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NON-PARAMETRIC STABILITY ANALYSES OF PROTEIN CONTENT IN MULTI-ENVIRONMENT TRIALS OF WHEAT (*T. aestivum* L.)

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According to literature, a detailed paper has not been published yet on using non-parametric stability statistics for evaluating genotypic stability in protein content (PC) of wheat. Thus, this study aimed to investigate the stability for PC of wheat using sixteen non-parametric stability measures (YSD-PC standard deviation, RM-Rank mean, RSD-Rank's standard deviation, RS-Rank Sum stability statistic, PA-Percentage of adaptability, R_1 and R_2-Range indexes, TOP-Ranking, $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$and $NP_i^{(4)}$ rank statistics, together with Y-PC mean). The study included 13 wheat genotypes, consisting of 5 registered cultivars and 8 breeding lines, selected from National Wheat Breeding Program of Turkey. The genotypes were grown in ten rain-fed environments, representative of major rain-fed wheat-growing areas of Turkey, during 2011-2013 cropping seasons. The ANOVA showed that the effects due to environments (E), genotypes (G) and GE interaction (GEI) were significant ($P < 0.01$). Spearman's rank correlation and principal component analyses (PCA) also revealed that two types of associations were found between the stability parameters: the first type included $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$, $NP_i^{(4)}$, RSD and YSD parameters which were related to static stability, whereas the second type consisted of the Y, RM, TOP, PA, RS, R1 and R2 parameters which are related to dynamic concept of stability. Among the 8 breeding lines, G7 and G8 were the best genotypes in terms of both high PC and stability. In conclusion it could be suggested that dynamic non-parametric stability statistics should be used for selecting genotypes with high PC and stable when tested across a wide range of environments.

Key words: Wheat (*T. aestivum* L.), protein content, non-parametric stability statistics

INTRODUCTION

Wheat is one of the world's most important food grains. It has the highest protein content (PC) among cereals, ranging from 8 to 16 % (BRANLARD *et al.*, 2001). Traditionally, wheat

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breeding concentrates largely on the improvement of protein quality due to the importance of protein in bread making, end-product quality, nutritional value, and economic impact (SUCHY *et al.*, 2007).

Achieving the standards of grain quality demanded is complex as it is usually influenced by G, E and GEI factors. The understanding of these effects is essential to help breeders to set proper objectives and strategies to develop wheat genotypes with high yield potential as well as with specific and consistent quality attributes to meet market needs (WILLIAMS *et al*., 2008). The importance of the effects of G, E and GEI is increasing for breeders, growers, grain traders and end-use processors (VAZQUEZ *et al.*, 2012).

While the magnitude of the comparative effects of G and E has been studied by several authors, there is no general consensus about which is more important for most quality characteristics (VAZQUEZ *et al.,* 2012). DENCIC *et al*. (2011) proposed that the relative importance of G and E effects depends on tested Gs and Es. Similarly, WILLIAMS *et al.* (2008) suggested that the amplitude of the variation between Es vs. Gs influences the observed results, and could be part of the explanation for the different magnitude of G, E and GEI found in several works.

Some studies concluded that G influence is the most important. SOUZA *et al.* (2004) cultivated seven Gs in nine Es, concluding that G selection is critical, while E effects were of secondary importance for the range of Es used. The wide variability of the Gs used by DENCIC *et al.* (2011) caused the G effect to be dominant. It is known that certain quality parameters are highly influenced by G factors (i.e. hardness is clearly genetically determined) (CARSON and EDWARDS, 2009; WRIGLEY, 2007) while other parameters are highly influenced by E (i.e. PC) (CARSON and EDWARDS, 2009; DENCIC *et al*., 2011; WRIGLEY, 2007). Other works found that E effects prevailed over the G ones (PETERSON *et al*., 1998; JOHANSSON *et al*., 2003; FINLAY *et al*., 2007; SAHIN *et al*., 2012; KAYA and AKCURA, 2014; KAYA and SAHIN, 2015).

It is well documented that PC is affected by G, E and GEI factors (PETERSON *et al*. 1998; JOHANSSON *et al*., 2003; FINLAY *et al*., 2007; WRIGLEY, 2007; CARSON and EDWARDS, 2009; DENCIC *et al*., 2011). For this reason, several statistical methods have been proposed to investigate G, E and GEI effects in multi-environment trials. They display different aspects of the stability approach, including parametric, non-parametric and multi-variate methods (EBERHART and RUSSELL, 1966; HUEHN, 1996; YAN and KANG, 2003). In our study, we were interested in using non-parametric measures of phenotypic stability of genotypes for PC. Because non-parametric measures of phenotypic stability do not depend on any assumptions about the distribution of phenotypic observations (HUEHN, 1996) one can easily estimate the variance or standard deviation of the ranks of a genotype in different environments (BECKER and LEON, 1988).

In principal, non-parametric procedures are based on the ranks of genotypes in each environment and genotypes with similar ranking across environments are classified as stable (BECKER and LEON, 1988). There are several non-parametric stability statistics. The percentage of adaptability (PA) is a measure for the capacity of a genotype (ST-PIERRE *et al.*, 1967). Two non-parametric stability measures (R_1 and R_2) were suggested by LANGER *et al.* (1979). HUEHN (1996) proposed four nonparametric measures of phenotypic stability $S_i^{(1)}$, $S_i^{(2)}$ $S_i^{(3)}$ and $S_i^{(6)}$. KETATA (1988) suggested rank mean (RM) against standard deviation of ranks (RSD) and also performance mean (Y) and standard deviation of performance mean (YSD) as nonparametric stability statistics. FOX *et al.* (1990) proposed a nonparametric superiority measure (TOP) for general adaptability. The Rank-Sum (RS) statistic was generated by KANG (1988). THENNARASU (1995) suggested as stability measures, $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ based on ranks of adjusted means of genotypes.

In this study, we used sixteen non-parameric stability statistics, already mentioned above, for detecting G, E and GEI effects on PC of thirteen wheat genotypes tested across ten rain-fed environments of Turkey. According to the literature, related with the context of our study, there were merely three papers published (HAZEN *et al*., 1997; ROBERT, 2002; MUT *et al*., 2010). However, they included only two non-parametric stability measures (viz. $S_i^{(1)}$ and $S_i^{(2)}$) proposed by HUEHN (1996). Indeed, since they failed to distinguish the genotypes with high PC and stabil vs. ones with low PC and unstable in our study, we excluded them from the recommended non-parametrics measures for selecting genotypes with high PC and stable, tested in the multi-environment trials.

The objectives of this study were to (i) identify wheat genotypes with high PC and stable tested across different environments representative for rain-fed areas of Turkey, (ii) study the relationships among nonparametric stability statistics, and (iii) determine the nonparametric stability statistics suitable for detecting the genotypes with high PC and stable.

MATERIALS AND METHODS

Thirteen wheat (*T. aestivum* L.) genotypes were grown in ten rain-fed environments, including 7 locations viz. Konya, Cumra, Karaman, Nigde, Altinova, Kocas and Bala, and 3 locations viz Kocas, Cumra and Karaman, during the two consecutive cropping seasons (2011-2012 and 2012-2013) at the Central Anatolian Region of Turkey. They comprised 5 registered cultivars and 8 advanced lines from National Winter Wheat Breeding Program, Turkey. The experimental layout was a randomized complete block design with 4 replications. Sowing was done with an experimental drill in 1.2 m x 7 m plots, consisting of 6 rows spaced 20 cm apart. The seeding rate was 550 seeds m^{-2}. Fertilizer application was 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} at the planting and 50 kg N ha^{-1} at the stem elongation stage. Harvesting was done with an experimental combine in 1.2 m x 5 m plots. Details of the 13 genotypes and 10 environments are given in Tables 1 and 2, respectively.

For determining protein content (PC), wheat grains were stored for 48 h at 14% moisture and milled using a Quadrumat Senior mill in order to make flours of approximately 65 % extraction. PC was determined by the Dumas method using the Leco FP-528 (AACC, 2000).

A combined analysis of variance (ANOVA) was applied to PC (%) data from combinations of locations with cropping seasons (hereafter referred to as Environment). Once ANOVA revealed that genotype (G) and environment (G) main effects and G x E interaction (GEI) were statistically significant, 16 non-parametric stability approaches were performed the multi-environment PC data, in order to measure the stability levels of 13 genotypes.

The ANOVA, Spearman's rank correlation and comparison of the means with LSD test ($P<0.05$) were performed using SAS© 9.1. SAS codes proposed by HUSSEIN *et al.* (2000) for nonparametric statistics $S_i^{(3)}$ and $S_i^{(6)}$ (HUEHN, 1996) and TOP (FOX *et al*. 1990) and by LU (1995) for $S_i^{(1)}$ and $S_i^{(2)}$ (HUEHN, 1996) were used in the stability analyses. The other nonparametric statistics RM, RSD and YSD (KETATA, 1988), PA (ST PIERRE *et al*., 1967), R_1 and R_2 (LANGER *et al*., 1979), RS (KANG, 1988), $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ (THENNARASU, 1995) were estimated using Microsoft Excel©. Principal components and biplot analyses were performed using Biplot and Singular Value Decomposition Macros for Excel© (LIPKOVICH and SMITH, 2002).

Table 1. Code, parentage and protein content of 13 wheat genotypes

Code	Genotype	Protein Content (%)
	Cultivar	
G1	BAYRAKTAR	12.56 d†
G2	GEREK	13.09 ac
G3	KARAHAN	13.39 a
G4	TOSUNBEY	13.44 a
G5	BEZOSTAYA	13.38 a
	Advanced Line	
G6	KARAHAN/KONYA	12.82 bd
G7	KRC/BEZ/3/1150-18/VGDWF/4/YE2453/5/BEZ/NAD//KZM (ES85.24)/3/F900K	13.11 ab
G8	PLK70/LIRA"S"/5/C126-5./4/KRC/7/NECORMP1/5/BEZ// TOB/ 8156/4/ON/3/TH*6/KF//LEE*6/K/6/TAST/SPRW (BDME-11/1K)	12.89 bd
G9	BLL/6/NAD/CO652643/4/NAI60/MY54//NAI60/KODOS/3 /NS220/5/HYS"S"/7/ALY00/8/ALY00 (BDME-11/2K)	12.55 d
G10	BOL-2973/6/CTK/3/ATL66/CMN//TX2607-6/4/SS8/LLFN/3/ BEZ/NAD//KZM74/BB//CC/CNO*2/3/TOP156/BB/5/GÜN-91 (BDME-11/3K)	12.83 bd
G11	SDY/3/NAI60/HN//BUC/4/KEA/TOW/5/YAN7875.128 (BDME-09/1K)	12.49 d
G12	F10S-1/CHISHOLM (BDME-09/2K)	12.67 cd
G13	GV/4/D6301/NAI//WRM/3/CNO*3/CHR/5/BL2973/6/ LOVRIN6/SAMSUN	12.60 d
	Mean	12.91
	LSD $_{(0.01)}$	0.419

†Lower case letters stand for genotype ranking based on LSD $_{(0.01)}$

Table 2. Codes, cropping season, protein content and precipitation amount for 10 environments

Code	Cropping Season	Location	Protein Content (%)	Precipitation (mm)	LTEP‡ (mm)
E1	2011-2012	Konya	12.80 bc†	223	316
E2	2011-2012	Cumra	12.85 bc	235	316
E3	2011-2012	Karaman	12.97 bc	259	327
E4	2011-2012	Nigde	14.07 a	281	330
E5	2011-2012	Altinova	13.08 b	258	325
E6	2011-2012	Kocas	13.99 a	301	344
E7	2011-2012	Bala	12.33 d	275	401
E8	2012-2013	Kocas	12.62 cd	271	344
E9	2012-2013	Cumra	11.26 e	302	316
E10	2012-2013	Karaman	13.12 b	315	327
LSD $_{(0.01)}$			0.367		

†Lower case letters stand for environmental ranking based on LSD $_{(0.01)}$

‡ Long term average precipitation

RESULTS

The ANOVA indicated that the effects of E, G, and GEI were highly significant ($P < 0.01$) on PC (Tables 1, 2 and 3). E effect accounted for most of the sums of squares (SS) of total variation, indicating the substantial effect of E on the PC performance of the thirteen genotypes evaluated in this study. On the other hand, significant GEI effects demonstrated that the genotypes responded differently to variations in environmental conditions.

Table 3. Combined analysis of variance for protein content of 13 wheat genotypes grown at 10 environments

Source	df	SS	MS	F	Model	Explained (%)†
Environment (E)	9	169.57	18.84	30.15**	Random	57.9
Replication (E)	10	6.25	0.63			
Genotype (G)	12	57.98	4.83	7.96**	Fix	19.8
G x E Interaction	108	65.56	0.61	3.07**	Random	22.4
Error	120	23.74	0.20			
Total	259	323.10				100.0

$CV_{(\%)} = 3.45$ $R^2 = 0.93$ Mean 12.91 (%)

** $P < 0.01$; CV, Coefficient of variance; R^2, Coefficient of determination; † Proportional to sums of squares (SS) of total variation

Mean values of PC across genotypes are shown in Table 1. In the present study, the PC ranged from 13.44 % for G4 to 12.49 % for G11, with over all mean of 12.91 %. Of thirteen genotypes, cultivars (from G1 to G5) used as checks for comparing with advanced lines (from G6 to G13) had higher PC (except G1) than that of the average genotype (12.91 %). Among the 8 advanced lines, solely G7 had higher PC than that of the rest.

Mean PC values of test environments are shown in Table 2. They varied from 14.07 % at environment E4 to 11.26 % at environment E9. ANOVA showed that PC range was wider among environments (11.26 to 14.07 %) than that among genotypes (12.49 to 13.44 %). On the other hand, the correlation between annual precipitation amounts and mean PCs across environments was not significant ($r = 0.02$).

Assessment of the genotypes based on the 16 different non-parametric measurements, together with PC means, is presented in Table 4. KETATA (1988) proposed four non-parametric methods: rank's mean (RM) and its standard deviation (RSD) and PC mean (Y) and its standard deviation (YSD). According to RM, genotypes G4 and G5 were the most desirable, while genotypes G3 and G4 were identified as the most stable considering Y. As for YSD, genotypes G12 and G13 were the most consistent, whereas genotypes G7 and G3 were the most desirable for RSD (Tables 4).

The nonparametric measure of FOX *et al.* (1990) consists of scoring the percentage of environments in which each genotype ranked in the top (TOP), middle (MID) and bottom (BOT) third of trial entries. A genotype usually found in the top third of entries across environments can be considered relatively well adapted and stable. Thus, G5 was an adapted genotype, because it ranked in the top third of genotypes in a high percentage of environments (high top value, 80 %),

and was followed by G2, G3 and G4 (50 %) (Table 4). The undesirable genotypes identified by this method were G8, G1 and G13.

Table 4. Protein content (Y) and 16 non-parametric stability statistics for 13 wheat genotypes tested across 10 environments

Genotype	$Y^{\dagger}$	YSD	RM	RSD	TOP	RS	PA	R_1	R_2
G1	12.56	0.73	8.4	3.4	10	14	30	1.97	1.97
G2	13.09	1.08	6.2	4.2	50	11	60	3.62	3.62
G3	13.39	1.18	5.7	3.9	50	14	60	4.14	4.14
G4	13.44	1.15	3.8	2.6	50	5	60	4.31	4.31
G5	13.38	1.13	3.1	3.7	80	12	80	4.19	4.19
G6	12.82	0.94	5.5	3.7	20	13	50	2.79	2.68
G7	13.11	0.86	4.7	3.2	40	11	50	3.06	2.30
G8	12.89	0.92	6.6	2.3	0	8	40	3.20	3.20
G9	12.55	1.10	7.4	4.1	20	25	50	3.23	2.61
G10	12.83	0.92	7.1	3.6	20	15	50	3.63	2.28
G11	12.49	0.83	8.1	3.5	20	24	20	2.66	1.71
G12	12.67	0.57	6.7	4.1	20	19	30	1.96	1.13
G13	12.60	0.73	8.6	2.4	10	11	40	2.68	2.45
Mean	12.91	0.93	6.3	3.4	30	14	48	3.18	2.81

Genotype	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$
G1	3.4	9.8	10.7	2.6	1.9	0.19	0.31	0.05
G2	4.3	13.2	23.1	5.2	2.8	0.62	0.54	0.03
G3	5.7*	24.7*	25.7	6.2	4.7	0.94	0.86	0.08
G4	4.6	15.1	15.9	5.5	3.4	0.76	0.92	0.17
G5	4.8	16.4	29.8	6.1	3.3	1.10	0.94	0.14
G6	4.7	16.1	17.5	4.4	3.3	0.55	0.55	0.02
G7	4.3	13.4	16.6	4.7	2.8	0.43	0.60	0.06
G8	3.8	10.1	6.8	2.8	2.6	0.43	0.42	0.04
G9	5.4*	22.4*	18.1	4.1	3.8	0.51	0.53	0.04
G10	4.7	15.7	15.2	3.8	3.3	0.44	0.52	0.03
G11	4.9	18.2	12.6	3.2	3.2	0.32	0.45	0.06
G12	4.8	17.2	18.9	4.1	3.2	0.34	0.49	0.02
G13	3.2	8.7	6.2	1.8	1.9	0.21	0.30	0.06
Mean	4.5	15.5	16.7	4.2	3.1	0.53	0.57	0.06

*P<0.05, †Symbols: Y-Protein content (%), YSD-Protein content standard deviation, RM-Rank mean, RSD-Rank's standard deviation (KETATA, 1988), RS-Rank Sum stability statistic (KANG, 1988), PA-Percentage of adaptability (ST-PIERRE *et al*., 1967), R_1 and R_2-Range indexes (LANGER *et al*., 1979), TOP-Proportion of environments in which a genotype ranked in the top third (FOX *et al*., 1990), $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$-Ranks of adjusted protein content means of genotypes (HUEHN, 1996), $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$and $NP_i^{(4)}$-Ranks of adjusted protein content means of genotypes (THENNARASU, 1995).

KANG's (1988) rank-sum (RS) nonparametric stability statistic uses both PC mean and stability variance (SHUKLA, 1972). The genotypes with the lowest RS are the most favorable ones. According to the RS statistic, G4 and G8 had the lowest values for RS and therefore the former was stable with high PC, but the latter was stable with low PC (Table 4). With respect to this statistic, the undesirable genotypes were G9 and G11.

A genotype can be evaluated for its adaptation using the percentage of adaptability (PA) (ST PIERRE *et al*., 1967). This method measures proportion of environments in which is a given genotype outperforms the average of all genotypes including in the trial (DUARTE and ZIMMERMANN, 1995). The genotype G5 had the highest PA value (80 %), which indicates that the its PC was superior to the overall mean PC of the 13 genotypes in the trials, while G11 had lowest PA value (20 %) (Table 4).

LANGER *et al.* (1979) suggested two indexes (R_1 and R_2) related to the ranges in productivity of genotypes as crude measures of production response. The first, denoted R_1, equals the difference between the minimum and maximum PCs of a genotype in a series of environments, and the second, denoted R_2, is the difference between the PCs of a genotype in the lowest and highest production environments. Based on statistics R_1 and R_2, the most stable genotypes were G3, G4 and G5 with higher PC, whereas G1, G11 and G12 were unstable ones with lower PC (Table 4).

According to the significance tests for $S_i^{(1)}$ and $S_i^{(2)}$ developed by HUEHN (1996), there were no significant differences in rank stability among the 13 genotypes (except G3 and G9) grown in 10 environments (Table 4). Those, however, were significantly unstable relative to others. Genotypes with fewer changes in rank are considered to be more stable (BECKER and LEON, 1988). The $S_i^{(1)}$ estimates are based on all possible pair-wise rank differences across environments for each genotype, whereas $S_i^{(2)}$ is based on variances of ranks for each genotype across environments (HUEHN, 1996). These two statistics ranked genotypes similarity for stability. For example, according to both $S_i^{(1)}$ and $S_i^{(2)}$, G13 had the smallest changes in ranks and is thus, regarded as the most stable genotype, unlike G3 and G9. The next most stable genotype was G1. However, the most stable genotypes had lower PC than the average genotype (12.91 %).

Two other nonparametric statistics of HUEHN (1996), $S_i^{(3)}$ and $S_i^{(6)}$ combine PC and stability based on PC ranks of genotypes in each environment. These parameters measure stability in units of the mean rank of each genotype. The lowest value for each of these statistics indicates maximum stability for a certain genotype. G13 followed by G8 were the most stable according to the $S_i^{(3)}$ and $S_i^{(6)}$ parameters. The mean PC of G13 followed by G8 was one of the lowest genotypes tested (Table 4).

Results of THENNARASU's (1995) nonparametric stability statistics, which are calculated from ranks of adjusted PC means, are shown in Table 4. According to THENNARASU's (1995) three methods ($NP_i^{(1)}$, $NP_i^{(2)}$ and $NP_i^{(3)}$) genotypes G1 and G13 were stable in comparison with the other genotypes, although they were among genotypes with the lowest mean PC (Table 4). Stability parameter $NP_i^{(4)}$ identified G6 and G12 as stable genotypes, followed by G2 and G10. The results of three NPs ($NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$) were very similar to each other and identified G3, G4 and G5 as unstable, although they had the highest maximum mean PC performances. According to $NP_i^{(1)}$, G3 followed by G4 and G9 were unstable genotypes, although G9 was one of genotypes with the lowest mean PC performances.

The thirteen genotypes were ranked based on the numerical values of the PC and sixteen non-parametric stability methods (Table 5), where the lowest rank for PC corresponds to genotype

with highest PC; regarding the stability statistics, the lowest rank means the most stable genotype across environments. According to the overall mean of genotypic ranks, G8, G13, G1, G4 and G7 were the most stable ones, respectively, but G1 and G13 had lower PC than that of the average genotype (12.91 %).

Table 5. Ranks of 13 wheat genotypes based on protein content (Y) and 16 non-parametric statistics

Genotype	Y†	YSD	RM	RSD	TOP	RS	PA	R_1	R_2
G1	11	2	12	5	5	6	5	12	11
G2	5	7	6	11	2	3	2	5	4
G3	2	11	5	9	2	6	2	3	3
G4	1	10	2	3	2	1	2	1	1
G5	3	9	1	8	1	4	1	2	2
G6	8	6	4	8	4	5	3	9	6
G7	4	4	3	4	3	3	3	8	9
G8	6	5	7	1	6	2	4	7	5
G9	12	8	10	10	4	10	3	6	7
G10	7	5	9	7	4	7	3	4	10
G11	13	3	11	6	4	9	6	11	12
G12	9	1	8	10	4	8	5	13	13
G13	10	2	13	2	5	3	4	10	8
Mean	7.0	5.6	7.0	6.5	3.5	5.2	3.3	7.0	7.0

Genotype	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$	Mean
G1	2	2	3	2	1	1	2	4	5.1
G2	4	4	11	9	3	9	8	2	5.6
G3	11	13	12	12	8	11	11	6	7.5
G4	6	6	6	10	6	10	12	8	5.1
G5	8	9	13	11	5	12	13	7	6.4
G6	7	8	8	7	5	8	9	1	6.2
G7	5	5	7	8	3	5	10	5	5.2
G8	3	3	2	3	2	5	3	3	3.9
G9	10	12	9	6	7	7	7	3	7.7
G10	7	7	5	5	5	6	6	2	5.8
G11	9	11	4	4	4	3	4	5	7.0
G12	8	10	10	6	4	4	5	1	7.0
G13	1	1	1	1	1	2	1	5	4.1
Mean	6.2	7.0	7.0	6.5	4.2	6.4	7.0	4.0	5.9

†Symbols: Y-Protein content (%),YSD-Protein content standard deviation, RM-Rank mean, RSD-Rank's standard deviation (KETATA, 1988), RS-Rank Sum stability statistic (KANG, 1988), PA-Percentage of adaptability (ST-PIERRE *et al*., 1967), R_1 and R_2-Range indexes (LANGER *et al*., 1979), TOP-Proportion of environments in which a genotype ranked in the top third (FOX *et al*., 1990), $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$-Ranks of adjusted protein content means of genotypes (HUEHN, 1996), $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$and $NP_i^{(4)}$-Ranks of adjusted protein content means of genotypes (THENNARASU, 1995).

On the other hand, G4, G7 and G8 had medium to highest PC (12.89-13.44 %). As for the least stable ones, G3 and G9 were the most unstable genotypes. The former was among genotypes with higher PC whereas the latter was one of those with lower PC.

Table 6. Spearman's coefficients of rank correlation for the mean protein content (Y) and 16 non-parametric stability measures of 13 wheat genotypes evaluated in 10 environments

	Y†	YSD	RM	RSD	TOP	RS	PA	R_1	R_2
Y	1.00								
YSD	-0.68**	1.00							
RM	0.85**	-0.65*	1.00						
RSD	0.07	0.26	-0.11	1.00					
TOP	0.68**	-0.70**	0.72**	-0.46	1.00				
RS	0.68**	-0.21	0.50	0.57*	0.18	1.00			
PA	0.79**	-0.83**	0.74**	-0.25	0.75**	0.44	1.00		
R_1	0.76**	-0.90**	0.64*	-0.08	0.68**	0.38	0.88**	1.00	
R_2	0.76**	-0.87**	0.68**	0.03	0.59*	0.60*	0.85**	0.84**	1.00
$Si^{(1)}$	-0.08	0.49	-0.28	0.63*	-0.46	0.61*	-0.21	-0.30	-0.08
$Si^{(2)}$	0.01	0.42	-0.21	0.64*	-0.40	0.67*	-0.12	-0.20	-0.02
$Si^{(3)}$	-0.45	0.58*	-0.61*	0.81**	-0.80**	0.16	-0.63*	-0.43	-0.40
$Si^{(6)}$	-0.77**	0.81**	-0.84**	0.48	-0.92**	-0.18	-0.79**	-0.71**	-0.66*
$NPi^{(1)}$	-0.34	0.76**	-0.44	0.52	-0.56*	0.34	-0.50	-0.60*	-0.41
$NPi^{(2)}$	-0.75**	0.92**	-0.81**	0.41	-0.82**	-0.25	-0.89**	-0.86**	-0.84**
$NPi^{(3)}$	-0.76**	0.80**	-0.91**	0.34	-0.89**	-0.24	-0.82**	-0.74**	-0.68**
$NPi^{(4)}$	-0.47	0.46	-0.33	-0.40	-0.49	-0.37	-0.34	-0.50	-0.50

	$Si^{(1)}$	$Si^{(2)}$	$Si^{(3)}$	$Si^{(6)}$	$NPi^{(1)}$	$NPi^{(2)}$	$NPi^{(3)}$	$NPi^{(4)}$
$Si^{(1)}$	1.00							
$Si^{(2)}$	0.99**	1.00						
$Si^{(3)}$	0.65*	0.63*	1.00					
$Si^{(6)}$	0.58*	0.52	0.85**	1.00				
$NPi^{(1)}$	0.90**	0.85**	0.65*	0.72**	1.00			
$NPi^{(2)}$	0.52	0.45	0.77**	0.91**	0.73**	1.00		
$NPi^{(3)}$	0.53	0.46	0.76**	0.95**	0.70**	0.90**	1.00	
$NPi^{(4)}$	0.06	0.03	0.02	0.37	0.19	0.32	0.42	1.00

*P< 0.05, **P<0.01 †Symbols: YSD-Protein content standard deviation, RM-Rank mean, RSD-Rank's standard deviation (KETATA, 1988), RS-Rank Sum stability statistic (KANG, 1988), PA-Percentage of adaptability (ST-PIERRE *et al*. 1967), R_1 and R_2-Range indexes (LANGER *et al*., 1979), TOP-Proportion of environments in which a genotype ranked in the top third (FOX *et al*., 1990), $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$-Ranks of adjusted protein content means of genotypes (HUEHN, 1996), $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$and $NP_i^{(4)}$-Ranks of adjusted protein content means of genotypes (THENNARASU, 1995).

One of the main concerns of this study was to determine the non-parameteric stability statistics associated with mean PC (Y), because they might be used in selecting genotypes with high PC and stable in the multi-environment trials. Therefore, spearman's coefficients of rank correlation between 16 non-parametric stability measures and mean PC were estimated for this purpose (Table 6). Correlations higher of 0.70 are discussed because lower correlations than that may be significant from the statistical point of view, but not from the biological point of view (ZIVANOVIC *et al*., 2012). Mean PC was significantly positively correlated with RM, TOP, RS, PA, R_1 and R_2, while it was significantly negatively correlated with YSD, $S_i^{(6)}$, $NP_i^{(2)}$ and $NP_i^{(3)}$. The correlations were not significant between mean PC and RSD, $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $NP_i^{(1)}$ and $NP_i^{(4)}$.
Generally speaking, the stability parameters RM, TOP, RS, PA, R_1 and R_2 were significantly positively correlated with each other, but these measures were significantly negatively correlated with HUEHN's (1996) ($S_i^{(1)}$, $S_i^{(2)}$ $S_i^{(3)}$ and $S_i^{(6)}$) and THENNARASU's (1995) ($NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$) parameters. On the other hand, relationships among HUEHN's (1996) and THENNARASU's (1995) statistics were positively significant. Among those, $NP_i^{(4)}$ was not significantly correlated with all other parameters used in this study.

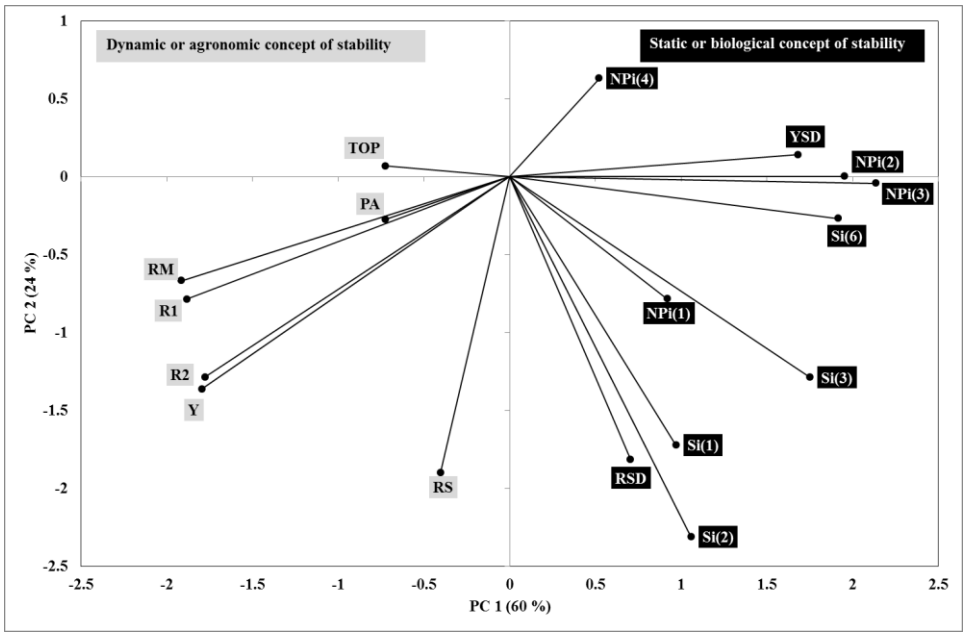

Figure 1. Biplot depicted by PCA1 versus PCA2 of principal component analysis conducted for ranks of stability of protein content, estimated by 16 non-parametric methods using protein content data from 13 wheat genotypes grown at 10 environments. Symbols: YSD-Protein content standard deviation, RM-Rank mean, RSD-Rank's standard deviation (KETATA, 1988), RS-Rank Sum stability statistic (KANG, 1988), PA-Percentage of adaptability (ST-PIERRE *et al*., 1967), R_1 and R_2-Range indexes (LANGER *et al*., 1979), TOP-Proportion of environments in which a genotype ranked in the top third (FOX *et al*., 1990), $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$-Ranks of adjusted protein content means of genotypes (HUEHN, 1996), $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$and $NP_i^{(4)}$-Ranks of adjusted protein content means of genotypes (THENNARASU, 1995).

Ranks of mean PC and 16 non-parametric stability parameters were subjected to principal components analysis (PCA) (Figure 1). PCA separated those parameters based on an agronomic (dynamic) concept of stability from those which are based on a biological (static) one (BECKER and LEON, 1988). The first and second principal components (PCs 1 and 2) extracted from PCA explained 84 % total variance of stability parameters.

For better visual, the two first PCs 1 and 2 were plotted against each other to generate a biplot (Figure 1), in which the first PC 1 separated the parameters Y, TOP, PA, RM, R_1, R_2, and RS (as those in the negative axis of PC 1) from the other methods (as those in the positive axis of PC 1). Figure 1 shows, on the right, methods corresponding to the biological (static) concept and, on the left, the methods based on the agronomic (dynamic) concept of stability. According to Biplot (Figure 1), concern was on the non-parametric statistics related with the agronomic (dynamic) concept of stability, since they could assist in discriminating genotypes with high PC and stable from ones with low PC and ustable, tested across a wide range of environments.

DISSCUSSION

The PC is influenced by both G and E factors (WILLIAMS *et al*., 2008). We found that PC was substantially influenced by E, rather than G and GEI effects. Our results were in agreement with those of PETERSON *et al*. (1992 and 1998), but in contrast with that of SOUZA *et al.* (2012). In case of E influence on PC, the relationship between mean PC and annual precitation amount across environments was not significant. But, we were expecting a positive correlation between them, because all environments received lower rainfall than that of the long term average during the crop cycles. Several authors reported that PC was higher in low rainfall environments and/or moisture stress increased it (RHARRABTI *et al*., 2003; HAILU *et al*., 2007; MUT *et al*., 2010; LI *et al*., 2013). In our case, genotypic differential responses to environments were irrelevant with annual rainfall. This lack of association showed that the magnitude of GEI effects for PC was unpredictable (MUT *et al*., 2010). Selection of genotypes for stability is needed in rainfed conditions, where the environment is variable and unpredictable. Therefore, genotype evaluation under variable environments and adoption of simultaneous selection for PC and stability is the most valuable selection index that can lead to desirable genotypes (JAMSHIDMOGHADDAM and POURDAD, 2013).

Comparing advanced lines with cultivars, used as checks, in respect of mean PC, the former, having lower PC, were obviously different from the latter, having higher PC. Hence, we should discuss the pros and cons of the selection strategy currently used for PC in our wheat breeding program. On the other hand, we should remember selecting genotypes not only for acceptable quality but also agronomic traits, including resistance to diseases in regular breeding cycles. We welcome new technologies that offer a means to predict processing and end-product quality of early generation lines that may reduce the investment (time and money) to bring a cultivar to the final stages of commercialization, because development of a commercial wheat cultivar requires 10 to 12 years at an estimated cost of US$ 2 million (SEABOURN *et al*.,2012).

Among the several objectives that breeding programs try to achieve simultaneously, the most important one is high yield. Good quality comes later in the list, although it is recognized that quality improvement is an essential objective to reach markets (VAZQUEZ *et al*., 2012). Obviously, there is a dilemma among dryland farmers in Turkey. In general, they demand neither genotypes with lower yielding and higher PC nor ones with higher yielding and lower PC, but do the ones combining higher yielding ability with higher PC capacity. From the breeding perpective, we are

not sure that their demand can be met for the time being. Hopefully, it can be materialized as a breeding goal for long term, as acceptable PC can be maintained while concomitantly increasing grain yield, due to the fact that there is a sufficient genetic variation to increase both grain yield and PC in wheat germplasm (DEPAUW *et al*., 2007). Even so, this is challenging because PC is a quantitatively inherited trait that is negatively correlated with grain yield and is greatly influenced by E factors (STEIGER *et al.*, 1996).

Turkey is one of the largest wheat producers in the world (http://faostat3.fao.org), but also recently has become an importer of high quality wheat for the domestic demand (http://www.tmo.gov.tr). Thus, it has been a driving force for our wheat breeding program in order to develop the genotypes with high PC and stable. Strictly speaking, we should compare our breeding stategy for quality improvement with that of BAENZIGER *et al*. (2001). In principal, both programs follow the bulk selection method during the breeding cycles. However, BAENZIGER *et al.* (2001)'s program starts evaluating breeding materials for quality at observation nurseries (F6), while our program does at the yield trials (F8). It means that they discard genotypes with unacceptable quality two generations earlier without advancing them upto yield trials. There are several reasons why we make quality analyses at very advanced generations, even if we know that selection efficieny would be lower at those generations, because genotypes with low quality could be discarded at the observation nurseries and/or preliminary yield trials, without promoting them to advanced tials. In practice, the most important reason is that our quality laboratory needs more personnel and equipments than what it has.

It is well-documented that GEI is important for most quality traits (BAENZIGER *et al.*, 2001). But, in our breeding program, quality assessment is regularly done at data obtained from one or rarely two locations, depending on the seasonel work loan at the quality laboratory. Our approach does not assume that GEI for quality traits (here PC) is absent. Rather, we recognize its presence and test promising genotypes in a wide range of environments for measuring both agronomic and quality traits (at one or two environments). We select them on the basis of their mean performance across all environments used and decide that the performance of the resulting genoypes is superior when averaged across all test environments (BERNARDO, 2002). In other words, our approach ignores GEI especially for quality evaluation.

Most of the studies, which aimed to detect stability for yield of genotypes tested across a wide range of environments, revealed a high correlation between stability rankings and yield, showing that Y, TOP, PA, RM, R_1, R_2, and RS were better indicators of the dynamic concept of stability (DUARTE and ZIMMERMANN, 1995; ADUGNA and LABUSCHAGNE, 2003; MOHAMMADI *et al*., 2007; MOHAMMADI and AMRI, 2008; SEGHERLOO *et al.*, 2008; ZIVANOVIC *et al*., 2012; JAMSHIDMOGHADDAM and POURDAD, 2013; MOHAMMADI and AMRI, 2013). Accordingly this study also showed that the results of rank correlation and biplot analyses for 16 non-parametric stability statistics, together with mean PC, were in complete agreement. But, from the perspective of PC stability, rather than that of yield, we found only three published studies, containing GEI detection for PC in the multi-environment trials using non-parametric stability measures in wheat (HAZEN *et al*., 1997; ROBERT, 2002; MUT *et al*., 2010). However, only two non-parametric stability statistics, HUEHN's (1996) $S_i^{(1)}$and $S_i^{(2)}$, were used in those papers. Moreover, they made a very limited contribution to the context of our paper. On the other hand, we were interested in dynamic non-parametric stability statistics (Y, TOP, PA, RM, R_1, R_2, and RS) because we found that they can be effectively used for simultaneously selecting genotypes high PC and stable.

In conclusion, we took the messages from this study given below:

1- Screening the breeding materials for quality in our breeding program should be commenced at observations nurseries (F6), but not at yield (F8) trials. By doing this, we can discard the genotypes with unacceptable quality, from the breeding materials, without advancing them to yield trials.
2- Due to the fact that quality traits are influenced by GEI, quality analyses should be conducted on multi-environment (year by location combinations) trials, but not on one year and/or location trials.
3- According to the current study findings, dynamic non-parametric stability statistics, Y, TOP, PA, RM, R_1, R_2, and RS should be used for selecting genotypes with high PC and stable in the meantime.

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NEPARAMETARSKA ANALIZA STABILNOSTI SADRŽAJA PROTEINA KOD PŠENICE (*T. aestivum* L.) GAJENE U MULTI EKOLOŠKIM USLOVIMA SPOLJNE SREDINE

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Izvod

Cilj eksperimenata je bio ispitivanje stabilnosti sadržaja proteina (PC) kod pšenice koristeći 16 ne-parametarskih pokazatelja stabilnosti (YSD-PC standardna devijacija, RM- sredina ranga, RSD- Standardna devijacija ranga, RS- Suma stabilnosti ranga, R_1 i R_2-indeksi ranga, TOP - rangiranje, $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$ i $NP_i^{(4)}$statistika ranga zajedno sa Y-PC sredine). Studija je uključila 13 genotipova pšenice, među kojima 5 registrovanih genotipova i 8 linija, odabranih iz Nacinalnog programa oplemenjivanja pšenice u Turskoj. Genotipovi su gajeni u 10 sredina sa dovoljno kiše, koji pretstavljaju glavne areale gajenja u Turskoj, u toku sezona 2011 – 2013. ANOVA je pokazala da su efekti spoljne sredine (E), genotipova (G) i GE interakcije (GEI) bili značajni ($P < 0.01$). Spearman's korelacija ranga i analiza glavnih komponenata prinosa (PCA) su takođe potvrdile da su nađena dva tipa asocijacije između parametara stabilnosti: prvi tip uključuje $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(1)}$, $NP_i^{(2)}$ $NP_i^{(3)}$, $NP_i^{(4)}$, RSD i YSD parametar koji se odnose na statičku stabilnost, dok se drugi tip sastojo od Y, RM, TOP, PA, RS, R1 i R2 parametara koji se odnose na dinamički koncept stabilnosti. Među 8 linija G7 i G8 linije su bile najbolji genotipovi u smislu visokog sadržaja i stabilnosti. Zaključeno je da se dinamička neparametarska statistika stabilnosti može koristiti za odabiranje genotipova sa visokim sadržajem proteina (PC) i stabilnih kada se vrši testiranje u velikom broju uslova sredine.

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