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Original article

Environment- and Genotype-Dependent Stability in the Common Wheat Grain Quality (*Triticum aestivum* L.)

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Abstract

The study was conducted to evaluate the stability of common wheat varieties in four locations with proven different environmental conditions. Three indexes of grain quality were studied: wet gluten content, (WGC); gluten index of grain (GI) and grain sedimentation value (Zeleny). The stability of varieties has been evaluated by many parameters that reflect different aspects of the complete picture for it. The indexes studied are strongly influenced by environmental conditions. The most genetically stable among them is the gluten index (GI), where the genotype has a decisive role of about 70% of the variation, and the most unstable is the wet gluten content (WGC), with only 17% of the effect. As a result of reliable GE, the ranking of the varieties according to the performance of each of the indexes is different in the individual locations. The ranking of varieties in terms of stability according to the ranks of each of the parameters is very different. Even a visual representation of the results, which clears the picture to the maximum extent, shows a different set of stable varieties in each of the quality indexes. Only a few of the varieties (G2, G6, G9, G13, G18, G20, G22) have a good balance between the size and stability of all quality parameters, with a moderate compromise with the grain yield level. The assessment of the stability of the variety in terms of quality can be made according to any of the indexes used. The stability of the variety depends to a large extent on the effect of the environment, which must be considered when selecting a specific index for assessment. The most suitable for this purpose is the gluten index (GI), where the influence of genotype is the strongest, with a significant GE interaction accounting for 25% of all variation. The stability of the variety does not depend on the magnitude of the quality indexes. Stable can be both quality (G2, G6) and varieties with very low grain quality (G18, G20, G22). Stability of quality, at high levels of indexes, is associated with low grain yield and vice versa. From this point of view, combining high yield stability and grain quality at the highest possible levels is a very rare exception (G2, G9).

Keywords: Wheat, Genotype by Environment, Stability, Grain Quality.

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INTRODUCTION

Wheat grain is a product whose quality is extremely important to the world. Wheat grain provides about 21% of the calories needed by humans (McFall & Fowler, 2009). The globally proven high nutritional value of wheat is inevitably related to the quality of the grain, not its quantity (Shewry, 2009). In several countries, grain quality is more important than yield because climatic conditions do not allow the realization of high productive potential (Bhatta et al., 2016; Bornhofen et al., 2017; De Santis et al., 2017). Where there are real opportunities for a successful compromise combination between yield and grain quality, breeding has a long-standing successful practice (Atanasova et al., 2012; Nehe et al., 2019; Ilin et al., 2022). Although grain yield is always paramount, quality has been systematically and thoroughly investigated (De Santis et al., 2017, Liu et al., 2022). The influence of environments on quality has been studied through a number of its different team-related indexes (Bornhofen et al., 2017; Penchev et al., 2019; Öztürk, 2022). The strength of the effects of the conditions (E), of the genotype (G) and the interaction between them (GE) for each index have different shares (Alemu and Gerenfes, 2021; Kyratzis et al., 2022, Tsenov et al., 2023). The influence of the environment on the basic indexes of the grain as Wet Gluten Content index (WGC), Gluten Index (GI), and Zeleny sedimentation index (Zeleny) in wheat without a doubt is significant (Karaman, 2020; Bosi et al., 2022; Öztürk, 2022). This makes the evaluation of varieties by grain quality very difficult, because each of them shows different performance, despite the existing positive correlations between them (Öztürk, 2022; Tsenov et al., 2023). Varieties differ in genetic potential, which is realized to varying degrees under the direct influence of environmental changes (Morgounov et al., 2014; Tsenov et al., 2021). Therefore, the assessment of the performance (stability) of the variety is directly related to the specific information about the relationship between individual indexes, their genetic conditioning and the peculiarities of their change due to environments (Desheva, 2016; Taneva et al., 2019; Kyratzis et al., 2022). What part of the grain quality the variety will realize depends on its genetic stability, which is different for individual indexes? In this regard, it is necessary to clarify the following few aspects relevant to the subject.

First, are there fundamental differences in changes in quality indexes in varieties that have different genetic potentialities? The reason for this judgment is the established regularities for a different reaction to changing conditions, in direct dependence on the genetics of quality (Morgounov et al., 2014; Tsenov et al., 2021). In this regard, it is imperative to gather enough information to establish realistic levels of trade-off between grain size and grain quality stability. This is important for metrics that could potentially be used in breeding.

Second, is the variation of a variety as measured by one of the quality indexes analogous to that of other ones? Divergence in variety stability measured by different indexes is more the rule than the exception (Stoeva, 2012; Öztürk, 2022). Discrepancy in the evaluation of the stability of cultivars by specific indexes is common, and yet there is progress in breeding (Mirosavljević et al., 2020). Establishing a specific set of indexes would increase the effectiveness of grain quality breeding since such analysis is already possible by spatially mapping the relationships between them and environmental conditions (Yan & Hunt, 2001; Yan et al., 2007).

Third, is it possible to mark varieties that, over a wide range of environments, possess a stable combination of yield plus grain quality? The most important task is to determine in practice a "stability" formula for the various combinations between grain yield and quality. High-yielding stables, what level of quality could they have - "low" "medium" or even "high"? Complex yield-quality relationships are generally negative but may change substantially by genotype x environment interaction (Öztürk, 2022; Tsenov et al., 2023). This is an indication the combination of the two aspects of the grain - yield and quality is possible, but at compromise levels of each of them to have progressed in terms of quality as well (Bosi et al., 2022; Ilin et al., 2022). The question of what combination of yield and quality should be aimed at in practical breeding remains open, because with productivity above 9 t/ha, a "balance" between them is difficult to achieve (Dencic et al., 2007; Atanasova et al., 2009). In this study, an attempt was made to seek answers to the questions posed in direct dependence on specific environmental conditions and an up-to-date set of varieties with different genetics for both aspects.

The purpose of the study is to evaluate the stability and adaptability of each variety according to basic quality indexes in a wide range of environmental conditions. The working hypothesis is related to gathering information on the three important aspects that were mentioned (Wet Gluten Content index, Gluten Index, Zeleny sedimentation index). An answer will be sought to the question up to what yield levels can the variety have high quality, which will be stable in various environments?

MATERIALS and METHODS

Field trails design

Twenty-four (24) varieties of common winter wheat were tested in four climatic zones of the country in the following locations: the village of Paskalevo (Dobrich), the village of Trastenik (Ruse), Plovdiv and Veliko Tarnovo, during three consecutive seasons - 2017-2019. The exact sites of the locations used as well as the specifics of the used factor levels are provided in Table 1. The main measures of cultivation technology include sowing in the optimal period for each region (October 10-20) and harvesting at full maturity at a standard grain moisture of 14%. Fertilization included N160-P100-K100 (in active substance, kg/hectare), with 30% of the nitrogen and all the phosphorus and potassium applied as the main pre-sowing fertilization. In the spring, the crops are fed with the rest of 70% of the mentioned amount of nitrogen. Plant protection is combined with the specific meteorological conditions of cultivation, and in each individual season it is similar for the individual points and the same for the investigated varieties in each point. The cultivars were grown in a randomized block consisting of 24 cultivars in three replicates, with a plot size of 10 $m^{2.}$ Variety quality samples were from each point and year, without replicates, and grain yield was analyzed from data from the three replicates.

Table 1. General information on the levels of the main factors of the conducted field experiments

Locations	Coordinates		Altitude, m	Years	Genotypes	
	N	E				
Dobrich, (Paskalevo)	43⁰38'47"	27⁰48'40"	248	2017	Varieties	22
Plovdiv, *AU (Experimental field)	42⁰08'13"	24⁰48'22"	155	2018	Checks	2
Trastenik, (Rousse)	43⁰37'40"	25⁰51'37"	170	2019		
V.Tarnovo, (Tsarevets)	43⁰36'30"	25⁰30'02"	110			

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Group of Varieties

The 24 varieties selected for research cover all levels of grain quality and at the same time differ in productivity (Table 2). Their ranking here is in descending order of quality from G1 to G22, according to preliminary data. The selected set of varieties is a small sample of winter common wheat varieties created in the last 15 years in Bulgaria. About two-thirds of them are newly developed (after 2017) and still little is known about the performance of their yield and grain quality under different growing conditions. Due to the unexpected loss of samples for technical reasons, 22 instead of the 24 cultivars from the whole group were analyzed.

Table 2. List and preliminary information on the genetic potentialities for grain yield and quality of the varieties studied

Designation	Variety name	Year of Release	*Group of Quality	**Yield Rank
G1	Tervel	2010	A	IV
G2	ABC Alfio	2017	A	II
G3	ABC Kolino	2018	A	IV
G4	Apogej	2004	A	IV
G5	ABC Navo	2018	A	III
G6	ABC Lombardia	2017	A	III
G7	ABC Zigmund	2017	AB	IV
G8	ABC Aldo	2019	AB	III
G9	ABC Grosso	2020	B	II
G10	ABC Clover	2019	B	IV
G11	ARO Redmat	2019	B	II
G12	Neven	2005	B	III
G14	Ognyana	2009	B	III
G13	Riana	2012	B	IV
G15	ARO Sankti	2019	C	II
G16	ABC Klauzius	2018	C	I
G17	ABC Veto	2021	C	I
G18	ARO Romans	2021	C	I
G19	ABC Speri	2017	C	II
G20	Faktor	2007	C	II
G21	Vyara	2005	D	II
G22	Aneta	2004	D	I

* - A > D high to low quality groups; **I > IV – high to lower grain yield ranking

Analyzes of grain yield and quality

Three quality indexes were studied: wet gluten content, (WGC); gluten index of grain (GI) and sedimentation value index (Zeleny). They were determined using the INFRAMATIC, 8600 Perten apparatus, which analyzes the grain without grinding it to flour (NIR) and is used daily in practice because its data show high correlations with classical methods of analysis (Alava et al., 2001; Zhang et al., 2022). Grain yield (GY) is the subject of attention with the sole intention of placing them simultaneously with variety quality for a combined assessment of both aspects of grain.

Statistical analyses

Varietal stability was represented by ranking the stability parameters (known as stability statistics) calculated using the statistical program PBSTAT (Suwarno et al., 2008). It includes all the necessary tools (modules) for a complete and objective evaluation of the performance of the varieties in the presence of the interaction of the genotype with the environment. The large number of 16 stability statistics (parametric and non-parametric) to estimate led to the application of Pearson correlations (Statgraphics Centurion) between them to simplify evaluation, like the approach of Pour-Aboughadareh et al. (2019). The arrangement of the varieties (rank) according to the analyzed parameters of stability is according to the statistical program: the most stable is 1, and the most variable - is 22. The combined evaluation of grain yield and quality of each variety and for all investigated indexes was made using the XLSTAT 2014 program. In it, the ranking is mirrored in relation to the previous analysis (the highest value is the most stable, and the lowest value - is the most variable). This was done for the most stable varieties in yield and quality (both together) to be more easily identified than the others in the group.

RESULTS and DISCUSSION

The correct evaluation of the variety stability in each group is directly related to an analysis of variance to determine whether the environment has a share in the change of the studied index (Table 3). The indexes included in the study show differences in the effects of the three main factors: environment (E), genotype (G) and interaction between them (GE). The latter (GE) causes from 20% (Zeleny) to 32% (WGC). This is a significant influence that changes the values of each variety in the group. There are differences between indexes regarding the effects of the environment (E). The environment is a significant factor in two of the indexes - WGC and Zeleny, where it accounts for about 50% of their changes. In the third one (GI), the environment has a very low influence of 5%. The role of genetic endowments of varieties (G) is very different: from 70% (GI), 30% (Zeleny) and 17% (WGC). The change in the environmental conditions causes a difference in the direction and magnitude of performance of the group of varieties. For two of the WGC and Zeleny, three significant components of variation (PC) were found, and for the gluten index - two. Non-linear variation (PC2, PC3) was found for all indexes, but it was most pronounced for the WGC index (44%). For the other two ones, this share is significantly lower, against the background of a strong linear change from 70% (Zeleny) to 79% (GI). This situation is favorable because when the indexes change drastically because of the conditions, the genetic differences between the varieties should be largely preserved. In turn, this is a prerequisite for a higher degree of predictability of changes in the various conditions of the locations. Changes in the WGC index are both linear (45%) and non-linear (44%), which reduces the probability of predicting its values. However, the mean values of the index of the group of varieties in the locations differ reliably (Table 4).

Table 3. Type III Sums of squares analysis of variance for grain quality parameters

Statistics	**Environment (E)**	**Genotype (G)**	**GE**	**PC1**	**PC2**	**PC3**
	WGS					
Sum Sq	1172.45	387.1	743.42	419.54	194.38	129.48
Mean Sq	390.81	18.43	11.80	18.24	9.25	6.81
F value	839.51	1.56	33.78	52.22	26.5	19.51
*PV%	51	17	32	45	27	17
Pr(>F)	*0.000*	*0.0890*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
	GI					
Sum Sq	958.27	14638.15	5290.72	3073.75	1299.63	917.33
Mean Sq	319.42	697.05	83.97	133.64	61.88	48.28
F value	1.09	8.30	3.43	5.47	2.53	1.98
PV%	5	70	25	79	13	5
Pr(>F)	*0.4051*	*0.0000*	*0.0000*	*0.0000*	*0.0006*	*0.0118*
	ZELENY					
Sum Sq	7705.53	11062.98	5568.80	3913.75	1048.44	606.59
Mean Sq	2568.51	526.80	88.39	170.16	49.92	31.92
F value	102.27	5.95	13.93	26.82	7.87	5.03
PV%	50	30	20	70	20	6
Pr(>F)	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
Df	3	21	63	23	21	19

* - PV - **P**roportion (%) of all **v**ariation

Table 4. Wet Gluten content index (WGC) means, ranks* by locations and genotype by environment interaction (GE) of every single variety

Genotype / Location	Dobrich	Plovdiv	Trastenik	V. Tarnovo	GE
G1	32.1/2*	30.7/2	26.5/3	27.4/3	*
G2	26.6/3	30.3/3	25.7/4	26.0/7	***
G3	28.8/1	31.6/1	27/2	24.4/12	***
G4	31.4/5	29.1/5	24.7/7	23.7/14	***
G5	27.4/10	27.8/10	22.2/15	26.0/8	***
G6	30.4/12	27.1/12	25.1/5	27.1/4	***
G7	26.5/7	28.6/7	21.2/8	22.7/18	***
G8	30.9/14	26.5/14	21.1/9	22.5/19	***
G9	29.4/13	27.1/13	22.3/12	23.1/17	***
G10	27.8/4	29.8/4	22.5/11	21.6/22	***
G11	27.9/16	26.1/16	22.9/10	23.1/16	***
G12	26.7/21	25.6/21	22.1/16	29.1/1	***
G13	30.5/20	25.8/20	21.4/17	25.6/10	***
G14	28.4/11	27.2/11	25.0/6	24.1/13	***
G15	27.8/19	26.0/18	21.1/19	26.2/6	***
G16	26.2/6	28.7/6	22.3/13	21.8/21	***
G17	28.5/18	26.0/19	20.9/20	27.5/2	***
G18	27.5/17	26.1/17	22.2/14	23.2/15	ns
G19	25.2/15	26.4/15	27.3/1	21.2/20	***
G20	26.9/9	27.9/9	21.3/18	26.0/9	***
G21	27.9/22	23.7/22	18.9/21	26.3/5	***
G22	24.6/8	28.3/8	18.3/22	24.9/11	***
Mean	28.2	27.6	23.0	24.8	
HG**	d	c	a	b	

* The number after the slash is the rank of the variety in the location,
** HG - Homogeneous groups by location (Multiple Range Tests in Statgraphics XVIII)

The arrangement of varieties according to the WGC shows a different rank for some of the varieties in the studied sites (Table 4). In some of them (G1, G9, G18) the ranks are similar, in others (G10, G22) they change significantly. It is likely this change in ranks is also due to the proven GE interaction, in each cultivar, with the only exception being cultivar G18, or to the strong proportion of non-linear change (PC2+PC3=44%). Significant changes in individual varieties in the conditions of the selected locations are a good prerequisite for analyzing their stability.

**Table 5. ***Rank of WGC means and ranks of stability parameters by varieties

	Statistics	**CVi**	**bi**	**s2di**	**Wi2**	**Di**	**StabVar**	**YSi**	**Si(1)**	**Si(2)**	**Si(3)**	**Si(6)**	**TOP**	**NPi(1)**	**NPi(2)**	**NPi(3)**	**NPi(4)**
Genotype	**Mean**	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*
G1	1	5	8	2	2	2	2	1	2	2	2	7	1	2	18	20	19
G2	5	2	20	12	14	12	14	5	13	14	20	21	6	14	21	18	18
G3	2	9	9	19	18	19	18	2	20	20	18	22	2	20	22	22	22
G4	4	16	16	11	11	11	11	4	12	12	13	19	6	9	19	19	21
G5	8	8	4	6	4	6	4	8	3	4	5	4	16	5	3	3	3
G6	3	3	12	7	8	7	8	3	10	11	8	13	16	7	20	21	20
G7	17	10	1	9	9	9	9	18	9	10	10	12	16	15	11	8	8
G8	10	19	18	19	15	16	15	10	16	15	15	18	6	16	15	17	17
G9	13	15	14	4	6	4	6	14	6	5	6	5	16	6	5	5	5
G10	14	20	19	19	16	15	16	15	18	17	16	11	16	14	12	15	15
G11	18	6	5	3	3	3	3	19	4	3	3	2	16	4	2	2	2
G12	7	11	21	19	21	21	21	7	14	18	19	15	6	12	7	11	10
G13	9	18	15	13	13	13	13	9	17	16	14	14	16	19	14	14	13
G14	6	1	13	5	5	5	5	6	6	6	4	3	16	3	8	9	9
G15	15	13	3	10	10	10	10	16	7	7	9	8	16	8	4	4	4
G16	19	17	11	14	12	14	12	20	11	9	11	9	16	10	6	6	6
G17	11	14	7	19	17	17	17	11	16	13	22	20	6	18	13	13	14
G18	20	7	2	1	1	1	1	14	1	1	1	1	16	1	1	1	1
G19	17	4	22	19	22	18	22	18	19	19	21	16	6	17	9	10	11
G20	12	12	6	8	7	8	7	12	8	8	7	7	16	12	10	7	7
G21	21	21	10	19	20	22	20	21	22	22	17	17	16	22	17	16	16
G22	22	22	17	19	19	20	19	22	21	21	12	10	16	21	16	12	12

* - ranking of cultivars by each stability statistic is according to PBSTAT 3.0

The calculated ranks scores of the index mean, as well as those of the 16 parameters analyzed to evaluate the stability of the variety, are shown in Table 5. There are several aspects that need to be addressed. *First*, the ranks between the appearance of the trait and its stability score, in many cultivars, differ significantly. *Second*, the rank evaluations of each variety differ, depending on the parameter on which they are performed. For example, for G3, the parameter ranks range from 2 (YSi, TOP) to 22 [(Si(6), NPi(3)]. *Third*, the scores for each variety on several parameters match perfectly—bi with Di and NPi(3) with NPi(4). Other parameters, such as Si(2), Si(3), and Si(6) have very similar values that do not differ significantly at the individual cultivar level. *Fourth*, with this colorful picture of numerical cultivar information, it is difficult to accept which of the parameters should be applied for stability assessment. In this regard, there are many studies that point out a different set of indices as effective, creating confusion as to which ones should be applied (Kang, 2020; Cheshkova et al., 2020; Öztürk, 2022; Tsenov et al., 2022a).The choice of parameter/s depends largely on the trade-off between the mean value and the stability of the variety that can be allowed (Olivoto et al., 2019, Tsenov et al., 2022b). It also depends on the correlation that a given parameter shows with respect to the index mean (Flores et al., 1998).

Table 6. Correlations between indexes means and rank of stability statistics

№	Parameter	WGC	GI	ZELENY
1	CVi	0.44*	0.48*	0.56**
2	bi	-0.19	-0.30	0.06
3	s2di	0.13	0.11	-0.25
4	Wi2	0.13	-0.29	-0.13
5	Di	0.16	0.11	-0.25
6	StabVar	0.13	-0.29	-0.13
7	YSi	0.97**	0.92**	0.93**
8	Si(1)	0.14	-0.40	-0.13
9	Si(2)	0.07	-0.38	-0.19
10	Si(3)	-0.05	-0.03	-0.46*
11	Si(6)	-0.31	-0.65**	-0.69**
12	TOP	0.58**	0.73**	0.68**
13	NPi(1)	0.21	-0.32	-0.24
14	NPi(2)	-0.50*	-0.90**	-0.80**
15	NPi(3)	-0.61**	-0.88**	-0.83**
16	NPi(4)	-0.59**	-0.88**	-0.82**

The ratio of the stability parameters to the means of each of the indexes (their ranks) is different (Table 6). The parameters could be separated into three main groups: with positive correlation (Cvi, YSi, TOP); with a negative correlation [NPi(2), NPi(3), NPi(4)] and all others showing no reliable correlation. For subsequent evaluation, three parameters [(Wi2, YSi, NPi(3)] were adopted, which are typical representatives of each of the mentioned groups and are well-known and applied. High values of YSi are directly related to specific stability, while those of Wi2, NPi(3) express high general stability and adaptability. In this situation, the evaluation of the stability in all three quality indicators was made by spatial representation of the performance of the varieties in two ways: 1) by direct comparison of their performance and change induced from the entire interaction of the genotype with the environment GGE (stability) and 2) by direct comparison of the adaptability of each cultivar as assessed by each of the stability parameters.

The results of the combination of magnitude and stability for the WGC index can be traced in Figure 1-I. For comparison of cultivar stability, the ranks of all parameters are shown, and comments will be specifically directed to the three selected parameters outlined in black lines (Figure 1-II).

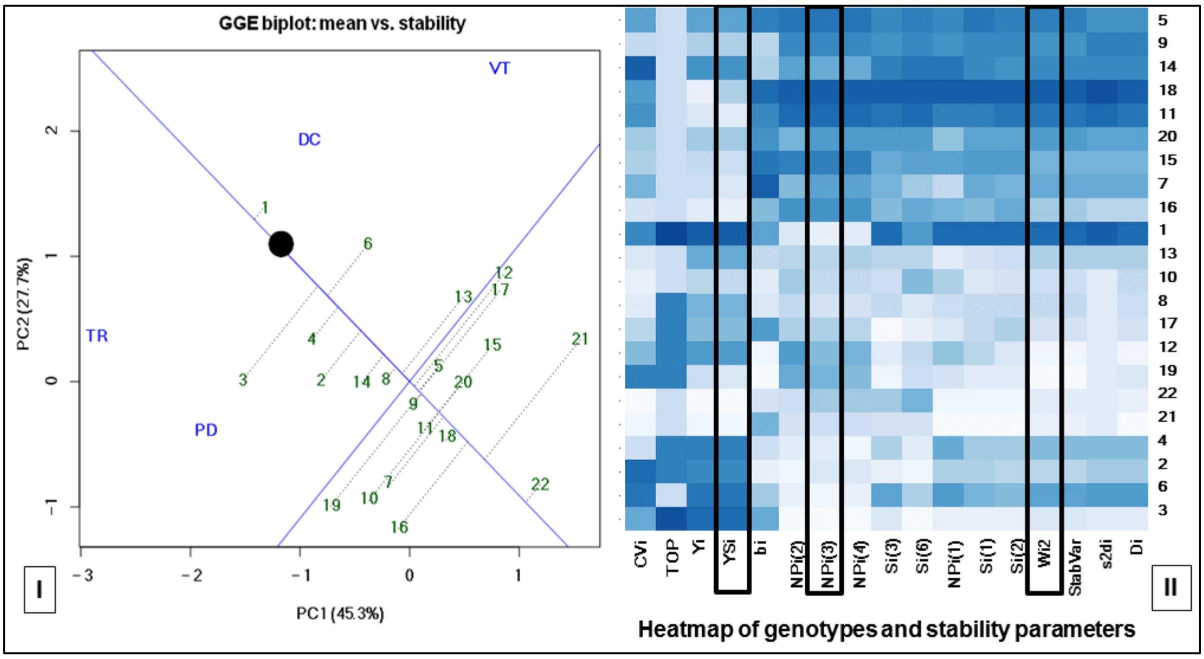

Figure 1. Wet gluten content index: GGE biplot of Means vs. Stability (**I**) and Heat map of variety and stability statistics (**II**)

The visual representation of the performance of the varieties and locations corresponds to almost 3/4 of the real picture (PC1+PC2=73%). Therefore, it can be assumed that the information extracted from it can be representative. The circle in black located on the right-bottom to left-top line represents (AEC=Average Environmental Coordination), the point to which the closer the variety is, the higher its means and stability as well. The stability of each variety is marked with a perpendicular dashed line, relative to the average stability of the variety group. The shorter this dotted line, the more stable the variety. The line parallel to these perpendiculars indicates the mean of the WGC for the whole group. Eight of the 22 cultivars showed higher means than the group mean (G1, G2, G3 G4, G6, G8, G13 and G14), of which G1, G2, G4, G8 and G14 were stable. The varieties G5, G9 and G22 are very stable, but their means are low. Therefore, the mean is not fundamentally related to the level of genotype stability. There are stable varieties whose means are high (G1 and G6) or varieties with low means (G18 and G22) which are very stable. There was a tendency for cultivars with a relatively lower WGC mean (G16, G17, G19, G21) to show greater variability than the top six, which had the highest means. The information about the stability of the mentioned varieties is fully confirmed by the intensity of staining in the left part of figure (II), with few exceptions (for the varieties G8 and G22), but specifying the high stability of the varieties G7, G11 and G20. The cultivars G1, G5 and G14, which are stable in most parameters, most likely have high general stability. The visualized evaluation of the cultivars and their parameters (Heatmap) fully confirms the picture of the performance of the varieties in Figure 1-I. It gives specific information about the magnitude of stability. The varieties G5, G9, G14 G18 possess both specific (Ysi) and general stability (Wi2). A few others are more general than specific (G7, G15, G16).

Table 7. Gluten index (GI) means and ranks* by locations

Genotype / **Location**	**Dobrich**	**Plovdiv**	**Trastenik**	**V. Tarnovo**	**GE**
G1	94/2*	100/1	90.3/3	100/1	ns
G2	92.3/4	96.7/3	90.2/4	95.3/2	ns
G3	94/3	96.6/2	88.6/5	92.3/3	ns
G4	85.7/8	93/4	92.7/1	87.7/4	ns
G5	94.7/1	77/11	87/6	85/5	**
G6	86.7/6	83/7	79.3/15	85/6	ns
G7	89.3/5	87.6/5	87/7	79.3/8	ns
G8	86.6/7	86.7/6	79.7/13	75.3/12	**
G9	72.3/21	70.3/16	77.3/19	74.3/13	ns
G10	74.3/17	82/8	77.6/18	73/14	ns
G11	77.2/14	67/21	91/2	75.6/11	**
G12	78/13	64.3/22	72.7/21	69.3/19	*
G13	73/19	74/14	83.7/10	76.7/9	*
G14	74.4/18	71.7/15	86.6/8	82.6/7	**
G15	72.7/20	69.3/17	84.3/9	75.7/10	**
G16	82.3/9	79.7/10	76.7/20	70.3/18	*
G17	76.2/15	68.2/19	79.7/14	70.7/16	ns
G18	78.6/11	75.5/13	78.3/17	66/21	ns
G19	76/16	81.2/9	71.3/22	62.3/22	**
G20	78.7/12	67.2/20	81.3/11	71.3/15	ns
G21	79.6/10	76.5/12	79/16	70.6/17	ns
G22	70/22	69.2/18	80/12	67/20	ns
Mean	81.2	79.1	82.5	77.5	
HG**	**c**	b	**c**	a	

* The number after the slash is the rank of the variety in the location,
** HG- Homogeneous Groups by Location (Multiple Range Tests in Statgraphics XVIII)

The performance of the varieties according to the GI in the locations is different, which is the reason why most of them have a different rank (Table 7). The change in the ranking of each cultivar is directly dependent on its interaction with environments (GE). Those of the varieties for which this interaction is absent (G1, G9 and G17) keep their ranks similar. Others such as G11, G14, and G19 change their place in the group strongly, probably because of their strong interaction with the conditions. There are a greater number of varieties (13) that show no interaction (GEns) than those that change significantly (9). The latter is probably related to the significant GE interaction found, for a group of cultivars. The established strong genetic control on the index (Table 3), which changes the means of the cultivars adequately to the change of conditions (PC1=79%), is probably the reason for these differences in their response.

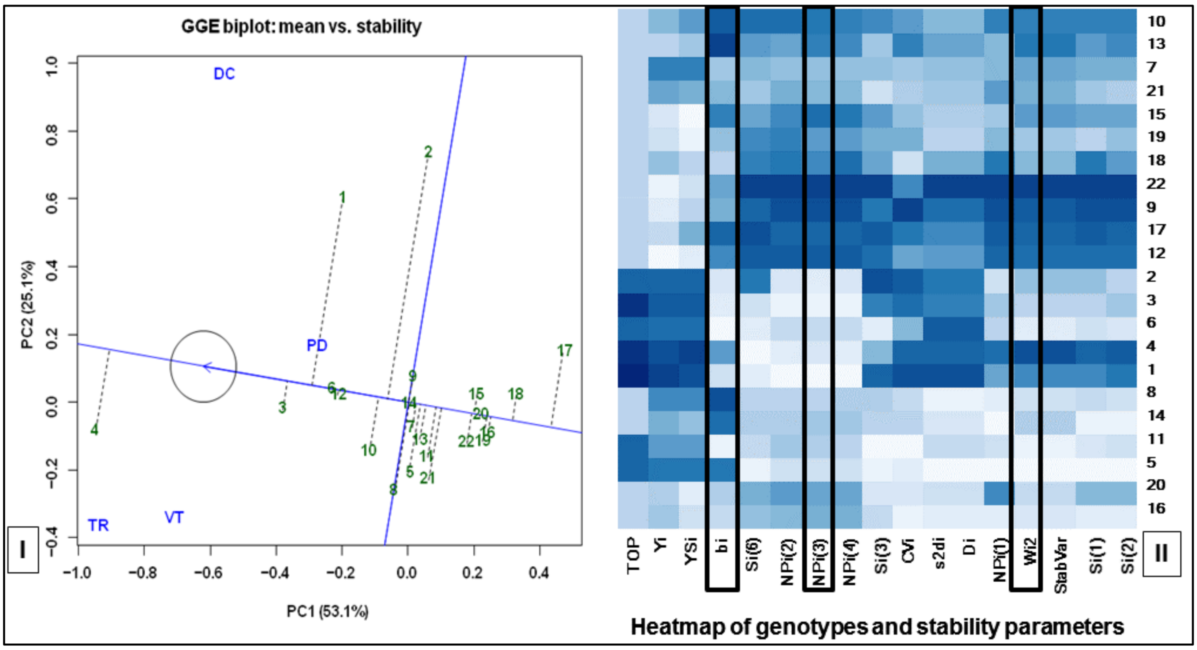

Figure 2. Gluten index: GGE biplot of Means vs. Stability (**I**) and Heat map of variety and stability statistics (**II**)

The appearances of the cultivars according to GI, as well as the parameters for evaluating their stability, are presented in Figure 2. In general, this trait varies significantly less than the others in the study. Only a few varieties showed a relatively strong change – G1, G2, G4, G8, G17 and G21. All others showed low variability, although some showed a reliable interaction with conditions (G12, G13, G14, G15, G16) (Table 6). Varieties with the highest values of the indicator appear to be the most variable (G1, G2, G4). Therefore, a combination of size and stability should be sought in varieties with medium grain quality (fillers). Typical representatives of such a combination are the varieties - G6, G7, and G9. Another group of varieties - G9, G12, G17 and G22 possess both specific and general stability (Figure 2-II).

Table 8. Zeleny sedimentation index (Zeleny) means and ranks* by locations

Genotype / Location	Dobrich	Plovdiv	Trastenik	V. Tarnovo	GE
G1	63/6	67/1	52/8	54/6	***
G2	59/10	54/11	62/3	55/3	*
G3	64/3	61/4	65/1	58/1	ns
G4	64/4	50/14	39/20	33/20	***
G5	61/9	55/7	53/6	43/11	***
G6	66/2	52/13	57/4	49/7	*
G7	64/5	54/12	54/5	55/5	***
G8	72/1	57/6	53/7	44/9	***
G9	63/7	62/3	51/9	55/4	ns
G10	56/13	47/18	63/2	57/2	ns
G11	48/21	55/9	49/10	40/15	ns
G12	63/8	55/8	48/11	44/10	ns
G13	57/12	55/10	44/13	39/17	ns
G14	54/16	48/15	43/15	40/14	**
G15	56/14	44/20	46/12	41/12	***
G16	58/11	60/5	42/17	28/21	ns
G17	56/15	47/19	38/21	35/19	*
G18	53/18	48/17	41/18	41/13	*
G19	51/19	65/2	42/16	38/18	***
G20	54/17	48/16	44/14	46/8	ns
G21	49/20	39/21	33/22	25/22	***
G22	46/22	35/22	40/19	40/16	ns
Mean	58.0	53.0	48.0	44.0	
HG**	d	c	b	a	

* The number after the slash is the rank of the variety in the location, ** HG- Homogeneous Groups by Location (Multiple Range Tests in Statgraphics XVIII)

In the case of the Zeleny index, the change in the ranking of the variety by location is not related to its interaction with the conditions (Table 8). Nine of all varieties are not affected by the conditions directly (G3, G9, G10, G11, G12, G13, G16, G20, G22) and yet their ranks change significantly (G11, G16). The performance of individual varieties does not affect the mean of the group at a given location. Despite the change in conditions, the group means differed significantly. With this complex picture of ranking varieties, it would be difficult to make even a simple comparison between the stability and the variety mean against the others. There is a tendency for varieties with higher means to change significantly (GE*), while for those with medium high means (G9, G10, G11, G12, G13) this is absent (GEns).

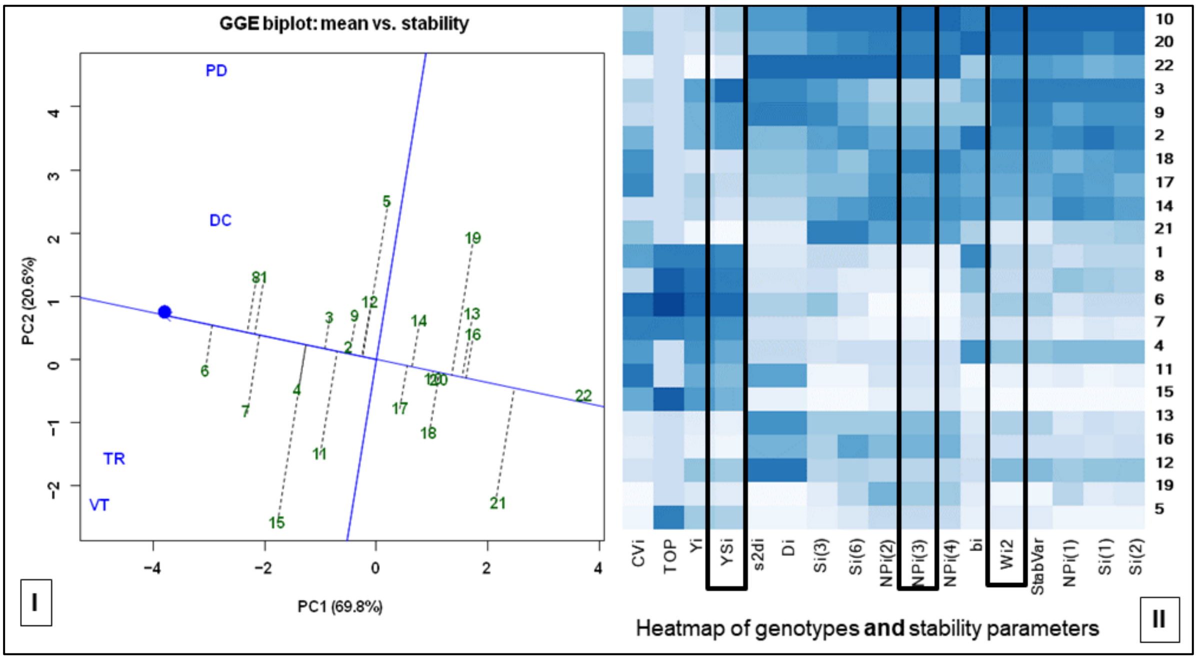

Figure 3. Zeleny test: GGE biplot of Means vs. Stability (**I**) and Heat map of variety and stability statistics (**II**).

The change in the index is strong for a relatively small number of varieties - G5, G15, and G19 (Figure 3-I). The most stable varieties can be defined as G2, G3, G4, G9, G10, G15, G20, G22, and all the others have an average degree of stability compared to the group mean. The stability of these varieties is fully confirmed if their staining intensity is observed against the three parameters accepted for evaluation (Figure 3-II). The varieties listed in order from top to bottom in the figure have the highest general stability - G10, G20, G22, G3, G9, G2, G18, G17, and G14.

The availability of dozens of parameters for stability evaluation has caused researchers to look for ways to use combinations of parameters. Such as KR (Pour-Aboughadareh et al., 2019; 2022), or AR6 (Gubatov & Delibaltova, 2020), which are derivatives of a group of single parameters, were applied because they show high correlations with both yield and stability. This was done at the level of a single trait, and here the situation is how to extract information about the stability of the variety using all three investigated quality indexes.

Table 9. Correlations between the 3-parameter stability average (~stab3) and the 16-parameter stability average (Stab-16), and the ranking of the parameters involved in the combination

Variables	*Stab-16	***Mean Rank	Wi2	YSi	NPi(3)
**WGC-stab3	**0.95**	0.28	**0.92**	0.37	**0.49**
p-values	*0.0001*	*0.2062*	*0.0001*	*0.0945*	*0.0209*
#R^2	**0.91**	0.08	**0.85**	0.13	0.24
**GI-stab3	**0.93**	-0.18	**0.96**	0.18	**0.56**
p-value	*0.0001*	*0.4222*	*0.0001*	*0.4297*	*0.0072*
R^2	0.87	0.03	**0.93**	0.03	0.31
**ZELENY-stab3	**0.95**	-0.02	**0.98**	0.27	**0.52**
p-value	*0.0001*	*0.9413*	*0.0001*	*0.2323*	*0.0131*
R^2	**0.90**	0.01	**0.95**	0.07	0.27
****WGS+GI+ZEL-stab3	**0.96**				
p-value	*0.0001*				
R^2	**0.93**				

* - Stability rank from average of all 16 stability parameters; **(~stab3) - Stability rank from three stability parameters [(Wi2, YSi, NPi(3)]; *** - Rank of respect index mean; ****WGS+GI+ZEL-stab3 - Stability rank from three quality indexes (WGC, GI, ZELENY); # R^2 – coefficient of determination; Values in **bold** are different from 0 with a significance level alpha=0.01.

A combined assessment including the stability of each of the three parameters would indicate which cultivars are stable in grain quality, regardless of their levels. An average rank score obtained by averaging the ranks of each of them [Wi2, Ysi, NPi(3)] can be used effectively because there are significant correlations with the average stability of the 16 parameters (Table 9). The parameter (Wi2) shows significant correlations with both integral evaluations (stab-3, stab-16) which shows its high applicability in stability evaluation even alone. Similar effectiveness for this parameter has been reported repeatedly in research (Verma et al., 2018; Vaezi at al., 2019; Mohammadi et al., 2021). When combining (averaging) the ranks of the three parameters for each index, an integral rank (WGS+GI+ZEL-stab3) will be obtained, the correlation of which is as high as the single ranks of each of the analyzed indicators (~stab). This integral rank can be used directly to derive information about the rank of each variety in terms of yield and quality within the group.

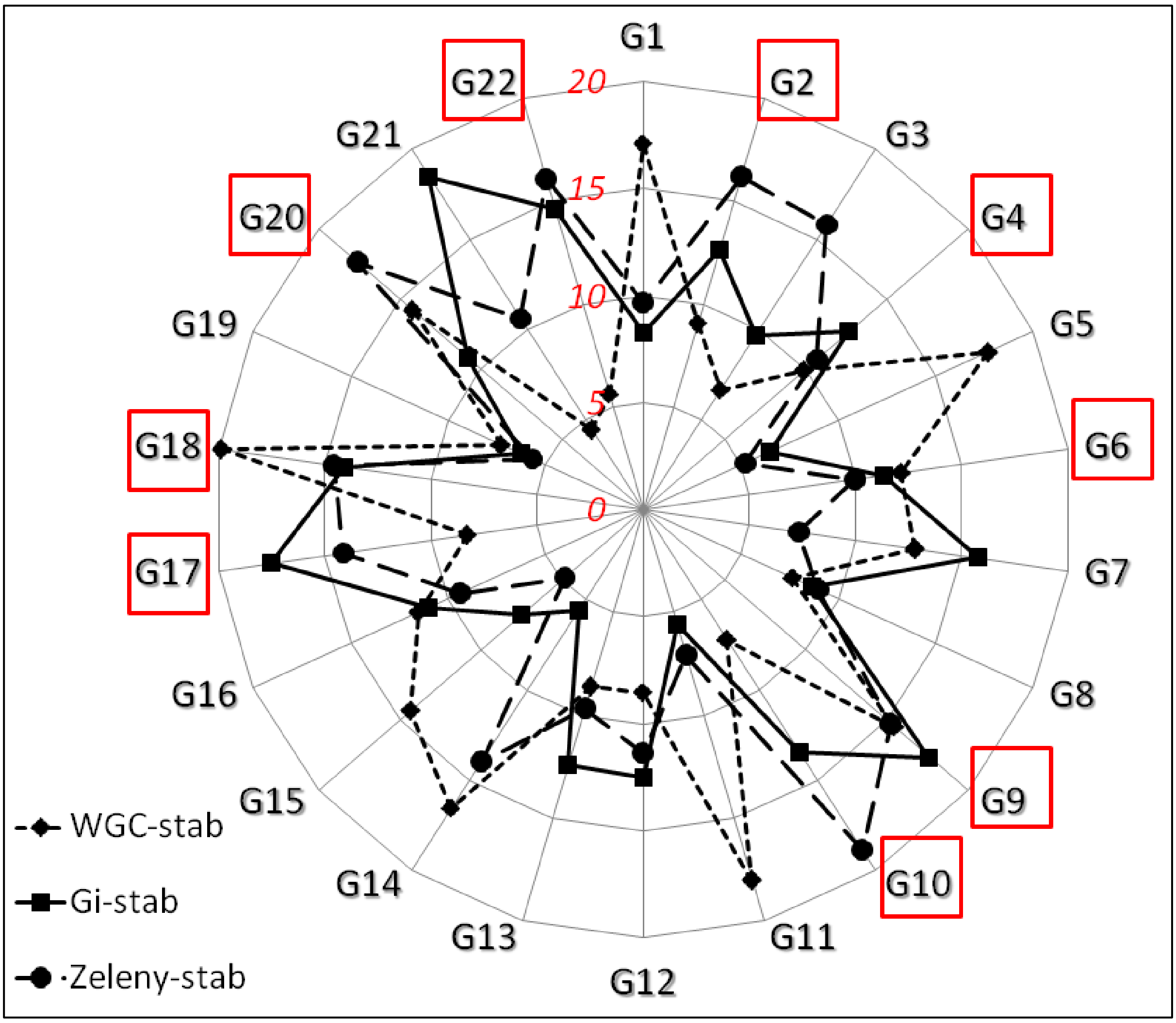

Figure 4. Ranking of varieties by stability according to the three indicators studied (high ranks indicate high stability)

Marking of the most stable varieties was done based on parameter values in the following order of importance: GI, Zeleny and WGS (Figure 4). It is related to the degree of genetic control of the indexes (Table 2), as well as to the interaction of each variety with environmental conditions (Tables 4, 7, 8). The varieties that have shown stability according to each of the three indexes are a total of about 40% of those studied. Three are the high-quality varieties that are stable against the background of the research locations (G2, G4 and G6). They are significantly affected by conditions (GE) in WGC, Zeleny indexes, but not in GI (Tables 4, 7, 8). In the absence of GE interaction on all three indexes, two more groups of varieties (G9, G10), with medium quality (fillers) and low grain quality (G17, G18, G20, G22) are stable. Therefore, the stability of varieties does not depend on their quality level. High-quality stability can be compromised with high grain yield, which is very rarely observed (Figure 5).

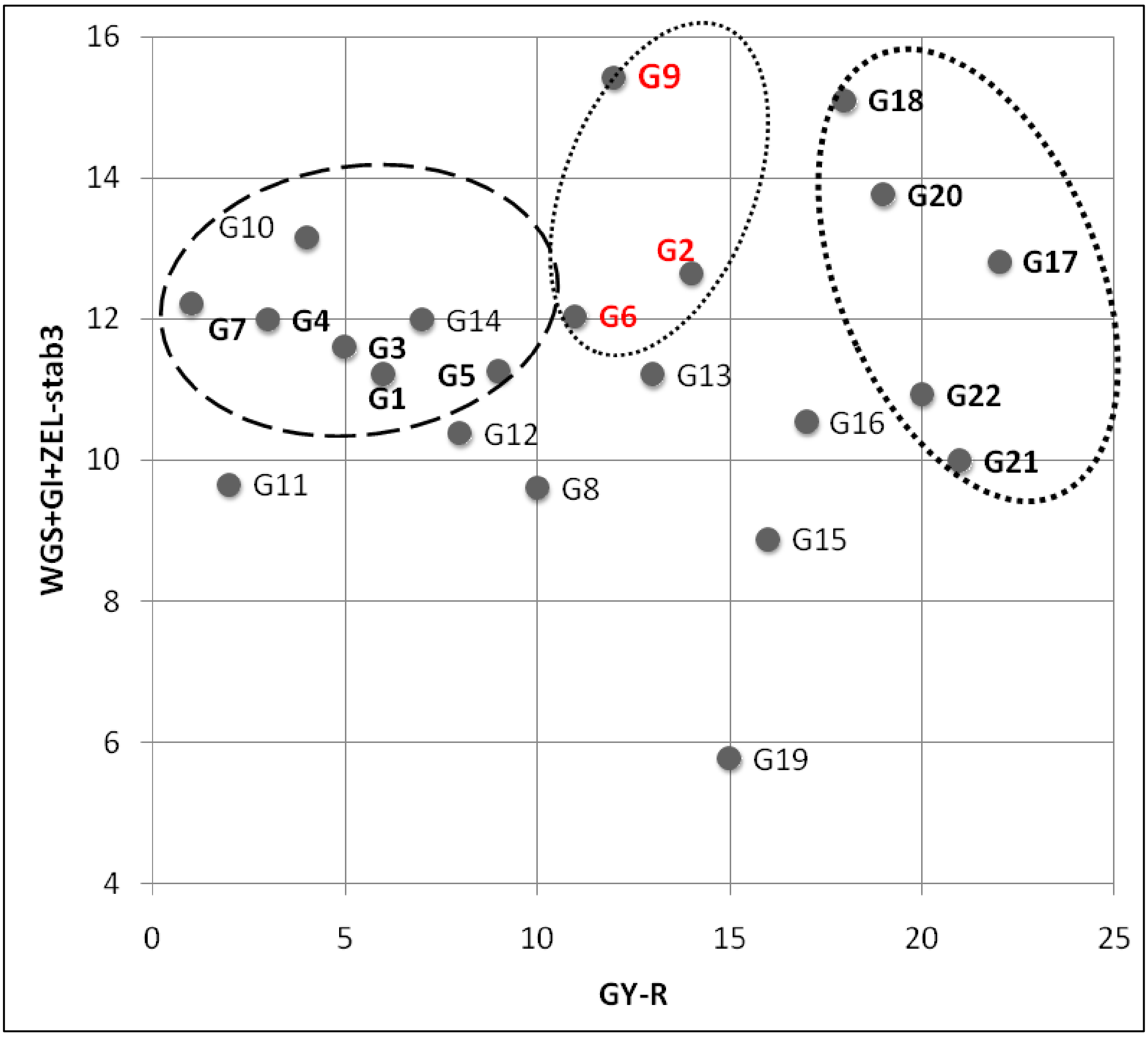

Figure 5. Scatter plot of grain quality stability versus grain yield ranks of the cultivars studied, (WGS+GI+ZEL-stab3 - Stability mean rank from three quality indexes (WGC, GI, ZELENY)

Varieties are mainly located in several groups: with high (right) and low grain yield (from left) and those with medium yield and quality. Only three varieties possess a combination between these two categories, at the highest possible level (G2, G6, G9). The first two are especially valuable because they are significantly influenced by the conditions of all three studied parameters (Tables 4, 7, 8). Relatively high stability of quality (rank 10-12) with medium-high grain yield (rank 5-10) shows the varieties G1, G3, and G5, whose quality in all three indicators is high.

Wet gluten content (WGC) is an index whose quantitative expression is related to the quality level of the grain. It is determined quickly and easily and is therefore mandatory for characterizing the quality of individual varieties. Environmental conditions alone and GE interaction strongly influence its variability. The data here broadly confirm the patterns established by Alemu & Gerenfes, (2021), but are in stark contrast to the results of Ma et al. (2021) who reported no GE interaction. On the other hand, Branković et al., (2018) show that it is possible to increase the WGC by breeding by 10-18%, against the background of relatively strong influences of genotype (G) and GE interactions and a significantly weaker influence of (E). Here, the influence of the environment is strongly emphasized (GE) even in each of the cultivars involved in the study, without G18 (Tables 4). The established high values of the principal components of interaction cause changes in different directions of the change of conditions, which further complicates the variation. Regardless of the established changes, the varieties managed to preserve their values in a large part of the locations. Some of them are stable regardless of the magnitude of their WGC means. Therefore, in a wide range of different environments, it is possible to combine a high index means with high stability (Figure 1). However, this stability is associated with a low mean of the indexes (Atanasova et al., 2009). Varieties with higher means averaged over the entire experiment (G1, G2, G4, G8, G14) showed high stability, while some of those with lower means (G15, G16, G17, G21) showed high variability. Therefore, the stability of the variety in the WGC index is not related to its size. The results here are analogous to the opinion of (Khazratkulova et al., 2015), according to which combining high index means with stability in a small number of varieties is possible.

The gluten index (GI) is a parameter whose value largely reflects the genetic potentialities of the grain quality level (Bonfil & Posner, 2012). The role of the "genotype" for its performance and change is predominant over environmental conditions (Öztürk, 2020; Alemu & Gerenfes, 2021; Vida et al., 2022). The data in this study fully support these views, with genetic control accounting for 70% of its variation. On the other hand, the interaction (GE) determines about ¼ of the index variation. It changes mostly adequately (linearly) to changing conditions (PC1=79%) and only 13% is due to non-linear change. This increases the probability of predicting its values by location. Nine of the 22 cultivars showed an interaction with conditions that are likely related to exactly this partition. In this group are those whose values are around and below the average for the group. The number of stable varieties is greater, compared to the variable ones, which are only six. The results presented here outline a trend for high stability in cultivars whose values were around the trial mean (G7, G9, G13, G14, G15) that showed a reliable interaction with conditions (Table 7). On the other hand, the cultivars with the highest (G1, G2, G4) and lowest values (G17, G21) turned out to be the most variable, which was unexpected because they did not show an interaction with the conditions (GEns). The probable explanation for this is their strong linear performance when the conditions change from favorable to unfavorable for the index in the different seasons of the locations.

Several cultivars have a favorable combination of GI stability and high grain yield (G9, G17, G21). The reported lack of correlation between this index and yield (Kasahun & Alemu, 2022; Öztürk, 2022) in proven interaction (GE) is a reason to assume that the gluten index can be used for direct selection in breeding. Research data in climatically similar regions of the Balkan Peninsula confirm this statement (Öztürk, 2022; Ilin et al., 2022). Changes occurring in quality (GI) can change correlations with grain yield, in a direction that allows a progressive increase of the latter, without negatively affecting the quality (Herrera, et al., 2020; Ilin et al., 2022)

The sedimentation value (Zeleny) is the most popular quality index because it has proven high positive correlations with a few other indexes (Chamurliyski et al., 2016; Branković et al., 2018). This makes it an effective breeding tool in efforts to successfully combine grain yield and quality (Tsenov et al., 2021). Research on the role of conditions on the metric is substantively different. Here, the change in the measure is strongly related to the changes in conditions, with the role of genotype accounting for about one-third of it, while the interaction with conditions is about one-fifth of the total variation. "Genotype" is the dominant factor on the index according to Desheva (2016), while Mut et al. (2010) showed that it is modified equally by “genotype” and “conditions” and the interaction between them. Pengpeng et al. (2022) reported a strong influence of the environment (E) and a significant interaction with genotype (GE). Clearly, the impact of the environment is significant and should be accounted for in the index analysis. Varieties interact with changing conditions in different ways, which is reflected in their stability. The stability does not depend on the genetic potential of the cultivar, which is contrary to previous studies (Tsenov et al., 2013; Morgounov et al., 2014). In their efforts to determine the stability of a group of cultivars, few authors pay attention to these details. Important in this regard are the varieties (G2, G3, G4, G8, G9), whose high means are realized with relatively small deviations in the test locations. In this index, this is illustrated by all the stability parameters and in particular, the three specifically analyzed their representatives (Figure 3-II). The Zeleny index can be used to assess quality stability because it allows cultivars to be easily identified. Practically except for G5 and G7, all other high-quality varieties show stability that can be considered sufficiently high. Analogous regularities in Italy for common and durum wheat were established by Bosi et al., (2022).

The combined (integral) evaluation of the quality through the three indexes proves to be a suitable tool for assessing the stability of the variety. When it is compared with the amount of grain yield of the varieties, those of them possess the combination of grain yield and grain quality that is constantly monitored in the breeding activities. This approach is easily applicable and gives sufficient information about the combinations in real varieties of the two most important aspects of the grain. An analogous approach could be applied to the combination of yield and quality in terms of their stability. However, this will direct attention not so much to the productive, as to the stable yielding varieties. After a direct comparison of data from different traits, an optimal combination between the two aspects of the grain in single varieties was found (Khazratkulova et al., 2015; Bosi et al., 2022).

Conclusion

Each of the three investigated quality indexes is affected by the genotype by environment interaction (GE). This strongly affects the variation of every variety, apart from a few varieties in the GI.

The most stable genetically is the Gluten Index, where the varietal variability is relatively the weakest compared to the other indexes.

For each individual quality character, groups with different stability of varieties have been established, which has no relation to the performance of their grain quality means.

A correct assessment of the quality stability of the variety can be made by each of the indexes used, which is illustrated by a series of correlations between stability statistics and quality parameters.

The stability of the variety could be established by combining the rank estimates of each index into a single "integral" rank, the performance of which approximates that of each of the indexes separately.

The applied approach for integrated evaluation of the yield and stability of the quality of each variety is an effective way to determine those that have a high breeding value in both aspects of the grain - yield and quality.

The most valuable for the breeding, as a performance and stability in grain quality, are the varieties G2, G6, and G9, the yields of which are higher than the mean of the group.

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