DOI: 10.22620/agrisci.2022.35.005

ANALYSIS OF GENOTYPE BY ENVIRONMENTAL INTERACTION OF COMMON WHEAT (*Triticum Aestivum* L.) BY NON-PARAMETRIC STABILITY METHODS

Nikolay Tsenov[1]*, Todor Gubatov[1], Ivan Yanchev[2]

[1] Department of Wheat Breeding and Technology, Agronom Breeding Company, Dobrich, Bulgaria
[2] Agricultural University, 4000 Plovdiv, Bulgaria
***e-mail: nick.tsenov@gmail.com**

Abstract

Data from the Multi Environmental Field Trail (MET), which examined 24 varieties of common wheat, were divided into three "datasets" related to three of the five study locations. Using meta-analysis, these three data sets were compared with those from the whole experiment. The aim of the study is to determine whether a 4-year growing period in three country-specific locations, it is possible to establish a significant impact of the environment on the stability of a group of varieties. The analysis of the genotype x environment interaction (GEI) was performed in parallel in the three groups of data, which were compared with the entire MET database. A direct comparison was made on them regarding the possibilities of non-parametric methods to assess the stability of the variety. The analysis of the results of the four "datasets" is done through a number of statistical approaches, allowing them to be correctly compared at different levels. Genotype x environment interaction was found in each of the studied locations. The variation in yield in them is a result of the direct effect of the "year" and the combined effect of the genotype x year. At all three locations, the GEI is broken down into four main components, which is evidence of the strong linear and non-linear nature of the dispersion of grain yield. These results are a prerequisite for an objective assessment of genotype stability. All applied parameters give completely similar stability information for each of them, regardless of the test location. Data from one location are sufficient to assess the stability of each variety in a group. This may be the case if significant differences between the seasons of the trial, are found. The applied non-parametric methods for stability assessment give correct information about the varieties, in the presence of GEI, regardless of the conditions from which the data for analysis are selected.

Keywords: wheat, genotype x environment, stability, nonparametric methods

Abbreviations: AMMI - Additional main effects and multilayer interaction, GEI - interaction of the genotype with the conditions, PCA - Analysis of the Principal (main) Components, PC1.2… i – Principal component 1… 2… i

INTRODUCTION

Genotype x environment interaction (GEI) should not be confused with phenotypic variation (Kang, 2020). Schlichting, (1986) defines phenotypic plasticity as the ability of a plant organism to change its physiology and/or morphology as a result of changes in environmental conditions. de Leon et al., (2016) present GEI as a unique and distinctive response of different genotypes to different environments. GEI occurs when the response rate of different varieties is not parallel, ie they intersect, diverge or merge, with similar changes in conditions (van Eewijk et al., 2016). The cross-interaction that causes a difference in the performance (variability) of the variety underlies its plasticity and/or adaptability (Cooper et al., 1999). The response of each genotype to changing environmental conditions (season, location or technological approaches) is measured by the degree and direction of change that GEI causes in it (Singh et al., 1999). Stability is the ability of a variety to realize its genetic makings in a wide range of different environmental conditions (Annicchiarico, 2002). For this reason, it is subject to evaluation because it reveals the complex and unique performance of each variety, provoked by changing conditions.

The assessment of stability or plasticity is done through specialized approaches, which, depending on their nature, are called parameters, indices or methods (Kang, 2020; Pour-Aboughadareh et al., 2022). The division of the evaluation parameters is made on the basis of several essentially different criteria. *The first* is the way in which the variety change is explained. Becker & Leon (1988) are the authors of the idea of grouping assessment methods known as "static" and "dynamic" concepts of stability. Minimal change in yield when conditions change is also called "biological" stability of the variety. The "dynamic" concept, also known as the "agronomic" concept, defines as stable those varieties that have a minimal deviation from the predicted average response of the whole group of varieties after changing environments. *The second* is the concept proposed by Flores et al. (1998) divides the assessment methods into three main groups according to the size of the trade-off between yield and stability, depending on the specific weight (criterion) chosen to assess yield stability, different sets of parameters belonging to these groups are applied.

The third criterion is related to the nature of the value. In addition, these methods can be divided into two groups: one-dimensional (univariate) and multidimensional (multivariate) (Mohammadi et al., 2021). A typical example of a single type of parameters are the indicators related to regression analysis: regression coefficient (bi) and deviation from the regression line (s^2d), coefficient of variation (CV), stability variance (σ^2), ecovalence (W^2). Multivariate are the parameters resulting from analysis by PCA and AMMI (ASV, ASI, EV), (Pour-Aboughadareh et al., 2022).

The fourth criterion for grouping evaluation parameters is based on the way their values are formed (Kang, 1997; Kang, 2020). There are two groups of parameters: parametric and non-parametric (Flores et al., 1998). Parametric is this statistical parameter in which specific assumptions are made for the distribution of a known population. The non-parametric parameter is defined as a hypothesis test that does not require the distribution of the population to be denoted by specific parameters.

Recently, researchers are increasingly using preferably the non-parametric type of methods for evaluating breeding materials (Mohammadi et al., 2021; Vaezi et al., 2022). Non-parametric models for evaluation of varieties and have some advantages over parametric: 1- reduction of deviations caused by extreme values as a result of the conditions, 2- no assumptions are needed for the normal distribution of the analysed values of the trait, 3- the presence of homogeneity of deviations and additivity (linearity) of reactions for which it is not necessary to comply with any restrictive requirements (Hühn, 1996).

Nonparametric methods are based on rank estimates of genotype representation under different conditions. In order to calculate them, the average yield data must be transformed into rankings for each genotype and/or environment. A genotype is considered stable if its ranks are similar in different environments. The lowest value of each of these parameters indicates high stability for a particular genotype (Nassar and Hühn, 1987).

Nonparametric parameters for stability assessment were first proposed by Huhn (1990) and Nassar and Huhn (1987), in four variants [S(1), S(2), S(3), S(6)]. A set of four [NP(1), NP(2), NP(3), NP(4)] are alternative nonparametric stability assessment methods defined by Thennarasu (1995). These are based on the series of corrected rank estimates of genotypes in each experimental environment, through the values of their arithmetic means and medians. The lower the values of this parameter, the higher the stability of the genotype they show.

Another non-parametric model of stability is ***TOR***. Fox et al. (1990) propose an ideal and simple parameter for a measure of the superiority of general adaptability. This method is a ranking approach that involves estimating the number of test environments in which each variety is ranked in the Low, Mid, and TOP thirds of the ranking in the experiment. The genotype, which occurs mainly in the upper third (high value), is considered a variety with wide adaptation (stability). The share of the environments in which the genotype appeared in each of these groups is determined in order to form the magnitude of the nonparametric rank.

The statistical parameter ***(KR***, Kang's Rank-sum) was introduced by Kang (1988) in an attempt to simultaneously select high-yielding and stable varieties of corn in different growing conditions. It uses yield variance and stability variance (σ^2_i) (Sukla 1972) as parts of the new combined index. Each variety receives a yield rank and a stability rank. The new index (rank) is obtained after adding the yield ranks and stability. In this way, the index (*KR*) gives the same weight of yield and stability of the variety. The lower the value of the parameter (the sum of the two ranks), the more valuable the variety is, because it has the desired compromise between yield and stability.

The yield stability index (***YSi***) is calculated by the method of Kang (1993), which compromises between yield and variety stability. Each variety receives a rank rating for yield and stability, separately. The variety with the highest yield value and the one with the least variation (high stability) receive a rank of 1. The ranking of each variety by yield is adjusted by assessing its stability. This adjustment is made according to the variation of stability (σ^2i) (Shukla 1972), as follows: when measured significant variation in stability, relative to the average level of experience, the values of 8, or 4, or 2 with statistical reliability are added to the yield rank the difference of $p < 0.01$, $p < 0.05$ and $p < 0.10$, respectively. When the variation is found to be lower, these correction values are added to the yield range and vice versa, they are subtracted, when the variation is higher, than average. In this way, a statistically adjusted grain yield is obtained, designated by the author as (*YSi* = Yield stability index). The final rank of each variety is obtained after arranging the values of this parameter, with the variety with the highest value of the parameter receiving rank 1.

A measure of total variety superiority (***Pi***), based on test location data is defined as the mean square of the difference between the variety yield and the maximum yield at each of the locations (Lin & Binns, (1988). The smaller the value of Pi, the smaller the distance to the genotype with maximum yield, i.e. so the variety is better.

The advantages of nonparametric methods have been highlighted many times (El-Hashash et al., 2019; Cubukcu et al., 2021), which makes them desirable and suitable for analysis of the data collected in this study. According to Segherloo et al., (2008) and Mohammadi et al., (2016) the rank estimates of some of these parameters are generally assigned to the biological concept of evaluation [NP(2-4)], some to the agronomic one (*Pi, YSi, KR, TOP*), (Flores et al., 1998; Vaezi et al., 2019). This is an additional motive for use, due to the existing contrast between them, which would enrich the information about their effectiveness.

The purpose of this study is to determine whether in field experiments conducted in one location it is possible to establish a significant influence of the environment on the stability of a group of varieties. In order to answer this important question for the selection, a direct comparison was made between the data from several grain production locations, conditionally divided into several "databases".

MATERIALS AND METHODS

Situation of experiment

In a multi environment field experiment, 24 varieties of winter common wheat were studied in five regions of the country (Dobrich, Trastenik, Veliko Tarnovo, Plovdiv and Yambol) over a period of four years (2009-2012). Some of the data collected by them, in connection with the possibilities for evaluation of varieties in grain yield and stability, have already been published (Tsenov et al., 2022a, 2022b). In this study, data from three (Dobrich, Trastenik and Yambol) out of a total of five locations were analysed separately to establish the interaction of genotype x environment in them. It is assumed that the year as a factor (four "environments") would have a similar effect to the factor on the yield. In other words, is it possible that four-year conditions in one place cause a proven interaction of genotype x environment in grain yield? To prove this claim, a direct comparison was made between the results of the GEI analyses from each location with those from the whole field experience.

Statistical analyses

The data from each item, as well as those from the whole experience, were "accepted" as separate "datasets". Therefore, four sets of data were compared: from all locations, from Dobrich, from Trastenik and from Yambol. The data grouped in this way are analysed in several main areas: 1) establishing the characteristics of the genotype x environment interaction, 2) analysing the possibilities for assessing the yield and stability of each variety, in the individual locations and 3) determining a set of stability assessment parameters as effective, regardless of the database selected for analysis.

Statistical analyses were performed after using the software packages GEA-R (Pacheco et al., 2015), PBSTAT 3.03 (Suwarno et al., 2008), Stabilitysoft (Pour-Aboughadareh et al., 2019). The analysis of the variants of the data groups, which was made through the GEA-R package, was considered to determine the situation. From the set of parameters for stability assessment from each of them are selected mainly those that are non-parametric indices (Table 1). The choice of this type of parameters was made in order to avoid the limitations accompanying the parametric type of models related to normal data distribution or homogeneity / heterogeneity of variation. This approach provides the most effective way to compare the varieties in the individual "datasets" by simply classifying (ranking) the varieties in them.

Table 1. Information on all the methods of stability analysis studied

Designation of parameters	Statistical name	Statistical program
bi	"Regression coefficient"	
Pi	"Superiority measure (index)"	GEA-R *
σ^2_i (*sigma*)	"Shukla's stability variance"	
W^2	"Wricke's ecovalence"	
NP(1), NP(2), NP(3), NP(4)	"Thennarasu's nonparametric stability parameters"	
S(1), S(2), S(3), S(6)	"Huehn's nonparametric stability parameters"	PBSTAT
TOP	"Fox's TOP"	
YSi	"Kang's yield and stability index"	
AR	"Average Rank of ranking"	
KR	"Kang's rank-sum index"	Stabilitisoft

* - GEA-R (Pacheco et al., 2015); PBSTAT (Suwarno et al., 2008); STABILITISOFT (Pour-Aboughadareh et al., 2019)

The statistical package META-R (Alvarado et al., 2020) was used to determine whether there are correlations between the different “conditions”. The correlations between the applied non-parametric methods were established by the program JASP 0.16, through the module for Bayesian type of data processing. With this program the regression analysis is made, the Stepwise model, this separates the most effective interactions of parameters up to the 3rd level (models), for each „database”. The application of Principled Component Analysis (PCA) and cluster analysis was done through the statistical program Minitab 17.

RESULTS

Each of the studied varieties shows a different ranking compared to the others, in the individual items (Table 2). How to determine the most valuable varieties in the group, since their order (rank) is so different when conditions change? The first-ranked variety № 24 has a large fluctuation in the yield in Trastenik (9th place). The next few varieties, ranked 2nd, 3rd, 4th and 5th, (17, 1, 6, 14), respectively, also have large fluctuations in their performance in different conditions of the locations. Grade № 12, which ranks only 23rd, on average from all locations, is in the top three in Dobrich. On the other hand, variety № 18, which is in the middle of the ranking with a rank of 10, in two of the locations is in last place in terms of yield (Dobrich and Trastenik), while in Yambol is in 7th place. It is quite clear that averaging the yield from all locations is not a correct way to determine the value of a variety in the group.

Such fluctuation in the ranking of each of the varieties, which is regularly observed in experiments, is a significant reason for the size and stability of their yield to be assessed using a set of statistical methods. However, their application is related to the basic requirement that there is an effect on yield due to the interaction between genotype and environment. Analysing its features in detail is a way to vary the yield in "size" and direction, not only for the whole experience, but also for each of the varieties in it.

Table 2. Grain yield rank of the varieties in the individual locations

Location Genotype	Dobrich Rank-D	Trastenik Rank-T	Yambol Rank-Y	**Mean Rank-M**
1	4	8	3	**3**
2	22	20	16	**24**
3	11	14	23	**21**
4	23	22	6	**6**
5	19	17	24	**18**
6	6	4	17	**4**
7	12	23	18	**12**
8	18	15	20	**22**
9	20	11	13	**9**
10	9	18	15	**19**
11	17	21	8	**15**
12	2	10	21	**23**
13	13	16	19	**20**
14	10	7	10	**5**
15	14	13	11	**11**
16	15	2	12	**13**
17	21	12	5	**2**
18	24	24	7	**10**
19	8	5	22	**14**
20	16	19	14	**17**
21	5	6	9	**16**
22	1	1	4	**8**
23	7	3	1	**7**
24	3	9	2	**1**

The results of the variance of the yield by groups of locations are presented in Table 3. The analysis of the variants was performed through the module (SREG) of the GEA-R program. The main reason for its application is that it provides significantly more objective information about the sources of variation, in cases where there is a significant difference between the locations in the manifestation of the sign. Such a difference was found and proven experimentally (Tsenov et al., 2022a), because there are at least four principal components of variation (PC1-PC4), and even five (PC5, for "all locations"). In addition, the value of the first component (linear variation) is approximately as large as the sum of the other components, which represent the part of the nonlinear type of variation. For example, the two groups of components have the following values by groups of calculation: in "all locations" - PC1 = 48.27 for PC1-5 = 51.73; to the point Dobrich - PC1 = 40.57 at PC1-4 = 59.43; at the Trastenik point - PC1 = 42.36 at PC1-4 = 47.63 and at the Yambol point - PC1 = 49.62 at PC1-4 = 50.37. And without testing for the presence of a cross-type GEI interaction, it is clear that it exists. The strongest is the effect of the conditions (share from 68.98 in Yambol to 81.37 in "all locations") on the variation of yield. The share of the variety is relatively weak and varies between 5 and 12% (5.31, at "all locations" up to 12.57, in Dobrich). The combined genotype x environment interaction accounts for about 13% of the total yield variation (lowest in Yambol - 11.94 and highest in Trastenik - 19.19). At the three test locations, the different seasons (conditions) provide significant variation in both magnitude and direction (linear and non-linear). Therefore, in each of the three locations selected for analysis, there is a tangible GEI, which can be statistically broken down into four principal components.

Table 3. Combined analysis of variances by Site regression (SREG) by GEA-R software

	All locations				Trastenik		
	PORCENT*	PORCENAC**	Df	PROBF***	PORCENT	PORCENAC	PROBF
ENV	81,37	81,37	4	*0,0000*	68,98	68,98	*0,0000*
GEN	5,31	86,68	23	*0,0000*	11,82	80,8	*0,0000*
GEN*ENV	13,32	100	92	*0,0000*	19,19	100	*0,0000*
PC1	48,27	48,27	26	*0,0000*	40,57	40,56	*0,0000*
PC2	21,29	69,56	24	*0,0000*	26,59	67,15	*0,0000*
PC3	14,10	83,66	22	*0,0000*	17,97	85,13	*0,0000*
PC4	9,51	93,17	20	*0,0004*	14,87	100	*0,0000*
PC5	6,83	100,00	18	*0,0084*			
	Dobrich				Yambol		
ENV	70,82	70,82	3	*0,0000*	80,63	80,63	*0,0000*
GEN	12,57	83,39	23	*0,0000*	7,42	88,05	*0,0000*
GEN*ENV	16,61	100	69	*0,0000*	11,94	100	*0,0000*
PC1	52,36	52,35	25	*0,0000*	49,62	49,62	*0,0000*
PC2	26,52	78,88	23	*0,0000*	22,55	72,18	*0,0000*
PC3	12,44	91,32	21	*0,0000*	17,60	89,77	*0,0003*
PC4	8,67	100,00	19	*0,0000*	10,22	100	*0,0336*

* **Percent**- percent of the total variability explain, ****Percenac**- percent of the total variability explain accumulative, *** **Prob F**- value of significance of the test ($p<0,001$)

Part of the reason for the large variance in yield data is the difference in its manifestation by seasons (Table 4). The phenotypic correlations between the yields of the individual years at each location show significant differences between them. Without having to analyse the meteorological situation during this test period, it is clear the conditions of the year at each of the locations are different, because the formed yield is different in size. An exception to this statement is the Dobrich, where one of the years (2011) has similar conditions with two others (2009, r = 0.49 and 2010 r = 0.48), but between which there is no reliable correlation (r = 0.38), for to be similar to each other. In Yambol, two of the years 2010 and 2011 are similar in terms of yield (r = 0.51). The similarity between the conditions of the year in terms of the manifestation of the yield can be said to be hinted at in these cases. This is because the correlation in yield between the mentioned years is about r = 0.50, which is not very convincing from a statistical point of view. Based on these data, it can be argued with a moderate degree of conviction that, in general, a 4-year period of time in one place of cultivation could lead to a dispersion of data to assess the stability of the varieties.

Table 4. Correlation between years at each test location for grain yield by META-R software

		Dobrich (‘D)		
D'09		*0,0645* **	***0,0134***	*0,836*
D'10	0,38 *		***0,0159***	*0,551*
D'11	**0,49 *****	**0,48**		*0,267*
D'12	0,05	-0,13	0,23	
		Trastenik (‘T)		
T'09		*0,646*	*0,841*	*0,449*
T'10	0,10		*0,188*	*0,232*
T'11	-0,04	0,28		*0,087*
T'12	0,16	0,25	0,36	
		Yambol (‘Y)		
Y'09		*0,835*	*0,992*	*0,777*
Y'10	-0,04		***0,011***	*0,615*
Y'11	-0,02	**0,51**		*0,087*
Y'12	0,06	0,11	0,35	

* **-** Below the diagonal- Spearman Correlation coefficient,
** - Above the diagonal – correlation significance (p-value)
*** **-** Values in bold are different from 0 with a significance level alpha=0,05

Is it possible to compare the data on the change in yield from the whole experience with those from the individual location? Such a comparison is important in terms of the goal of the study. Yes, this is possible if it is found that there is a correlation between the data (Table 5).

The correlations between the data from "all locations" and those from the single locations are significantly high. The weakest correlation is between the yield from "all locations" and that from Dobrich (r = 0.54, p = 0.0307), the strongest in Yambol (r = 0.80, p <0.000), and the Trastenik location occupies according to its correlation of r = 0.60, at p = 0.0022, intermediate position relative to the other two locations. There is no significant correlation between the data from the individual single locations. The relatively low positive correlation at Dobrich compared to the other two locations, compared to "all locations", is probably due to the similarity between the years of testing. Such similarity in general could be a reason for weak or even no GEI in the data from one study site.

Table 5. Spearman Rank Correlations between GY by locations

Variables	R-D	R-T	R-Y	R-M
R-D		*0,3535***	*0,0809*	***0,0307***
R-T	0,20*		*0,1908*	***0,0022***
R-Y	0,34	*0,28*		***0,0000***
R-M	**0,54****	**0,60**	**0,80**	

* **-** Below the diagonal- Spearman Correlation coefficient,
** - Above the diagonal – correlation significance (p-value)
*** **-** Values in bold are different from 0 with a significance level alpha=0,05

Once the GEI has been established for each 'database' analysed, it is now possible to assess the stability of the varieties (Figure 1). For the purpose of the study, it is important to determine whether the stability assessment parameters applied to the data from the locations provide similar experience information for each of the varieties. The parameters outlined with different line colours are related to the yield as follows: with a red solid line - those with a positive correlation - *Pi, YSi, TOP, KR, AR* and bi, with a blue solid line: *[S (6), NP 2), NP (3), NP (4)]* have a negative correlation with the yield and all others, delineated by a black solid line: [*CV, StabVar, W2i, S (3), NP (1)]*, which do not show with obtains a correlation relationship. With very few exceptions, the picture available to the groups formed is similar in each "database". With over 70% representativeness of the PCA data (PC1 +

PC2), it can be assumed that the informativeness of each of the analysed parameters is similar in each studied "database". This statement is confirmed in principle by the location of the parameters as a result of cluster data analysis (Figure 2).

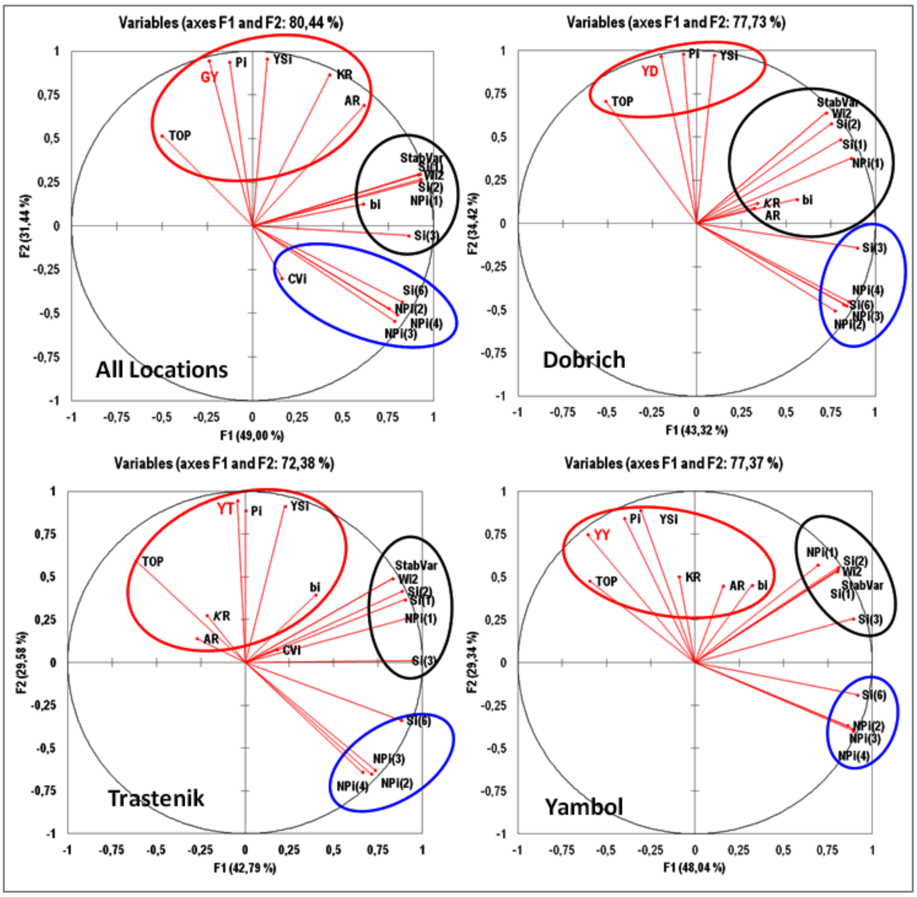

Figure 1. Spatial presentation of the data from the Principal component analysis of the parameters in the four groups "database"

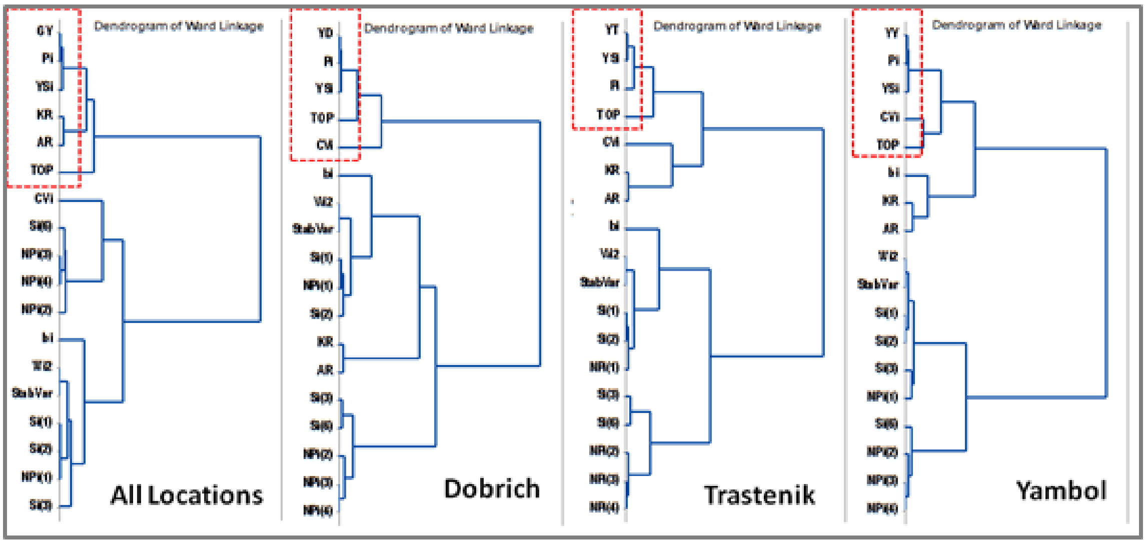

Figure 2. Cluster analysis of statistical parameters in the individual groups "database"

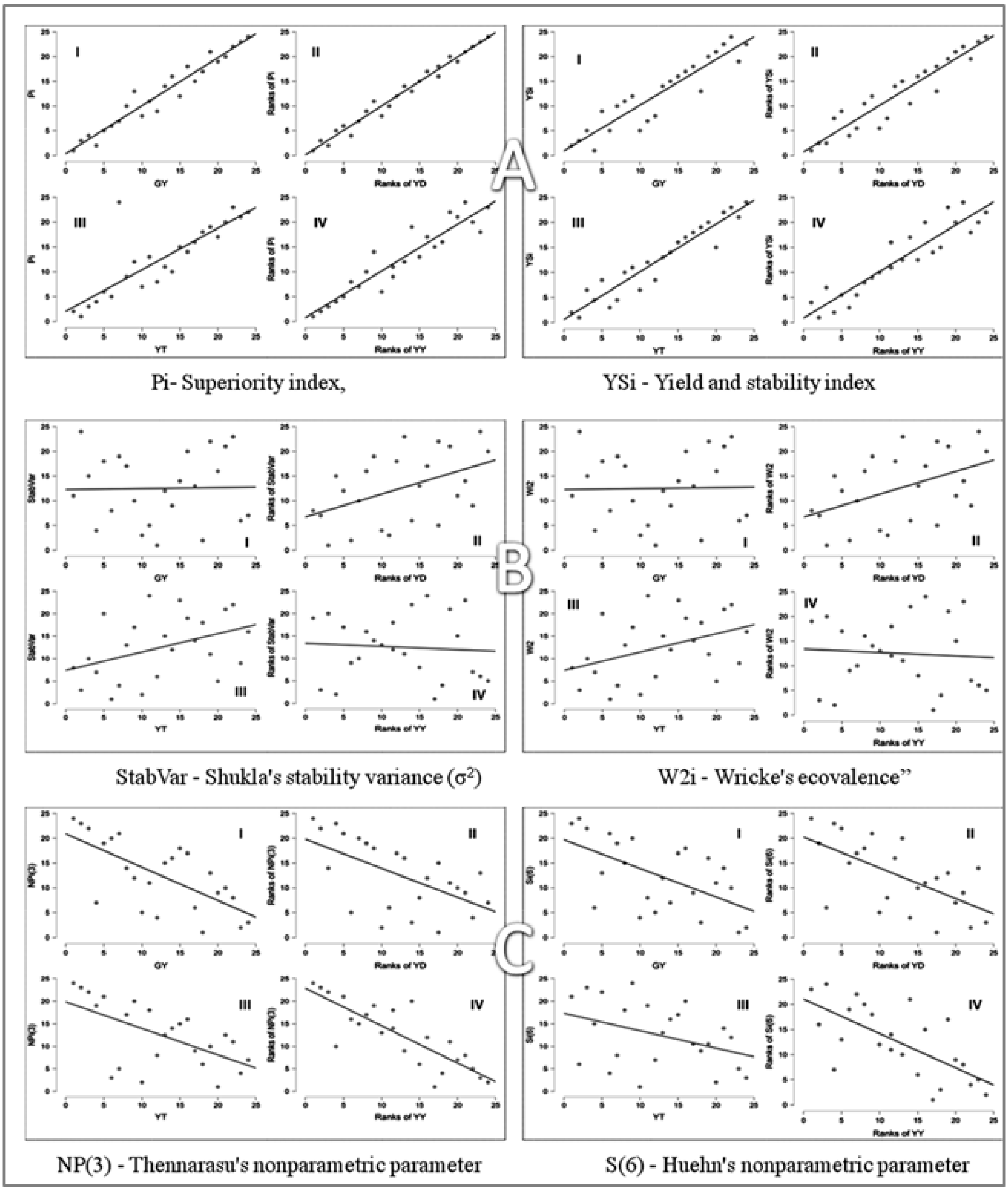

Figure 3, Non-parametric methods having different correlation with yield: **A**- with positive correlation, **B**- without correlation, **C**- with negative correlation, in different test locations: **I-** on average from all locations, **II-** at Dobrich location, **III-** at Trastenik location, **IV-** at Yambol location

In a detailed comparison of the effectiveness of some basic groups of parameters, a completely similar picture was observed for each of the "databases" (Figure 3). It analysed six parameters, two from each group according to their correlation with yield. Non-parametric methods, *Pi* and *YSi* provide fundamentally similar information on yield stability (Figure 3A). In the popular and most used parameters, such as *StabVar* and W^2i, there is some difference between the compared groups. In the group "all locations" and Yambol the straight line of correlation with the yield of both parameters is horizontal (Figure 3B). At the Dobrich and Trastenik check locations the correlation is positive, but very weak and unreliable. The arrangement in analogous figures with the parameter (*bi*) (data not shown) is identical, because it is the strongest expression of the average manifestation of the group of varieties, on the model of which most of the parametric evaluation models are built. This type of relationship between the two parametric methods in relation to yield is the reason why they are so widely used for evaluation in studies in different cultures (Georgieva & Kirchev, 2020; Desheva & Deshev, 2021; Stoyanov & Baychev, 2021).

According to Baker & Leon (1988), the regression coefficient (bi) should not be used to assess stability as a parameter, but should be included in the set of parameters because it provides additional information on the mean genotype response in favourable environmental conditions. The third selected tandem of parameters [*NP (3), S (6)]* is representative of those that have a negative correlation with yield (Figure 3C). The location of the line between the ranks of the parameters and the yield is descending. A slight difference from this trend is the data at the point Trastenik at the parameter S (6), where the line is almost horizontal.

The spatial representation of the data for the main sets of evaluation parameters fully confirms their similar effectiveness, regardless of the "database". This principle of kind with information must be further confirmed by comparing the rank correlations between yield and parameters, for each "database" separately. Additional analysis includes only non-parametric assessment methods. Correlations between rankings were calculated using two of the known approaches: Sperman and Kendall. An additional comparison was made between the correlations calculated using the classical approaches of the two models, as well as their Bayesian versions (Table 6).

Table 6. Rank correlations between yield and non-parametric stability statistics overall and by locations

Statistical parameters	All Locations		Dobrich		Trastenik		Yambol	
	Spearman *rho*	Kendall *tau B*	Spearman *rho*	Kendall *tau B*	Spearman *rho*	Kendall *tau B*	Spearman *rho*	Kendall *tau B*
bi	**-0,058**	**-0,036**	**0,022**	**-0,008**	**0,234**	**0,159**	**0,047**	**0,033**
YSi	**0,925*** **	**0,803*** **	**0,938*** **	**0,836*** **	**0,951*** **	**0,849*** **	**0,928*** **	**0,799*** **
Si(1)	0,061	0,062	0,307	0,218	0,314	0,219	-0,069	-0,029
Si(2)	0,027	0,025	0,383	0,256	0,354	0,243	-0,063	-0,033
Si(3)	-0,252	-0,138	-0,339	-0,229	-0,068	-0,058	-0,348	-0,225
Si(6)	**-0,577** **	**-0,428** **	**-0,624** **	**-0,435** **	**-0,384**	**-0,265**	**-0,683*** **	**-0,519*** **
TOP	**0,566** **	**0,482** **	**0,723*** **	**0,626*** **	**0,531** **	**0,449** **	**0,681*** **	**0,586*** **
NPi(1)	0,027	0,037	0,175	0,14	0,2	0,129	0,027	0,037
NPi(2)	**-0,571** **	**-0,419** **	**-0,619** **	**-0,461** **	**-0,573** **	**-0,442** **	**-0,778*** **	**-0,592*** **
NPi(3)	**-0,672*** **	**-0,514*** **	**-0,548** **	**-0,436** **	**-0,587** **	**-0,454** **	**-0,827*** **	**-0,657*** **
NPi(4)	**-0,64*** **	**-0,5*** **	**-0,566** **	**-0,44** **	**-0,61** **	**-0,464** **	**-0,813*** **	**-0,635*** **
Pi	**0,97*** **	**0,884*** **	**0,988*** **	**0,93*** **	**0,837*** **	**0,754*** **	**0,938*** **	**0,809*** **
KR	**0,714*** **	**0,525*** **	**-0,021**	**0,032**	**0,268**	**0,181**	**0,289**	**0,18**
AR	**0,457***	**0,319***	**-0,048**	**-0,022**	**0,135**	**0,076**	**0,073**	**0,054**

Significant at: * p <0,05, ** p < 0,01, *** p < 0,001

First, it must be said that the correlations between the two approaches (classical and Bayesian) are exactly the same. There are differences in absolute values according to Spearman and Kendall, but they are basically completely similar. According to the values of the correlations and their statistical reliability, the parameters are grouped into three groups: those with positive (proven), those with no correlations and those with proven negative correlation. ***Second***, there are several significant differences between the four "databases" studied. Only at "all locations" the parameters *AR* and *KR* show a positive correlation with the yield, while at the others there is no such connection. For all other parameters the correlation at different locations is completely similar. Those with a positive correlation with yield (*Pi*, *YSi*, *TOP*), those with a negative correlation with yield [*Si (6), NPi (2), NPi (3), NPi (4)]* and those with no yield relationship [*Si (1), S (2), S (3) NPi (2)]* are the same as shown in Figures 1 and 2. Therefore, each of the applied methods for grouping parameters according to yield correlation and efficiency for evaluation provides information that does not differ significantly by study items.

However, there are some differences, which are probably due to several reasons: first, in the group "all locations" the analysed data come from five locations, not from the three with which they are compared; second, the fundamental similarity between the locations is the result of specific differences between the proportions of the conditions and the genotype (Table 2); thirdly, the set of years is the same, but the conditions in each of them at the individual locations clearly have a different impact on the respective "database", fourthly, the ranking estimates of the yield at each point have different correlations with those of all locations. reflects the effectiveness of each parameter separately; fifth, the absolute values of the yield differ significantly in the individual locations, which is why the ranks of the parameters AR and KR have a different weight in them, compared to "all locations".

In the course of the analyses, the idea arose to assess the stability by a combination of a group of parameters. The most effective combinations of parameters (models) for each "database" were identified using Stepwise Linear regression (**Table 7**).

Table 7. The best Stepwise Linear regression models by location

All Locations		Dobrich		Trastenik		Yambol	
Models	R^2 #	Models	R^2	Models	R^2	Models	R^2
YSi*Si(6)***TOP**	0,991	YSi*Si(2)*Si(6)	0,997	NPi(3)	0,979	NPi(3)*Pi*AR	0,996
TOP*NPi(1)*Pi	0,989	YSi*Si(1)***Pi**	0,997	**Pi**	0,974	YSi***TOP***AR	0,995
YSi*NPi(3)*AR	0,986	YSi*Si(3)*Si(6)	0,996	**YSi**	0,901	YSi*Pi	0,992
YSi	0,962	**YSi**	0,993			**YSi**	0,962
Pi	0,939	**Pi**	0,977			**Pi**	0,874

#- Adjusted coefficient of determination (R^2), * - means interaction

It is noteworthy that the combinations involve parameters that are of different types (according to the correlation with the yield). Although the models in the individual locations differ in composition, they are invariably based on those with the highest correlation with the yield: *Pi*, *YSi* and *TOP*. The three parameters alone and in combination with the highest negative correlations Si(6), NPi(3), determine a sufficiently high yield (H^2 = 0.87 - 0.99). As a "moderator" they include parameters without correlation with the yield: Si(3) in Dobrich and NPi(1) in "all locations". The direct comparison of the individual models provides information in several main aspects: ***first*** - a set of parameters is determined, through which at each point of extraction could be provided as much as possible, ***second*** - indicates the existence of similar or similar models for this in individual locations and ***third*** - shows that the combinations include different parameters. The latter is very important because the use of such combinations fully complies with both concepts of stability assessment (static and dynamic).

DISCUSSION

The interaction of genotype x environment is a prerequisite for multilayer evaluation of varieties in different growing conditions (Kang, 2020; Pour-Aboughadareh et al., 2022). The collection of such information requires the organization of many factorial field trials at different locations, which have significant differences in growing conditions with each other (Yan et al., 2021). These conditions are expected to be not only different, but also to have a significant impact on the size and variation of yield, or any quantitative feature (Vulchinkov et al., 2020). Organizing such field trials is a complex event that requires large financial and human resources. Researchers prefer to organize specific and small-scale studies of quantitative traits in one place over a period of several predominantly consecutive years (Stoyanova et al., 2020; Uhr et al., 2021). In their desire to study the stability of groups of varieties, breeders encounter variation that is caused solely by seasonal conditions and / or in combination with genotype (Chamurliyski et al., 2015; Desheva & Deshev, 2021; Marcheva, 2021; Uhr et al., 2021). The absence of yield variance as a result of the direct influence of climate differences (locations) may not be sufficient to assess the stability of the variety. The differences between the conditions of the individual seasons are "proven" in some cases by analysing the meteorological situation during the specific test period with the presumption that they affect the yield in some way (Chamurliyski & Tsenov, 2013; Raykov et al., 2016; Stoyanov, 2020 ; Uhr & Samodova, (2020). Stoyanov & Baychev, 2021). Yes, such an effect exists and it must be proven statistically, otherwise there is a simple variation, not a multi-layered variance of yield.

In this study, an attempt was made to determine whether the conditions of several consecutive seasons in the regions characteristic of the country's grain production (Dobrich, Ruse and Yambol) would provoke GEI. For comparison, an experiment was used in which there is a proven strong and multi-layered interaction of the genotype with the environmental conditions (Tsenov et al., 2022a; Tsenov et al., 2022b). Comparisons made using different statistical approaches show unequivocally that there are real possibilities for assessing the impact of conditions at one point. This implies a correct assessment of the stability of the varieties, against the background of their productivity. At each of the study locations, the variation in yield was due to the direct effect of the "year" and the combined effect of the year x genotype. The latter has a share of only 12-20% of the variation, which causes differences in the reaction of individual varieties. However, it is a serious prerequisite for assessing their stability at any point studied, without exception. The variation in the individual locations is decomposed into four main components, as the linear and non-linear types of change in yield are approximately the same in size. Strongly similar to this study, variation was found in studies in different crops (El-Hashash et al., 2019; Sitaresmi et al., 2019; Aberkane et al., 2021; Mohammadi et al., 2021; Pour-Aboughadareh , et al., 2022). The divergent high degree of yield variance makes genotype assessment a serious challenge. Therefore, here it was performed by a set of a dozen non-parametric methods. To establish their effectiveness, each of them was analysed and subjected to a critical comparison with the established for year’s parametric methods.

In general, the effectiveness of the parameters is like the information already known about them in previous studies (El-Hashash et al., 2019; Sitaresmi et al., 2019; Cubukcu et al., 2021). In addition, the whole set of parameters gives a similar assessment of the varieties grown in different conditions (locations). Therefore, the use of non-parametric methods is the right choice for grading varieties when there is a strong GEI. The approach for assessing stability by using a combined interaction of the most efficient parameters at each point, by regression, also proved to be effective. This way of applying the different parameters provides an opportunity to predict the classification of the genotype in the group, with a high statistical probability of over 90%

CONCLUSION

The study confirmed the thesis that there is a real possibility to establish and prove GEI, which is the result of changes in yield caused by significant annual fluctuations in the conditions of one place of the experiment. The absence of variation as a result of the effects of the locations in a trail is not an obstacle to assessing the stability of the variety. Unfortunately, the stability found in this way is useful from a breeding point of view, only for a direct comparison between the varieties in the group. It cannot be used even for elementary zoning, which is a disadvantage of this approach. The stability of the variety could hardly be extrapolated to other, even very similar climatic conditions.

The application of non-parametric methods is a good approach to the evaluation of the variety, because the information about it depends largely on its manifestation in changing growing conditions. Some of the published information on the effectiveness of non-parametric methods is contradictory in terms of their direct correlation with yield. Using interaction between several effective parameters is an approach that could possibly solve this problem.

REFERENCES

Aberkane, H., Amri, A., Belkadi, B.; Filali-Maltouf, A., Valkoun, J., & Kehel, Z. (2021) contribution of wild relatives to durum wheat (*Triticum turgidum* subsp. *Durum*) yield stability across contrasted environments. *Agronomy*, 11, 1992. https://doi.org/10.3390/agronomy11101992

Annicchiarico, P. (2002). Defining adaptation strategies and yield-stability targets in breeding programmes. In *Quantitative genetics, genomics and plant breeding*, 365–383. https://doi.org/10.1079/9780851996011.0365

Becker, H.C., & Leon, J. (1988). Stability analysis in plant breeding. *Plant Breeding,* 101, 1-23. https://doi.org/10.1111/j.1439-0523.1988.tb00261.x

Chamurliyski, P. & Tsenov, N. (2013). Stability of grain yield in modern Bulgarian winter wheat varieties (*Triticum aestivum* L.) in Dobrudzha, *Agricultural Science and Technology*, 5(1), 16-21

Chamurliyski, P., E. Penchev, & Tsenov, N. (2015). Productivity and stability of the yield from common winter wheat cultivars developed at IPGR, Sadovo under the conditions of Dobrudzha region, *Agricultural Science and Technology*, 7(1), 19-24.

Cooper, M., Rajatasereekul, S., Immark, S., Fukai, S., & Basnayake, J. (1999). Rainfed lowland rice breeding strategies for Northeast Thailand. *Field Crops Research*, 64(1–2), 131–151. https://doi.org/10.1016/s0378-4290(99)00056-8

Cubukcu, P., Kocatürk, M., İlker, E., Kadiroğlu, A., Vurarak, Y., Şahin, Y., Karakuş, M., Akgün Yildirim, M., Göksoy, A., & Sincik, M. (2021). Stability analysis of some soybean genotypes using parametric and non-parametric methods in multi-environments. *Turkish Journal of Field Crops, 26(2), 262-271*. https://doi.org/10.17557/tjfc.1033363

de Leon, N., Jannink, J. L., Edwards, J. W., & Kaeppler, S. M. (2016). Introduction to a Special Issue on Genotype by Environment Interaction. *Crop Science*, 56(5), 2081–2089. https://doi.org/10.2135/cropsci2016.07.0002in

Desheva, G., & Deshev, M. (2021). Evaluation of the stability and adaptability of yield in varieties and breeding lines of common winter wheat. *Rastenievadni nauki*, 58(1), 3-13 (*Bg*).

El-Hashash, E. F., Tarek, S. M., Rehab, A. A., & Tharwat, M. A. (2019). Comparison of Non-parametric Stability Statistics for Selecting Stable and Adapted Soybean Genotypes under Different Environments. *Asian Journal of Research in Crop Science, 4(4)*, 1–16. https://doi.org/10.9734/ajrcs/2019/v4i430080

Flores, F., Moreno, M. T., & Cubero, J. I. (1998). A comparison of univariate and multivariate methods to analyze G x E interaction. *Field Crops Research*, 56(3), 271–286. https://doi.org/10.1016/S0378-4290(97)00095-6

Fox, P. N., Skovmand, B., Thompson, B. K., Braun, H. J., & Cormier, R. (1990). Yield and adaptation of hexaploid spring triticale. *Euphytica*, *47*(1), 57–64. https://doi.org/10.1007/bf00040364

Georgieva, R. G., & Kirchev, H. K. (2020). Ecological plasticity and stability of some agronomical performances in triticale varieties (x *Triticosecale* Wittm). *Ecologia Balkanica*, 12(1), 93-98.

Huehn M. 1996. Non-parametric analysis of genotype × environment interactions by ranks. In: Kang MS, & Gauch HG, (editors). *Genotype by environment interaction.* Boca Raton: CRC Press. 213–228.

Huehn, M. (1990). Nonparametric measures of phenotypic stability. Part 1: Theory. *Euphytica*, *47*(3), 189–194.

Kang, M. S. (1993). Simultaneous selection for yield and stability in crop performance trials: consequences for growers. *Agronomy Journal*, *85*(3), 754–757. https://doi.org/10.2134/agronj1993.00021962008500030042x

Kang, M. S. (1997). Using genotype-by-environment interaction for crop cultivar development. *Advances in Agronomy*, 62, 199–252. https://doi.org/10.1016/S0065-2113(08)60569-6

Kang, M.S. (1988). A rank-sum method for selecting high-yielding, stable corn genotypes. *Cereal Research Communication*, 16, 113–115.

Lin, C. S. & Binns MR (1991). Assessment of a method for cultivar selection based on regional trial data. *Theoretical and Applied Genetics*, 82, 379-388. http://dx.doi.org/10.1007/BF02190626

Lin, C. S. & Binns, M. R. (1988). A superiority measure of cultivar performance for cultivar × location data. *Canadian Journal of Plant Science*, 68(1), 193–198. https://doi.org/10.4141/cjps88018

Marcheva, M. (2021). Yield and quality stability of common winter wheat varieties. *Bulgarian Journal of Agricultural Science*, 27(Suppl. 1), 111–118.

Mohammadi, R., Farshadfar, E. & Amri, A. (2016) Comparison of rank-based stability statistics for grain yield in rainfed durum wheat, *New Zealand Journal of Crop and Horticultural Science*, 44(1), 25-40, https://doi.org/10.1080/01140671.2015.1100126

Mohammadi, R., Sadeghzadeh, B., Poursiahbidi, M.M., & Ahmadi, M.M. (2021). Integrating univariate and multivariate statistical models to investigate genotype × environment interaction in durum wheat. *Annals of Applied Biology*, 178(3), 450-465. https://doi.org/10.1111/aab.12648

Nassar, R., & Huhn, M. (1987). Studies on Estimation of Phenotypic Stability: Tests of Significance for Nonparametric Measures of Phenotypic Stability. *Biometrics*, *43*(1), 45. https://doi.org/10.2307/2531947

Pour-Aboughadareh, A., Barati, A., Koohkan, S. A., Jabari, M., Marzoghian, A., Gholipoor, A., Shahbazi-Homonloo, K., Zali, H., Poodineh, O., & Kheirgo, M. (2022). Dissection of genotype-by-environment interaction and yield stability analysis in barley using AMMI model and stability statistics. *Bulletin of the National Research Centre*, 46(1). https://doi.org/10.1186/s42269-022-00703-5

Raykov, G., P. Chamurliyski, S. Doneva, E. Penchev, & Tsenov, N. (2016). Productivity performance of bread winter wheat genotypes of local and foreign origin *Agricultural Science and Technology* *8*(4), 276 – 279. https://DOI.org/10.15547/ast.2016.04.052

Schlichting, C. D. (1986). The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, *17*(1), 667–693. https://doi.org/10.1146/annurev.es.17.110186.003315

Segherloo, A. E., Sabaghpour, S. H., Dehghani, H., & Kamrani, M. (2008). Non-parametric measures of phenotypic stability in chickpea genotypes (Cicer arietinum L.). *Euphytica*, 162(2), 221-229.

https://doi.org/10.3923/pjbs.2007.2646.2652

Shukla, G. K. (1972). Some statistical aspects of partitioning genotype-environmental components of variability. *Heredity*, *29*(2), 237–245. https://doi.org/10.1038/hdy.1972.87

Singh, M., Ceccarelli, S., & Grando, S. (1999). Genotype x environment interaction of crossover type: Detecting its presence and estimating the crossover point. *Theoretical and Applied Genetics*, 99(6), 988–995. https://doi.org/10.1007/s001220051406

Sitaresmi, T., Suwarno, W.B., Gunarsih, C., Nafisah Y., Nugraha, Y., Sasmita, P. & Daradjat, A.A., (2019). Comprehensive stability analysis of rice genotypes through multi-location yield trials using PBSTAT-GE, *SABRAO Journal of Breeding and Genetics*, 51 (4) 355-372.

Stoyanov, H. (2020). Response of Bulgarian triticale cultivars to unfavorable environments. *Rastenievadni nauki*, 57(6), 17-29.

Stoyanov, H. (2021). Tendencies in the reaction of the yield of Bulgarian triticale cultivars under contrasting environments. *Rastenievadni nauki*, 58(1), 14-25.

Stoyanov, H., & Baychev, V. (2021). Triticale lines combining high productivity with stability and adaptability under contrasting environments. *Rastenievadni nauki*, 58(5) 3-15 (Bg).

Stoyanova A., Georgiev M., Atanasova S., Emurlova, F., & Mineva, R. (2020). Study of productivity and stability of yield of common wheat varieties, *Journal of Mountain Agriculture on the Balkans*, 23 (5), 75-88.

Thennarasu, K. (1995). On certain non-parametric procedures for studying genotype-environment interactions and yield stability. *PhD thesis*, PJ School, IARI, New Delhi, India.

Tsenov, N., Gubatov, T, & Yanchev, I, (2022a), Comparison of statistical parameters for estimating the yield and stability of winter common wheat varieties, *Agriculture Science and Technology* 14, *in press*

Tsenov, N., Gubatov, T. & Yanchev, I. (2022b). Indices for assessing the adaptation of wheat in the genotype x environment interaction. *Rastenievadni nauki*, 59(2), *accepted*

Uhr, Zl., & Samodova, A. (2020). Agrobiological study of advanced winter common wheat varieties in the Pazardzhik region of southern Bulgaria. *Rastenievadni nauki*, 57(1), 27-31 (Bg).

Uhr, Zl., Dimitrov, E., & Delchev, G. (2021). Characteristics of perspective lines common winter wheat. 1. Yield and stability. *Rastenievadni nauki*, 58(4), 3-10 (Bg).

Vaezi B., A. Pour-Aboughadareh, R. Mohammadi, A. Mehraban, T. Hossein-Pour, E. Koohkan, S. Ghasemi, H. Moradkhani, & Siddique, K. H. M. (2019). Integrating different stability models to investigate genotype × environment interactions and identify stable and high-yielding barley genotypes. *Euphytica*, 215, 63. https://doi.org/10.1007/s10681-019-2386-5

Vaezi, B, Sabgahnia, N., Mehraban, A., & Hatami-Maleki, H. (2022). Nonparametric analysis of genotype grain yield performance of barley trials based on ranks, *Romanian Agricultural Research*, 39, 1-11.

van Eeuwijk, F. A., Bustos-Korts, D. V., & Malosetti, M. (2016). What Should Students in Plant Breeding Know About the Statistical Aspects of Genotype × Environment Interactions? *Crop Science*, 56(5), 2119–2140. https://doi.org/10.2135/cropsci2015.06.0375

Vulchinkov S., Reseleshka, L., Vulchinkova, P., Ilchovska, M., Petrovska, N., & Valkova V., (2020). Stability assessment of maize hybrids by different methods in relation to their zoning, *Perspectives on agricultural science and innovations for sustainable food systems, Jubilee scientific international conference of 75 years of Agricultural University – Plovdiv*, pp. 65-76. http://agrarninauki.au-plovdiv.bg/2021/issue-29/8-29/

Yan, W. (2021). A Systematic Narration of Some Key Concepts and Procedures in Plant Breeding. *Frontiers of Plant Sciences*, 12, 724517. https://doi.org/10.3389/fpls.2021.724517