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RESEARCH PAPER

Nonparametric methods for interpreting genotype×environment interaction in bread wheat genotypes

Ezatollah Farshadfar[1*], Nasrin Mahmudi[1], Azadahe Sheibanirad[1]

Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran

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Key words: Bread wheat, phenotypic stability, non-parameric procedures.

Abstract

In order to investigate phenotypic stability of bread wheat genotypes using non-parametric stability statistics an experiment was conducted in Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran for three years under two environments (rainfed and irrigated conditions). Parametric and non-parametric combined analysis of variance exhibited significant genotype × environment interaction, therefore it was possible to calculate phenotypic stability of genotypes. Non-parameric stability statistics $S_i^{(1)}$, $S_i^{(2)}$ and $S_i^{(3)}$ introduced G2 as the most stable genotype. According to $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ genotype G12 was identified as the most stable. The rank sum (RS) of all the genotypes investigated distinguished genotypes no. 12 as the most stable genotype with high grain yield, hence it can be used for improvement of adaptation in wheat.

***Corresponding Author:** Ezatollah Farshadfar ✉ farshadfar@razi.ac.ir

Introduction

The genotype by environment interaction is a major problem in the study of quantitative traits because it complicates the interpretation of genetic experiments and makes predictions difficult. Therefore, the first goal of plant breeders in a crop breeding program is the development of cultivars or genotypes which are stable or adapted to a wide range of diversified environments (Farshadfar and Sutka, 2006; Abdulahi *et al.*, 2009; Pimsaen *et al.*, 2010). The study of genotype by environment interaction (GEI) has assumed great importance in genotype testing programs because yield performance of a genotype is a result of the interaction between the genotype and environment. Environmental factors, such as rainfall, temperature and soil structure play an important role in genotype performance, grain yield and quality. GE interaction reduces the association between genotypic and phenotypic values and thereby reduces the genetic progress resulted from selection (Kearsey and Pooni, 2004). In semi–arid areas where climate is unpredictable, production of varieties with high yield and wide adaptation is one of the most important goals of plant breeding programs. When discussing unpredictable changes in the yield we use the term phenotypic stability which is the changes occurred in the phenotypic expression of yield (Becker and Leon, 1988).

On the basis of this idea, genotypes with a minimal variance for yield across different environments are considered stable. This idea of stability may be considered as a biological or static concept of stability (Becker and Leon, 1988). This concept of stability is not acceptable to most breeders and agronomists, who prefer genotypes with high mean yields and the potential to respond to agronomic inputs or better environmental conditions (Becker, 1981). The high yield performance of released varieties is one of the most important targets of breeders, which explains why they prefer a dynamic concept of stability (Becker and Leon, 1988).

Huehn (1990) indicated that there are two major approaches to studying G × E interaction and determining adaptation of genotypes. The first and most common approach is parametric, which relies on distributional assumptions about genotypic, environmental, and G×E effects. The second major approach is the nonparametric or analytical clustering approach, which relates environments and phenotypes relative to biotic and abiotic environmental factors without making specific modeling assumptions. For practical applications, however, most breeding programs incorporate some elements of both approaches (Becker and Leon, 1988).

The parametric stability methods have good properties under certain statistical assumptions, like normal distribution of errors and interaction effects; however, they may not perform well if these assumptions are violated (Huehn, 1990). That means parametric tests for significance of variances and variance-related measures could be very sensitive to the underlying assumptions. Thus, it is wise to search for alternative approaches that are more robust to departures from common assumptions, such as nonparametric measures (Nassar and Huehn, 1987; Huehn and Nasar, 1989).

Nonparametric measures for stability based on ranks provide a viable alternative to the above existing parametric measures based on absolute data. For many applications, including selection in breeding and testing programs, the rank orders of the genotypes are the most essential information. Stability measures based on ranks require no statistical assumptions about the distribution of the phenotypic values. They are easy to use and interpret and, compared with parametric measures, are less sensitive to errors of measurement. Furthermore, addition and deletion of one or a few observations is not as likely to cause great variation in the estimates as would be the case for parametric stability measures (Nassar and Huhn, 1987).

Several nonparametric procedures proposed by

Huehn (1979), Nassar and Huehn (1987), Kang (1988), and Thennarasu (1995) are based on the ranks of genotypes in each environment and genotypes with similar ranking across environments are classified as stable. Huehn (1979) and Nassar and Huehn (1987) proposed four nonparametric measures of phenotypic stability Si $^{(1)}$, Si $^{(2)}$, Si $^{(3)}$ and Si $^{(4)}$:

Kang (1988) assigned ranks for mean yield, with the genotype with the highest yield receiving the rank of 1, and ranks for the stability variance of Shukla (1972), with the lowest estimated value receiving the rank of 1.

Thennarasu (1995) proposed as stability measures the nonparametric statistics NPi $^{(1)}$, NPi $^{(2)}$, NPi $^{(3)}$, and NPi $^{(4)}$ 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. Huehn and Leon (1995) compared four nonparametric analyses of interactions and grouped them into two different concepts of interactions. While the Bredenkamp, Hildebrand, and Kubinger procedures depend on usual interactions, the van der Laan–de Kroon method depends on crossover interactions. Truberg and Huehn (2000) studied five statistical methods for the analysis of G × E interactions and suggested that for analysis of usual noncrossover interactions, the methods of Hildebrand and Kubinger are closely connected with the ANOVA. If some of the necessary assumptions are violated, the validity of the inferences obtained from the standard statistical techniques, for example, ANOVA, may be questionable or lost. In such cases, however, the results of nonparametric estimation and testing procedures, which are based on ranks, can be more reliable (Truberg and Huehn, 2000).

The objectives of this study were to (i) to identify wheat genotypes that have both high mean yield and stable yield performance across different environments for semiarid areas of Iran, (ii) to apply nonparametric tests to investigate of crossover and noncrossover interaction in multi-environments trials (METs), and (iii)to study the relationships among nonparametric stability statistics.

Materials and methods

This research data set involves 14 wheat genotypes tested in six environments (year–location combinations) during 2006– 2009. At each location, a completely randomized block design with three replications was used. Mean grain yield, was estimated for each genotype at each location (environment).

Statistical analysis

In this investigation, four nonparametric statistical methods were applied to test the significance of G×E interaction. The methods of Bredenkamp (1974), Hildebrand (1980), and Kubinger (1986) 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 (1981) was used for testing crossover G×E interactions. The test statistics of above methods are approximately χ^2 distributed with (k-1)(n-1) degrees of freedom, where k = number of genotypes, and n= number of environments. These statistical methods have been described in detail by Huehn and Leon (1995) and Truberg and Huehn (2000). Huehn (1979) and Nassar and Huehn (1987) proposed four nonparametric measures of phenotypic stability Si $^{(1)}$, Si $^{(2)}$, Si $^{(3)}$ and Si $^{(4)}$ as follows:

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

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

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

$$S_i^{(6)} = \sum_{j=1}^{m}\left|r_{ij} - \bar{r}_{i.}\right| / \bar{r}_{i.}$$

Where Si $^{(1)}$ = mean of the absolute differences among the classification l-th cultivar in j-th environment, Si $^{(2)}$ = variance of classification l-th cultivar in j-th environment, Si $^{(3)}$ = sum square of classification l-th cultivar in all environment divide to mean classification of cultivar in all environment and Si $^{(6)}$ = sum of mean absolute deviations in yield units of each classification relatives to mean classification, l = number of genotyoes, m= number of environments, rij = the rank of the ith genotype in the jth environment and $\bar{r}_{i.}$ = the mean rank across all environments for the ith genotype.

Thennarasu (1995) proposed nonparametric statistics NPi $^{(1)}$, NPi $^{(2)}$, NPi $^{(3)}$, and NPi $^{(4)}$ 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.

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

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

$$NP_i^{(3)} = \frac{\sqrt{\sum\left(r_{ij}^{*} - \bar{r}_{i.}^{*}\right)^2 / m}}{\bar{r}_{i.}}$$

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

In the above formulas,r^*_{ij} is the rank of X^*_{ij} = Xij – Xi., $r^*_{i.}$ and M^*_{di} are the mean and median ranks for adjusted values, where $r_{i.}$ and Mdi are the same parameters computed from the original (unadjusted) data. The data were subjected to nonparametric analysis using SAS software (SAS, 1996).

Results and discussion

Parametric and non-paramteric combined analysis of variance

Analysis of variance was conducted to determine the effects of year, location, genotype and interactions. Parametric combined analysis of variance displayed significant differences between years (Y), locations (L), Y × L interaction and genotypes indicating the presence of fluctuations between years and locations and genetic variability between accessions (Table 1). Methods of Hilderbrand, Kubinger and van der Laan-de Kroon didn't show significant interaction, whrease method of Bradenkamp revealed significant genotype × environment interaction for grain yield (Table 2), hence we can proceed an calculate phenotypic stability of genotypes (Farshadfar and Sutka, 2003; 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 thatboth non-significant non-crossover and crossover interactions were found in these multienvironment trials (MET) according to Kubinger and Hildebrand procedures (for non-crossover) and the *De* Kroon and *Van* der Laan test (for crossover). This result is in agreement with the ANOVA, but provides more specific information about the nature of GEI action (Sabaghnia *et al.*, 2006; Mohammadi *et al.*, 2007).

Table 1. Parametric combined analysis of variance over environments.

Source	Df	Grain yield
Year (Y)	2	174507.57**
Location (L)	1	453152.966**
Y×L	2	241447.286**
R (Y×L)	10	15211.872
Genotype (G)	13	105626.867**
G × Y	26	1418.135
G × L	13	4774.733
G × Y × l	26	2097.884
Error	172	13860.088

Non-parametric stability statistics

The results of nonparametric stability measures of genotypes for grain yield, are shown in Table 3. 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 (Becker and Leon, 1988). 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 $Si^{(2)}$, G2 had the smallest changes in ranks and is thus regarded as the most stable genotype unlike G3, which was significantly ($P<0.01$) unstable (Table 3). Two other nonparametric statistics of Huehn (1979), $Si^{(3)}$ 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 $Si^{(2)}$, G2 was the most stable according to the $Si^{(3)}$ parameter, but according to $Si^{(6)}$, G13 was the most stable genotype. The most unstable genotype according to $Si^{(3)}$ and $Si^{(6)}$ was G14.

Table 2. Non-parametric combined analysis of variance over environments.

Bredenkamp	885.771^{**}
Hildebrand	0.700
Kubinger	0.169493
Laan-Kroon	0.01

*P<0.05; **P<0.01.

Results of Thennarasu's nonparametric stability statistics, which are calculated from ranks of adjusted yield means, are exhibited in Table 3, and the ranks of genotypes according to these parameters are given in Tables 4. According to $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$ genotypes G12 was identified as the most stable. G3 was discriminated as the most ustable entry according to $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$. Therefore G12 and G3 can be used for the genetic analysis of phenotypic stability in wheat.

Table 3. Mean value and non-parametric stability parameters for grain yield of 14 wheat genotypes.

Genotypes	Mean	$Si^{(1)}$	$Z^{(1)}$	$Si^{(2)}$	$Z^{(2)}$	$Si^{(3)}$	$Si^{(6)}$	$NPi^{(1)}$	$NPi^{(2)}$	$NPi^{(3)}$	$NPi^{(4)}$
1	460.0771	110	102.634	16.571	0.008	20.622	3.297	2.000	0.500	1.008	0.494
2	490.3879	50	44.183	1.905	-0.339	7.432	1.297	4.000	0.800	1.677	0.684
3	467.0379	151	142.576	20.810	0.108	25.780	4.927	6.500	3.250	3.057	1.398
4	628.1521	114	106.531	19.238	0.071	21.000	1.813	4.714	0.363	1.544	0.421
5	510.5521	57	51.002	4.286	-0.283	10.457	1.391	2.000	0.286	0.733	0.261
6	525.0593	102	94.841	8.143	-0.192	13.719	2.035	2.143	0.214	0.860	0.287
7	523.8307	102	94.841	8.571	-0.182	13.596	2.723	3.000	0.333	1.283	0.458
8	537.5879	85	78.280	5.952	-0.243	13.000	1.515	3.000	0.375	0.967	0.303
9	528.5736	136	127.963	14.619	-0.039	18.136	2.644	5.500	0.917	1.891	0.632
10	572.5664	85	78.280	7.143	-0.215	13.177	1.935	3.857	0.351	1.440	0.448
11	666.4379	85	78.280	6.333	-0.234	14.833	1.333	5.571	0.398	1.814	0.492
12	450.6093	102	94.841	15.238	-0.024	25.600	4.067	0.000	0.000	0.000	0.000
13	604.7807	64	57.822	9.143	-0.168	15.789	1.263	1.286	0.107	0.429	0.119
14	419.9736	85	78.280	13.952	-0.054	35.000	6.556	1.000	1.000	0.926	0.833

Ranking procedure

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. 12 as the most stable genotype with high grain yield, hence it can be used for improvement of adaptation in wheat (Table 4). The same procedures have been employed for screening stability criteria and quantitative indicators of drought tolerance in wheat (Mohammadi *et al.*, 2007; Farshadfar *et al.*, 2012) and in chickpea (Mahtabi *et al.*, 2013; Zali *et al.*, 2011).

Relationship among different stability statistics

The Spearman's rank correlations between each pair of nonparametric stability parameters were calculated (Table 5). A high significant ($P<0.01$) rank correlation was observed between $Si^{(1)}$ with $Si^{(2)}$, $Si^{(3)}$, and $NPi^{(1)}$

the same results was reported by Zali *et al.* (2011) in chickpea. Scapim *et al.* (2000), Ebadi *et al.* (2008) and Mohammadi *et al.* (2007) also reported positive and significant correlations between $S_i^{(1)}$ and $S_i^{(2)}$. NPi $^{(1)}$, was negatively correlated with NPi $^{(2)}$. The stability parameters NPi $^{(2)}$, was positively correlated with NPi $^{(3),}$ but the NPi $^{(4)}$ measure was negatively correlated with NPi $^{(4)}$.

Table 4. Ranking of mean yield and stability statistics and their rank sum (RS) over environmental fluctuations.

Genotype	Si $^{(1)}$	Si $^{(2)}$	Si $^{(3)}$	Si $^{(4)}$	NPi $^{(1)}$	NPi (2)	NPi (3)	NPi(4)	RS
1	7	7	3	11	4	13	5	10	3.00
2	5	5	4	2	10	2	2	12	5.00
3	14	13	14	13	14	1	1	14	4.00
4	6	6	7	6	11	9	12	6	13.00
5	3	3	5	4	5	6	9	3	6.00
6	11	12	11	8	6	12	13	4	8.00
7	12	11	10	10	7	8	6	8	7.00
8	8	8	9	5	8	5	8	5	10.00
9	13	14	13	9	12	3	4	11	9.00
10	9	9	8	7	9	4	3	7	11.00
11	10	10	12	3	13	7	10	9	14.00
12	4	4	2	12	1	14	14	1	2.00
13	1	1	6	1	3	11	11	2	12.00
14	2	2	1	14	2	10	7	13	1.00

The Si$^{(6)}$ parameter was positively correlated with Si $^{(1)}$, and Si $^{(3)}$, was negatively correlated with NPi $^{(2)}$, NPi $^{(3)}$, and NPi $^{(4)}$. Parameters NPi $^{(1)}$, NPi $^{(2)}$, NPi $^{(3)}$, and NPi $^{(4)}$ were positively correlated with each other. The statistics with positive significant correlation introduce phenotypically stable genotypes in the same manner.

Table 5. Spearman's rank correlation coefficients between the different nonparametric stability parameters.

Stability parameters	Si $^{(1)}$	Si $^{(2)}$	Si $^{(3)}$	Si $^{(6)}$	NPi$^{(1)}$	NPi$^{(2)}$	NPi$^{(3)}$	NPi$^{(4)}$
Si $^{(1)}$	1							
Si $^{(2)}$	0.991**	1						
Si $^{(3)}$	0.864**	0.864**	1					
Si $^{(6)}$	0.284	0.257	0.159	1				
NPi$^{(1)}$	0.701**	0.688**	0.733**	-0.196	1			
NPi$^{(2)}$	-0.442	-0.415	-0.499	0.204	-0.767**	1		
NPi$^{(3)}$	-0.398	-0.354	-0.261	-0.174	-0.468	0.745**	1	
NPi$^{(4)}$	0.402	0.371	0.204	0.371	0.503	-0.530	-0.785**	1

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