype has the strongest influence on the trait with a share of 56% (Stoeva, 2012), up to 77% (Desheva, 2016). In other cereal crops, the data shows the exact opposite, the conditions have a share of 79 % in triticale (Stoyanov, 2020), which in durum wheat can reach 90 % (Panayotova et al., 2021). Environments reliably influence the trait as a main factor without interacting with the “genotype” as factor in a number of other studies as well (Stoeva, 2012; Penchev et al., 2019). On the other hand, the data of Gouzmán al. (2016) and Tsenov et al. (2022c) in field experiments with common and durum wheat, show the presence of complex interactions between all the investigated factors (variety, year, location), against the background of their credible independent influence. In this study, the picture is basically analogous, and the GE interaction (61.5%) exceeds the main effects of both “genotype” (11.2%) and “environments” (27.4%). This combination of factors and interactions between them is rare against

a number of reports of either a complete absence of GE (Penchev et al., 2019; Ma et al., 2021) or weak effects, GE=13% (Tsenov et al., 2022; Uhr et al., 2022) or GE=20% (Desheva, 2016). A low but positive correlation between grain yield and test weight has also been reported in other wheat of wheat (Tayyar, 2010; Marcheva, 2021).

*Wet gluten yield (WGC)* is important for grain quality because it expresses the share of gluten in its protein composition (Kaplan et al., 2020). The strong influence of location conditions in this study coincides with the data of Taneva et al. (2019a), in durum wheat, but it is in complete contradiction to the opinion of (Ma et al., 2021; Tsenov et al., 2022), according to which the trait is influenced only by the underlying factors, without interactions among themselves. The change of this trait is related more to the “environments” than to the “genotype” (Table 2). The published by Stoeva, (2012) and Plavšin et al. (2021) data show that the strongest effect is GE (70%), followed by that of G (21%), and the influence of conditions is negligible (7%). There are different opinions about the effect of “genotype” on variation of WGC are Alemu & Gerenfes, (2021), according to which it is 12% and Živančev et al. (2021), who reported a significantly higher effect of 45%. A number of researchers (Alemu & Gerenfes, 2021; Živančev et al., 2021), reported a weaker effect of GE, compared to the direct effects of E and G, thus confirm the data here (Figure 1). As can be seen, information at WGC regarding the effects of G, E, GE is too controversial and multidirectional to be used as a starting point, so it is important to conduct research to study in detail the mentioned groups of factor contributions under specific conditions and with specific cultivars (Williams et al., 2008). Researchers have different opinions regarding the correlation between the trait and grain yield. Tsenov et al. (2014) reported showed a reliable positive correlation of WGC with both yield and quality parameters. Analogous are the data of Ma et al. (2021), according to which this trait is directly strongly related to yield and to the analyzed quality parameters. This is an indication of its important role in characterizing grain quality. In other studies of common wheat, Nazarenko et al. (2020) and Kasahun & Alemu (2022) found that there is no correlation between yield and WGC, which coincides with the opinion of Taneva et al. (2019b) for durum wheat. On the other hand, a strong negative correla-



tion between the trait and grain yield has been reported in spring (Gómez-Becerra et al., 2010) and winter wheat (Tayyar, 2010; Surma et al., 2012). These radically different relationships are common against the background of the huge variety of conditions and genotypes involved in the studies. In terms of breeding, it is important to know that if this parameter is monitored in parallel with the yield in the breeding process, then its quantitative expression is quite possible to be preserved at the reached level (Kaplan et al., 2020).

**The content of protein in the grain (PC)** is a major indicator of its quality. Protein quantity is a prerequisite for high quality (Malik et al., 2013). The genotype effect (30.1%) in trait variation approaches the opinion of Dencic et al. (2011), Živančev et al. (2021), according to which in Serbian wheat varieties it is between 30 and 40 %. However, in these studies the influence of “environments” is 56-59%, while here it is almost twice as weak (30%). In the study by Lemma et al. (2022) in durum wheat, the share of “genotype” is almost negligible (5.4%) against the background of all the variation, and the GGE combination is only about 20%. Analogous are the results of the study by Alemu & Gerenfes, (2021), where the genotype effect is only 2.5%, at 14% for GGE. According to the large-scale study by Williams et al. (2008) all quality parameters related to protein quantity (PC) are very strongly influenced by conditions, while “genotype” has a much stronger influence on protein quality parameters. Despite the large divergence in effect shares, the first principle component has a similar share of trait change in common wheat from 42 to 45 %, (Alemu & Gerenfes, 2021; Plavšin et al., 2021), at 49.3% in the present study. Compared to the conflicting data on the effect of the “genotype” of PC, two aspects are interesting for breeding: 1) the correlation with grain yield and 2) the heritability (H<sub>2</sub>) under changing environmental conditions. The data here indicate that the correlation between GY and PC changes substantially by location conditions. Research in this direction gives radically opposite opinions: in one group there is no correlation (Nazarenko et al., 2020; Tsenov et al., 2022), in another it is negative to varying degrees (Nankova et al., 2020; Marcheva, 2021; Živančev et al., 2021). Both statements practically confirm the information provided here. The degree to which the performance of the trait can be predicted by the heritability coefficient (H<sub>2</sub>)

in this study varies from 0.85 to 0.95 (data not provided) and is completely analogous to that found by (Gómez-Becerra et al. (2010), Živančev et al. (2021) and Kyratzis et al. (2022). Therefore, on the amount of protein in the grain, targeted selection could be carried out without strongly negatively affecting the grain yield, naturally in certain environmental conditions (Eichi et al., 2020). If this is followed together in yield breeding success is guaranteed (Dencic et al., 2007; Nehe et al., 2019), otherwise a progressive decrease in protein is reached in the grain (Maich et al., 2020; Mirosavljević et al., 2020).

**Gluten index of grain (GI)** is genetically related to its quality (Clarke et al., 2010; Bonfil & Posner 2012; Vida et al., 2022), making it a desirable trait for cultivar characterization. Torbica et al. (2007) suggest a modification of the test for this trait, in case of damage to the grain by harmful wheat bug or extreme conditions of grain pouring. All environmental factors and their interactions have a significant impact on the performance of this trait (Table 2). Analogous in this respect are the regularities revealed by Zečević et al. (2009) in Serbia, Öztürk et al. (2020) in Turkey and Alemu & Gerenfes (2021) in Ethiopia. Vida et al. (2014) showed that in durum wheat the trait is mainly influenced by the “genotype” and very little by environmental conditions, the latter having the smallest share in the variation, which is also valid for common wheat (Sekularac et al., 2018), (Figure 1). When growing common wheat using extensive (low input) and organic technology, Rakszegi et al. (2016) investigated the change of a series of quality parameters of 37 cultivars in Hungary and Austria. The gluten index, as well as the grain yield, is the only traits that are changed by all the investigated factors: variety, year, location and technology. At the same time, its variation (14%) approaches that presented here (Table 1). Under those conditions, they found that GI exhibited a positive correlation with grain yield, but a negative correlation with PC. The likely reason for this according to Sekularac et al. (2018) is that the gluten index is highly influenced by the specific weather conditions of the season, especially the temperature and rainfall during grain maturation. When the season is close to the climatic norm, its positive relationship with bakery qualities is guaranteed. If quality preservation is pursued, then its values must be monitored during selection. Oikonomou et al. (2015) and Kasahun & Alemu (2022) found no correlation be-

tween yield and GI. Mirosavljević et al. (2020) outline the picture of yield and quality change in the breeding process over a period of almost a century in Serbia. Their observation leads to the conclusion that GI values decrease quantitatively, but not qualitatively, because of the selected new combinations of glutenin (Glu) allelic configurations.

**Grain yield (GY)** is always the most important factor for wheat grain production. As a quantitative trait, it is formed directly as a result of a different combination between its three components: grain size, number of grains in the ear and number of spike-bearing stems per m<sup>2</sup>, (Tsenov et al., 2021). Each of them, separately, is influenced to a different degree by the environmental conditions (Gubatov et al., 2016; Quintero et al., 2018; Tsenov et al., 2020), which in turn causes its complex and unpredictable performance against the background of the main meteorological parameters- temperature and precipitations (Mohammadi et al., 2020; Tsenov et al., 2022a). There is a huge number of studies in cereal crops, and wheat in particular, regarding the regularities of GE and GGE interactions (Yan & Hunt, 2001; Sanchez-Garcia et al., 2012; Roostaei et al., 2022). No less controversial than the characteristics related to grain quality discussed above are the published data on the relative contribution to the variation of the factors environment (E), genotype (G), their interaction (GE), and the combined interaction (GGE) in grain yield (Saltz et al., 2018; Negash & Birr, 2022; Pour-Aboughadareh, et al., 2022; Tsenov et al., 2022b). The genotype's share of variation is low along with its interaction with conditions, accounting for only about 10% (Negash & Birr, 2022). In this study, the share of the genotype (G) is low (6%, Figure 1), if we compare it with a share of the same of 29% (Desheva & Deshev, 2021), 40% (Dimitrov et al., 2022) or 63 % (Marcheva, 2021) in studies of other groups of varieties in Bulgaria. The main emphasis for the causes is placed on the specific conditions of the locations, as well as on the investigated varieties, such as number and significant biological or physiological differences between them (Yan, 2021). The published information on the nature of trait change expressed by the number of factors from PCA is extremely diverse. Four main factors of PCa were recorded in this study (Figure 2). In publications, they vary widely from three (PC<sub>1,3</sub>) (Yashavanthakumar et al., 2022) to ten (PC<sub>10</sub>) (Tsenov et al., 2022a), which is evidence of the complex

nature of post-interaction change “ genotype x environment”.

Increasing grain yield through breeding is generally associated with a tendency to lower grain quality (Kiszonas & Morris, 2018; Johansson et al., 2020; Wieser et al., 2020). Under different combinations of environments, correlations between yield and quality-important parameters sometimes change fundamentally (Tsenov et al., 2022c). This dynamic is the reason for launching the thesis that it is possible to make parallel selection of yield and quality (Jernigan et al., 2018; Maich et al., 2020). Changes in the magnitude and direction of genetic correlations with grain quality traits is the likely reason that grain yield is gradually increased (Herrera, et al., 2020; Tsenov et al., 2020) and the quality level is preserved through parallel selection (Khazratkulova et al., 2015; Nazarenko et al., 2020 Nehe et al., 2022) or through technological means during cultivation (density, varietal composition or fertilization) (Bhatta et al., 2018; Horvat et al., 2021).

Finally, it can be said that the established combinations of the influence of the conditions on the performance of the studied traits do not provide fundamentally new information. These regularities give a concrete idea of the changes of each in a wide range of combinations of conditions (locations + years). They show the principles of the analysed GE and GGE interactions, in the context of the variation of the traits from the point of view of the assessment of the single variety in the composition of the studied group. Stability and plasticity are characteristics of the variety that are most correctly assessed in the presence of a serious “genotype x environment” interaction, especially when it is a “cross-type” (linear and non-linear change). In the event there is no statically proven “GE” or the linear change of the group of varieties prevails (PC<sub>1</sub>>>PC<sub>n</sub>), then a simple “linear” variation of the trait is measured, which in no case can characterize the variety directly from the others as stable or plastic. The regularities established in this research provide a real opportunity for a similar assessment of the plasticity and adaptability of the variety, according to each of the trait analysed here.

## CONCLUSIONS

All traits related to grain yield and quality are significantly influenced by the main factors envi-



ronment (E), genotype (G) and the interaction between them (GE).

As a rule, the influence of “environment” exceeds the effect of “genotype” in three of the five traits. Exceptions to this statement are the PC (protein content) and GI (gluten index) traits, where the situation is diametrically opposed.

The “genotype-environment” interaction has a lower proportion of variation compared to the single effects of both factors, with the only exceptions being PC (protein content) and WGC (wet gluten content).

The general change of the cultivars in the traits caused by the various environmental conditions exhibits both linear and non-linear character, except for GI (gluten index), where the linear part is significantly more pronounced.

The location mean of the group of varieties is significantly different, although the data for the Trastenik location approaches one of the others for some of the parameters.

The identified differences resulting from the GGE interaction caused a change in the magnitude and direction of correlation between grain yield and each of the quality traits.

The change of correlations, especially towards their absence, is a serious prerequisite to express the opinion that it is quite possible under specific conditions to make a parallel selection of high yield and compromised high grain quality.

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