



## Evaluation of Genotype × Environment Interaction in Landraces of Common Wheat using Non-parametric Stability Indicators

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**ABSTRACT:** In order to determine stability of bread wheat genotypes field experiments were conducted for three consecutive years. Combined analysis of variance displayed significant differences between genotypes (G), environments (E) and genotypes × environments interaction (GEI) indicating the presence of fluctuations between environments and genetic variability between accessions. According to the parameters  $Si^{(1)}$ ,  $Si^{(2)}$ ,  $Si^{(3)}$  and  $Si^{(6)}$  G18 had the smallest changes in ranks and is thus regarded as the most stable genotype. Biplot clustered the stability measures in 3 groups. Group 1 (G1) included yield and  $NPI^{(3)}$ . The PCs axes separated  $Si^{(1)}$ ,  $Si^{(3)}$ ,  $NP^{(1)}$  and  $NPI^{(2)}$  in group 2 and  $Si^{(2)}$  and  $Si^{(6)}$  were classified as Group 3. G1 introduced G12 as stable with high mean yield. All of the stability indices in G2 discriminated accession G8 as stable with average grain yield. Non-parametric stability measures in G3 identified G8 as the most stable genotype with average grain yield. The rank sum (RS) method distinguished genotypes no. 19 as the most stable genotype with high grain yield, hence it can be used for improvement of adaptation in wheat.

**Key words:** Bread wheat, Stability, non-parametric statistics, rank sum.

### INTRODUCTION

Increasing the genetic potential of yield is an important objective of both bread and durum wheat breeding programs in Iran and other countries. Bread wheat genotypes are generally evaluated in multi-environment trials (MET) to test their performance across environments and to select the best genotypes for specific environments. In most cases, GEI is significant, complicating yield improvement studies, but the release of a genotype with consistent performance over a wide range of environments should lead to stability in production (Akçura and Kaya, 2008).

There are two major approaches to study genotype by environment interactions and determining the adaptation and stability of genotypes (Becker and Leon, 1988; Huehn, 1990). The most common approach is parametric analyses, which are based on statistical assumptions about the distribution of genotypic, environmental and GEI effects. Another approach is nonparametric analytical clustering, which makes no specific modeling assumptions when relating environments and phenotypes relative to biotic and abiotic environmental factors (Adugna and Labuschagne, 2003).

According to Huehn (1990) nonparametric procedures have the following advantages over parametric stability methods: (i) they reduce the bias caused by outliers, (ii) no assumptions are needed about the distribution of

observed values. (iii) they are easy to use and interpret. (iv) addition or deletion of one or more genotypes does not cause much variation in results.

The studies conducted against this background by Rao and Prabhakaran (2000) have shown that when a number of genotypes is fairly large, the power efficiency of the nonparametric measures will be quite close to those of parametric measures. So in situations, which are commonly encountered, i.e. those involving a good number of genotypes being performance tested in a set of environments whose number is neither too small nor too large, the risk of selecting inferior genotypes from use of nonparametric measures is minimal (Rao and Prabhakaran, 2000).

The following four nonparametric measures of phenotypic stability have been proposed by Nassar and Huehn (1987):  $Si^{(1)}$ , the genotype absolute rank difference mean as tested over  $n$  environments;  $Si^{(2)}$ , the between-ranks variance over the  $n$  environments;  $Si^{(3)}$ , the sum of the absolute deviations of the squares of ranks for each genotype; and  $Si^{(6)}$ , the sum of the squares of ranks for each genotype relative to the mean of ranks.

Thenarasu (1995) proposed as stability measures then non-parametric statistics based on ranks of adjusted means of the genotypes in each environment and defined stable genotypes using Nassar and Huehn's definition (1987).

Bredenkamp, Hildebrand and Kubinger (Sabaghnia *et al.*, 2013) proposed nonparametric tests based on the usual linear model for interactions or non-crossover interactions. de Kroon and van der Laan and Azzalini and Cox (Sabaghnia *et al.*, 2013) introduced nonparametric tests for evaluation of crossover GE interactions. The objectives of this study were to identify bread wheat genotypes that have both high grain yield and stable performance across different environments for semiarid areas of Iran and study the relationships between different nonparametric stability statistics.

## MATERIALS AND METHODS

### A. Experimental layout and genetic materials

In order to determine stability of 19 bread wheat genotypes field experiments were conducted for three consecutive years (2011-2013) under two different conditions (irrigated and rainfed) (Table 1) at Kermanshah (34° 21' N latitude, 47° 9' E longitude and 1319 m altitude).

**Table 1: Genotype code and name of 20 bread wheat genotypes.**

No.	Code	Name
1	G1	Geravandi-17
2	G2	WC-47536
3	G3	WC-4919
4	G4	WC-4868
5	G5	WC-5046
6	G6	WC-4995
7	G7	Pishgam-1
8	G8	WC-4536
9	G9	Pishgam-2
10	G10	WC-47582
11	G11	WC-47359
12	G12	WC-47403
13	G13	WC-47388
14	G14	WC-4611
15	G15	WC-4515
16	G16	Pishtaz
17	G17	Moghan-3
18	G18	WC-47472
19	G19	WC-4968

The experimental layout at each environment was randomized complete block design with three replications. Climate of the region is classified as semi-arid with mean annual rainfall of 379.3 mm. Minimum and maximum temperatures at the research station were 5.9 and 22.6°C, respectively. Each plot consisted of five rows with 5 meter length. Rows distance was 20cm with seed density 400 per m<sup>2</sup>. Data on seed yield were taken from the middle two rows of each plot. The seed yield was determined for each genotype at each test environments. The environments were considered as random factors while genotypes as fixed factors.

### B. Statistical analysis

In this investigation, four nonparametric statistical methods were applied to test the significance of G×E interaction. The methods of Bredenkamp, Hildebrand and Kubinger (Sabaghnia *et al.*, 2013) are based on the usual linear model for interactions: Interactions are defined as deviations from the additivity of main effects. The procedure of the de Kroon and van der Laan (Sabaghnia *et al.*, 2013) was used for testing crossover G×E interactions. The test statistics of above methods are approximately <sup>2</sup> distributed with (k-1)(n-1) degrees of freedom, where k = number of genotypes, and n = number of environments. Nassar and Huehn (1987) proposed four nonparametric measures of phenotypic stability Si<sup>(1)</sup>, Si<sup>(2)</sup>, Si<sup>(3)</sup> and Si<sup>(4)</sup> as follows:

$$S_i^{(1)} = 2 \sum_j^{m-1} \sum_{j'=j+1}^m |r_{ij} - r_{ij'}| / [m(m-1)]$$

$$S_i^{(2)} = \sum_{j=1}^m (r_{ij} - \bar{r}_i)^2 / (m-1)$$

$$S_i^{(3)} = \sum_{j=1}^m (r_{ij} - \bar{r}_i)^2 / \bar{r}_i$$

$$S_i^{(6)} = \sum_{j=1}^m |r_{ij} - \bar{r}_i| / \bar{r}_i$$

Where Si<sup>(1)</sup> = mean of the absolute differences among the classification l-th cultivar in j-th environment, Si<sup>(2)</sup> = variance of classification l-th cultivar in j-th environment, Si<sup>(3)</sup> = sum square of classification l-th cultivar in all environment divide to mean classification of cultivar in all environment and Si<sup>(6)</sup> = sum of mean absolute deviations in yield units of each classification relatives to mean classification, l = number of genotypes, m = number of environments, rij = the rank of the i<sup>th</sup> genotype in the jth environment and  $\bar{r}_i$  = the mean rank across all environments for the ith genotype. Thenarasu (1995) proposed nonparametric statistics NP<sub>i</sub><sup>(1)</sup>, NP<sub>i</sub><sup>(2)</sup>, NP<sub>i</sub><sup>(3)</sup>, and NP<sub>i</sub><sup>(4)</sup> based on ranks of adjusted means of the genotypes in each environment, and defined stable genotypes as those whose position in relation to the others remained unaltered in the set of environments assessed.

$$NP_i^{(1)} = \frac{1}{m} \sum_{j=1}^m |r_{ij}^* - M_{di}^*|$$

$$NP_i^{(2)} = \frac{1}{m} \left( \sum_{j=1}^m |r_{ij}^* - M_{di}^*| / M_{di}^* \right)$$

$$NP_i^{(3)} = \frac{\sqrt{\sum (r_{ij}^* - \bar{r}_i^*)^2 / m}}{\bar{r}_i}$$

$$NP_i^{(4)} = \frac{2}{m(m-1)} \left( \sum_{j=1}^{m-1} \sum_{j'=j+1}^m |r_{ij}^* - r_{ij'}^*| / \bar{r}_i \right)$$

In the formulas,  $r_{ij}^*$  is the rank of  $X_{ij}^* = X_{ij} - X_i$ ,  $r_i^*$  and  $M_{di}^*$  are the mean and median ranks for adjusted values, where  $r_i$  and  $M_{di}$  are the same parameters computed from the original (unadjusted) data. The data were subjected to nonparametric analysis using SAS software.

## RESULTS AND DISCUSSION

### A. Parametric and non-parametric combined analysis of variance

Analysis of variance was conducted to determine the effects of genotype, environments and interactions. Parametric combined analysis of variance displayed significant differences between genotypes (G), environments (E) and genotypes  $\times$  environments interaction (GEI) indicating the presence of fluctuations between environments and genetic variability between accessions (Table 2). Methods of Hilderbrand, Kubinger and van der Laan-de Kroon didn't show significant interaction, whereas method of Bradenkamp revealed significant genotype  $\times$  environment interaction for grain yield (Table 3), hence we can proceed to calculate phenotypic stability of genotypes (Farshadfar and Sutka, 2006). The null hypothesis for Hildebrand and Kubinger is no non-crossover GEI and for De Kroon and Van der Laan is no crossover GEI. Results of these indicated that both non-significant non-crossover and crossover interactions were found in these multi-environment trials (MET) according to Kubinger and Hildebrand procedures (for non-crossover) and the De Kroon and Van der Laan test (for crossover).

**Table 2: Parametric combined analysis of variance for grain yield across 6 environments.**

S.O.V	Df	Sum of squares	Mean of squares
Genotypes (G)	19	881132.014	48951.779**
Environments (E)	5	11103204.426	2220640.885**
Interactions (G $\times$ E)	90	2120305.515	23558.950**
Pooled error	228	3636455.24	15949.36

\*\* : significant at 1% probability level

**Table 3: Non-parametric combined analysis of variance over environments.**

Methods	df	$\chi^2$
Bredenkamp	90	979.3**
Hildebran	90	4.5 <sup>ns</sup>
Kubinger	90	2.85 <sup>ns</sup>
Van der laan and Dekroon	90	0.000072 <sup>ns</sup>

This result is in agreement with the ANOVA, but provides more specific information about the nature of GEI action (Mohammadi *et al.*, 2007). Mean comparisons revealed that maximum grain yield was related to G18, while minimum grain yield was attributed to G2.

### B. Non-parametric stability statistics

The results of nonparametric stability measures of genotypes for grain yield, are shown in Table 4. Test of significance  $Z^{(1)}$  for  $Si^{(1)}$  was significant for grain yield. The  $Si^{(1)}$  and  $Si^{(2)}$  statistics are based on ranks of the genotypes across environments and they give equal weight to each environment. Genotypes with fewer changes in rank are considered to be more stable (Mohammadi *et al.*, 2011). The  $Si^{(1)}$  estimates are based on all possible pair-wise rank differences across environments for each genotype, whereas  $Si^{(2)}$  is based on variances of ranks for each genotype across environments (Nassar and Huehn, 1987). Nevertheless, these two statistics ranked genotypes similarly for stability. For example, according to both  $Si^{(1)}$  and  $Si^{(2)}$ , G18 had the smallest changes in ranks and is thus regarded as the most stable genotype unlike G8 with maximum  $Si^{(1)}$  and  $Si^{(2)}$  which was significantly ( $P < 0.01$ ) unstable (Table 4). Two other nonparametric statistics  $Si^{(3)}$  and  $Si^{(6)}$  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. Like  $Si^{(1)}$  and  $Si^{(2)}$ , G18 was the most stable according to the  $Si^{(3)}$  and  $Si^{(6)}$  and the most unstable genotype according to  $Si^{(3)}$  and  $Si^{(6)}$  was G8. Results of Thennarasu's nonparametric stability statistics, which are calculated from ranks of adjusted yield means, are exhibited in Table 4, and the ranks of genotypes according to these parameters are given in Table 4. According to  $NPi^{(1)}$ , G2 and G8;  $NPi^{(2)}$ , G11;  $NPi^{(3)}$ , G11 and  $NPi^{(4)}$ , G12 was identified as the most stable, therefore these results are not in agreement with that of Thennarasu's nonparametric stability statistics.

### C. Relationship among different non-parametric stability statistics

To better understand the relationships, similarities and dissimilarities among non-parametric stability estimates, principal component analysis (PCA), based on the rank correlation matrix was used. The main advantage of using PCA over cluster analysis is that each statistics can be assigned to one group only (Khodadadi *et al.*, 2011).

The PCA1 and PCA2 axes which justify 71.75% of total variation, mainly distinguish the stability estimates in different groups. One interesting interpretation of biplot is that the cosine of the angle between the vectors of two indices approximates the correlation coefficient between them. The cosine of the angles does not precisely translate into correlation coefficients, since the biplot does not explain all of the variation in a dataset.

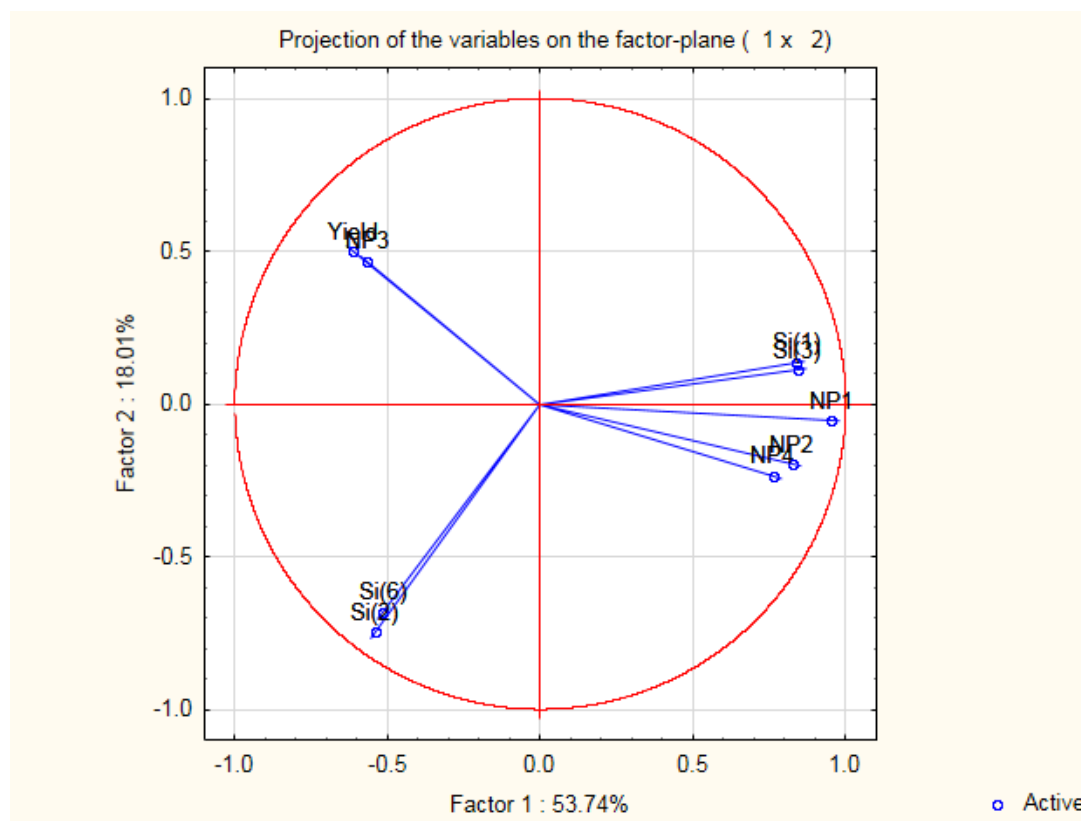
**Table 4: Mean values and nonparametric stability statistics for grain yield.**

Code	Yield	$S_i^{(1)}$	$Z_i^{(1)}$	$S_i^{(2)}$	$Z_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP <sub>1</sub>	NP <sub>2</sub>	NP <sub>3</sub>	NP <sub>4</sub>
1	281.6	2.50	46.02	7.86	2.74	5.13	1.478	-0.66	0.38	3.09	0.06
2	252.8	5.77	0.92	27.57	0.03	19.01	2.27	-1.83	0.95	4.54	0.088
3	255.6	7.16	2.26	36.64	0.24	25.86	4.3	-0.66	0.93	4.75	0.091
4	304.2	5.54	1.89	37.06	0.28	27.8	2.6	1.16	1	4.63	0.07
5	289.3	6.56	0.2	28.97	0.005	16.55	1.14	-1.33	0.46	3.4	0.08
6	276.6	6.40	0.02	30.16	0.0001	25.85	3.31	-1.66	1.16	4.95	0.10
7	282.5	5.61	1.58	25.9	0.094	13.63	1.7	0.83	0.45	2.73	0.03
8	233.4	20.8	671.17	491.4	1194.4	184.3	6.2	-1.83	1.25	6.28	0.17
9	308.6	6.33	0.0013	27.4	0.035	16.48	2.8	0.66	0.43	3.29	0.05
10	350.4	4.42	11.38	15.1	1.24	6.04	1.2	2.16	0.27	2.61	0.019
11	308.5	4.42	11.38	16.3	1.05	8.57	1.8	-1.33	0.19	2.163	0.056
12	296.4	3.82	19.64	11.06	2.01	6.38	1.4	0.33	0.235	1.66	0.017
13	296.5	4.42	11.38	14.96	1.26	6.90	1.56	-1.16	0.21	2.14	0.047
14	339.6	5.11	4.57	17.84	0.82	7.08	0.5	3.166	0.327	3.606	0.029
15	328.5	6.6	0.25	31.06	0.006	12.59	2.2	1.33	0.21	2.40	0.02
16	349.8	4.29	13.02	12.7	1.67	5.08	1.36	1.16	0.33	3.002	0.024
17	410.6	7.06	1.76	42.16	0.83	16.42	2.59	1.5	0.29	3.418	0.033
18	412.4	1.78	65.25	2.3	4.303	0.65	0.4	4	0.27	3.51	0.02
19	403.6	8.58	16.27	64.66	6.74	24.25	3.1	1.83	0.38	4.69	0.03
Mean	5980.9										
		117.1	878.9	941.1	1217.7	428.5	41.9	7.6	9.7	66.7	1.03
		Test statistics									
		$Z_i^{(1)} = 879.05$									
		$Z_i^{(2)} = 1217.861$									
		$V(S_i^{(1)}) = 2.39$					$V(S_i^{(2)}) = 179$				

$S_i$ , Huehn's (1979) non-parametric stability statistics. NP<sub>i</sub>, Thennarasu's (1995) non-parametric stability statistics.

Nevertheless, the angles are informative enough to allow a whole picture about the interrelationships among the stability estimates. Biplot clustered the stability measures in 3 groups. Group 1 (G1) included yield and NP<sub>i</sub><sup>(3)</sup>. The PCs axes separated  $S_i^{(1)}$ ,  $S_i^{(3)}$ , NP<sub>i</sub><sup>(1)</sup>NP<sub>i</sub><sup>(2)</sup> and NP<sub>i</sub><sup>(4)</sup> in group 2 and  $S_i^{(2)}$  and  $S_i^{(6)}$  were

classified as Group 3. G1 introduced G12 as stable which showed almost high mean yield. All of the stability indices in G2 discriminated accession G8 as stable with average grain yield. Non-parametric stability measures in G3 identified G8 as the most stable genotype with average grain yield.



**Fig. 1.** Biplot analysis of non-parametric stability statistics and grain yield of landraces of bread wheat over rainfed and irrigated conditions.

The vector view of the biplot (Fig. 1) provides a summary of the interrelationships among the stability indicators. The lines that connect the stability estimates to the biplot origin are called stability vectors. The cosine of the angle between the vectors of two stability indices approximates the correlation between them. For example, G2 stability measures were positively correlated (an acute angle), the same conclusion was obtained for the G3 stability estimates, while G1 was negatively correlated with G2 indices (an obtuse angle) and independence no correlation (right angle) between G1 and G3 stability measures. This procedure was also employed in chickpea (Khodadadi *et al.*, 2011) for clustering stability statistics and in wheat Mohammadi *et al.*, (2011) for screening selection criteria of different climate and water regime conditions.

#### *D. Ranking procedure*

The estimates of stability indicators (Table 4) exhibited that the identification of stable genotypes based on a single criterion was different. Therefore different groups introduced different stable genotypes. To have an overall judgement based on mean yield and all stability statistics ranking method was used. The rank sum (RS) of all the genotypes investigated distinguished genotypes no. 19 as the most stable genotype with high grain yield, hence it can be used for improvement of adaptation in wheat (Table 5). The same procedures have been employed for screening stability criteria and quantitative indicators of drought tolerance in wheat (Mohammadi *et al.*, 2007) and in chickpea (Zali *et al.*, 2011).

**Table 5: Ranks of yield, stability estimators, mean of ranks, standard deviation of ranks and rank sum of bread wheat accessions over three environments.**

Code	Yield	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP <sub>1</sub>	NP <sub>2</sub>	NP <sub>3</sub>	NP <sub>4</sub>	SDR	Mean of ranks	RS
1	15	18	3	18	4	17	13	12.5	9.5	5.31	11.72	17.04
2	18	9	15	9	16	6	8	18.5	4	5.53	10.31	15.85
3	17	3	11	5	13	3	2	12.5	5	5.28	7.04	12.32
4	10	11	12	4	12	2	6	7.5	3	3.62	7.13	10.756
5	13	6	17	8	18	7	17	15.5	6	5.06	11.13	16.20
6	16	7	18	7	19	4	3	17	2	7.13	8.81	15.95
7	14	10	14	11	14	10	11	9	7	2.39	11.5	13.89
8	19	1	1	1	1	1	1	18.5	1	7.18	4.2	11.40
9	8	8	19	10	15	8	5	10	8	3.85	10.09	13.94
10	4	14	8	14	8	16	16	3	14.5	5.17	11.86	17.04
11	9	14	8	13	9	12	10	15.5	19	3.80	12.22	16.03
12	12	17	4	17	5	15	14	11	16	5.14	13.54	18.69
13	11	14	8	15	7	14	12	14	17.5	3.55	12.77	16.33
14	6	12	10	12	11	13	18	2	12	4.31	10.63	14.95
15	7	5	16	6	17	11	9	6	17.5	5.11	11.54	16.65
16	5	16	6	16	6	18	15	7.5	11	4.77	11.68	16.45
17	2	4	13	3	10	9	7	5	13	3.89	7.818	11.71
18	1	19	2	19	3	19	19	1	14.5	8.07	11.09	19.16
19	3	2	5	2	2	5	4	4	9.5	3.31	4.81	8.13

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