World Journal of Agricultural Sciences 3 (2): 137-242, 2007
ISSN 1817-3047

Nonparametric Methods for Evaluating of Winter Wheat Genotypes in Multi-environment Trials

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Abstract: The objective of this study was to compare nonparametric stability procedures and apply different nonparametric tests for genotype×environment interaction (G×E) on grain yield data of 20 winter wheat genotypes selected from Iran/ICARDA joint project grown in 18 rainfed environments during 2003-05 in Iran. Results of nonparametric tests of G×E and a combined ANOVA across environments indicated the presence of both crossover and usual crossover interactions and genotypes varied significantly for grain yield. In this study, low values of sum of ranks of mean yield and Shukla's stability variance (rank-sum) were associated with high mean yield, but the other nonparametric stability methods were not positively correlated with mean yield but they characterized a static concept of stability. The results of Principal Component (PC) analysis and correlation analysis of nonparametric stability statistics and yield indicated that only rank-sum methods would be useful for simultaneous selection for high yield and stability. According to the rank-sum statistic, G18 (Fengkang15/Sefid), G17 (Anza/3/Pi//Nar/Hys/4/Sefid), G1 (Unknown-1), G10 ('Sardari'//Ska/Aurifen) and G4 (Unknown-1) had the minimum value for rank-sum and therefore were stable genotypes with high yield.

Key words: Winter wheat · nonparametric methods · stability · genotype-by environment interaction

INTRODUCTION

The nonparametric methods have some advantages over the parametric stability methods [1]. They reduce the bias caused by outliers and no assumptions are needed about the distribution of the observed values. They are easy to use and interpret and additions or deletions of one or few genotypes don't cause much variation of results. Huehn [1] and Nassar and Huehn [2] proposed four nonparametric measures of phenotypic stability (1) $S_i^{(1)}$ is the mean of the absolute rank differences of a genotype over the n environments, (2) $S_i^{(2)}$ is the variance among the ranks over the n environments, (3) $S_i^{(3)}$ and (4) $S_i^{(6)}$ are the sum of the absolute deviations and sum of squares of ranks for each genotype relative to the mean of ranks, respectively.

Kang [3] 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 [4], with the lowest estimated value receiving the rank of 1. The sum of these two ranks provides a final index, in which the genotype with the lowest rank-sum is regarded as the most desirable. Fox *et al*. [5] suggested a non parametric superiority measure for general adaptability. They used stratified ranking of the cultivars and ranking was done at each environment separately: the proportion of environments at which the genotype occurred in the top, middle and bottom third of the ranks was computed to form the nonparametric measures TOP, MID and LOW, respectively. A genotype that occurred mostly in the top third (high value of TOP) was considered as widely adapted genotype. Thennarasu [6] proposed as stability measures the nonparametric statistics $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ based on ranks of adjusted mean of the genotypes as those whose position in relation to the others remained unaltered in the set of environments assessed.

Statistical procedures [7, 8]. Most of these procedures, however, fail to distinguish between significant crossover and noncrossover (usual) interaction [9]. Many nonparametric statistical procedures have been proposed to study crossover and noncrossover G×E interactions. These methods have been developed in the field of medicine and can be

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applied to G×E interactions in MET [10]. These procedures are the Brdenkamp method [11, 12], the Hildebrand method [13, 12], the Kubinger method [14, 12] and theVan der Laan-De Kroon method [15]. These methods for the test of G×E provide a useful alternative to parametric methods such as the ANOVA currently used, which is based on original data value. The objectives of this study were to (i) identify winter wheat genotypes that have both high mean yield and stable yield performance across different environments (ii) apply nonparametric tests to investigate the crossover and noncrossover interaction in MET and (iii) study the relationship among nonparametric stability statistics.

MATERIALS AND METHODS

Data source: This study was carried out with 20 winter wheat genotypes in 18 environments (year-location combinations during 2003-2005) including six dryland agricultural research stations, i.e., Sararood, Maragheh, Shirvan, Zanjan, Kordestan and Ardebil in six provinces during 2003-05 in Iran. The studied genotypes selected from winter wheat improvement program of Iran/ICARDA joint project. Experimental layout was a randomized complete blocks design with four replications in each environment. Sowing was done by an experimental drill in 1.2×6 m plots, consisting of six rows with 20 cm between the rows. Seeding rate was 400 seeds m^{-2} for each location. Fertilizer application was 41 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} at planting. Yield (kg ha^{-1}) was obtained by converting the grain yields obtained from plot to hectare.

Statistical analysis procedures: In this study, statistical methods of Brdenkamp and van der Laan-de Kroon in comparison with ANOVA method (based on original data set) were applied to test the significance of GxE. The method of Bredenkamp [11, 16] is based on the usual model for interactions: interactions are defined as deviations from the additivity of main effects. The procedure of the van der Laan-de Kroon [15, 16] was used for test of crossover G×E [where, G×(E) is rank changes of genotypes within environments and E×(G) is rank changes of environments within genotypes [15]. The test statistics of above methods are approximately X^2-distributed with (l-1)(m-1) degrees of freedom, where l = number of genotypes and m = number of environments. These statistical methods have been described in detail by Huehn and Leon [16].

The four nonparametric stability statistics ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$) that combine mean yield and stability have been described in detail by Huehn [17] and Nassar and Huehn [2] and nonparametric stability measures ($Np_i^{(1)}$, $Np_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$) that described in detail by Thennarasu [6] and Sabaghnia *et al*. [18] were measured. Also, Kang's [23] rank-sum (RS) and parameters of Fox *et al*. [5] (TOP, MID and LOW) are other nonparametric stability procedures that were utilized in this study.

RESULTS

Analysis of GxE: The numerical values of the test statistic for the different statistical procedures to determine the effects of genotype, environment and G×E on grain yield of winter wheat genotypes are presented in Table 1. F-value with (l-1)(m-1) and lm (n-1) degrees of freedom for ANOVA method and X^2-values with (l-1)(m-1) degrees of freedom for Brdenkamp and van der Laan-de Kroon at the levels probability were tested. The null hypothesis for Brdenkamp is no noncrossover G×E and for van der Laan-de Kroon is no crossover G×E. The results indicated that both significant noncrossover and crossover interactions [G×(E) and E×(G)] were found according to Brdenkamp and the van der Laan-de Kroon procedures. These results were in agreement with the ANOVA method.

Stability analysis procedures: Evaluation of the genotypes based on the 10 different nonparametric measurements and genotypes mean yield are presented in Table 2. The significant tests (Z_1 and Z_2) for $S_i^{(1)}$ and $S_i^{(2)}$ were developed by Nassar and Huehn [2]. For each genotype, Z_1 and Z_2 values were calculated based on the ranks of adjusted data and summed over genotypes to obtain Z values (Table 2). It is seen that Z_1 sum = 23.23 and Z_2 sum = 26.86. Since both of these statistics were less than the critical value $X^2_{0.05,\,df=19}$ = 30.1, no significant differences in rank stability were found among the 20 genotypes grown in 18 environments. On inspecting the individual Z values, it was found that the genotypes were significantly unstable relative to others, because they showed large Z values, in comparison with the critical value $X^2_{0.05,df=1}$ = 3.84. The $S_i^{(1)}$ and $S_i^{(2)}$ statistics are based on ranks of genotypes across environments and they give equal weight to each environment. Genotypes with fewer changes in rank are considered to be more stable [19]. Nevertheless, these two statistics ranked genotypes similarity for stability. For example, according to both $S_i^{(1)}$ and $S_i^{(2)}$, G11 had the smallest changes in ranks and is

Table 1: The tests statistics for the effects of G, E and G×E interaction using parametric (ANOVA) and nonparametric (Bredenkamp and Laan-Kroon) methods on 20 genotypes grown in 18 environments

			Non parametric method		
			Bredenkamp	Laan-Kroon (Crossover interaction)	
		Parametric method	(Non-crossover interaction)		
Source	Df	ANOVA(F)†	X^2-statistic	G×(E) X^2-statistic	E×(G) X^2-statistic
Environment (E)	17	20.52**	1016**	1052**	1052**
Genotype (G)	19	208.85**	1023**	1049**	1049**
G×E	323	2.35**	2159**	1394**	1275**

**Significant at 0.01 level, †In combined analysis environment (year and location) and genotype were considered as random and fixed factors, respectively

Table 2: Mean values (Y) and nonparametric stability parameters for grain yield and tests of nonparametric stability measures (Z_1 and Z_2) for 20 bread wheat genotypes across environments

Code	Name of genotypes	Y	$S_i^{(1)}$æ	$Z_1^{(1)}$æ	$S_i^{(2)}$æ	$Z_2^{(2)}$æ	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$†	$NP_i^{(2)}$†	$NP_i^{(3)}$†	$NP_i^{(4)}$†	TOP‡	MID‡	LOW‡	RS¥
G1	Unknown-1	2210	6.05	0.60	27.31	0.63	43.75	9.58	4.11	0.39	0.83	0.99	17	44	39	10
G2	Unknown-2	1848	7.31	0.71	38.85	0.56	62.57	10.61	5.22	0.50	0.42	0.50	39	22	39	25
G3	Unknown-9	1952	5.71	1.47	23.44	1.72	35.69	6.96	4.11	0.39	0.36	0.43	22	33	44	18
G4	Unknown-11	2315	7.51	1.22	41.66	1.26	68.18	11.04	5.67	0.54	1.30	1.55	39	22	39	12
G5	135U8.01	1813	7.67	1.71	42.68	1.59	70.60	11.16	5.78	0.55	0.45	0.54	28	28	44	35
G6	5294 Karaj 98-99	1666	7.38	0.88	39.82	0.77	68.07	14.05	5.39	0.51	0.36	0.44	39	28	33	22
G7	1-27-6149/Sabalan//84.40023	1989	6.24	0.28	29.05	0.32	48.84	7.60	4.17	0.40	0.47	0.56	28	50	22	27
G8	Manning/Sdv1//Dogu88	2043	8.86	8.09	59.18	12.01	97.36	12.39	7.11	0.68	0.79	0.94	44	6	50	29
G9	Recttal/TIA.2//TRK13	1799	6.42	0.09	30.12	0.18	46.55	9.27	4.78	0.46	0.33	0.40	33	17	50	20
G10	Sardari//Ska/Aurifen	2237	6.52	0.03	30.82	0.11	49.13	9.59	4.67	0.44	0.88	1.06	22	39	39	14
G11	Unknown-3	2018	5.37	2.70	20.93	2.71	32.67	6.43	3.89	0.37	0.40	0.49	17	39	44	21
G12	Unknown-7	2036	6.69	0.00	32.24	0.02	53.03	8.13	4.67	0.44	0.57	0.69	28	39	33	22
G13	Pf 82200/Sardari	1820	6.51	0.03	30.46	0.14	52.36	10.68	4.78	0.46	0.37	0.45	39	22	39	24
G14	Ghafghaz//F9.10/Maya"s	1952	7.08	0.30	37.04	0.26	58.72	9.67	4.94	0.47	0.45	0.54	22	44	33	23
G15	Khazar/3/Jcam/Emu"s"//Dove"	2022	7.22	0.53	37.41	0.31	61.55	8.92	5.17	0.49	0.59	0.72	28	39	33	26
G16	Kvz/Tm71/3/Maya"s"//Bb/Inia/4/Sefid	1952	6.97	0.17	35.18	0.07	57.87	9.26	5.00	0.48	0.47	0.57	33	33	33	26
G17	Anza/3/Pi//Nar/Hys/4/Sefid	2195	5.98	0.74	29.86	0.21	43.30	10.43	4.94	0.47	0.80	0.90	22	11	67	11
G18	Fengkang15/Sefid	2171	6.26	0.26	28.61	0.39	46.81	9.82	4.61	0.44	0.84	1.01	33	28	39	9
G19	'Sardari' (National check)	2242	7.95	2.81	46.74	3.25	73.34	11.77	5.78	0.55	1.03	1.23	28	28	44	22
G20	'Azar-2 '(National check)	2131	7.25	0.59	37.91	0.39	67.84	10.33	5.22	0.50	0.80	0.97	39	28	33	24
		Sum		23.23		26.86										

Test statistics

$E(S_1^{(1)}) = 6.65$ $E(S_2^{(2)}) = 33.25$

$Var(S_1^{(1)}) = 0.605$ $Var(S_2^{(2)}) = 55.99$

X^2 Sum ? = 30.1 X^2 Z_1, Z_2 ? = 3.84

Grand mean = 2020 kg ha^{-1}

ӾS$_1^{(1)}$ statistics measures the mean absolute rank difference of a genotype over environments and $S_2^{(2)}$ is the common variance of the ranks; the Z-statistics are measures of stability. ; ¥X^2 Z_1, Z_2: Chi-square for $Z_1^{(1)}$, $Z_2^{(2)}$; X^2 Sum: chi-square for sum of $Z_1^{(1)}$, $Z_2^{(2)}$, † NP = nonparametric stability parameters; ‡ TOP, MID and LOW are the parameters of Fox *et al*. [5]; ¥ RS is the rank-sum of Kang [3]

Table 3: Ranks of 20 bread wheat genotypes after yield data from 18 environments were analyzed for G×E interaction and stability using 10 different nonparametric methods

Genotype	Y	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$	TOP	RS
G1	4	4	3	4	8	2	2	16	16	6	2
G2	18	15	15	14	14	10	10	6	6	2	12
G3	15	2	2	2	2	2	2	2	2	5	6
G4	1	17	17	17	16	12	12	20	20	2	4
G5	17	18	18	18	17	13	12	7	7	4	16
G6	20	16	16	16	20	11	11	3	3	2	9
G7	12	5	5	7	3	3	3	9	9	4	14
G8	9	20	20	20	19	14	13	13	14	1	15
G9	19	7	7	5	7	6	6	1	1	3	7
G10	2	9	9	8	9	5	5	18	18	5	5
G11	11	1	1	1	1	1	1	5	5	6	8
G12	8	10	10	10	4	5	5	11	11	4	9
G13	16	8	8	9	15	6	6	4	4	2	11
G14	14	12	12	12	10	7	7	8	8	5	10
G15	10	13	13	13	5	9	9	12	12	4	13
G16	13	11	11	11	6	5	8	10	10	3	13
G17	6	3	6	3	13	7	7	14	13	5	3
G18	5	6	4	6	11	4	4	17	17	3	1
G19	3	19	19	19	18	13	12	19	19	4	9
G20	7	14	14	15	12	10	10	15	15	2	11

Table 4: Spearman's rank correlation coefficients between the different nonparametric stability parameters for grain yield of 20 genotypes

Parameter	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$	TOP	RS
$S_i^{(2)}$	0.99**									
$S_i^{(3)}$	0.99**	0.98**								
$S_i^{(6)}$	0.74**	0.75**	0.73**							
$NP_i^{(1)}$	0.91**	0.95**	0.90**	0.84**						
$NP_i^{(2)}$	0.93**	0.96**	0.92**	0.80**	0.98**					
$NP_i^{(3)}$	0.25	0.24	0.26	0.23	0.19	0.22				
$NP_i^{(4)}$	0.27	0.26	0.29	0.24	0.21	0.23	0.99**			
TOP	-0.62**	-0.60**	-0.63**	-0.59**	-0.60**	-0.64**	0.04	0.01		
RS	0.52*	0.52*	0.56*	0.14	0.42	0.46*	-0.30	-0.28	-0.38	
Y	0.01	0.01	-0.01	-0.03	0.01	0.02	-0.92**	-0.91**	-0.23	0.49*

*, ** Significant at the 0.05 and 0.01 levels, respectively

thus, regarded as the most stable genotype unlike G8. The next most stable genotype was G3. Two other nonparametric statistics of Huehn [17], $S_i^{(3)}$ and $S_i^{(6)}$ combine yield and stability based on yield ranks of genotypes in each environment. These parameters measure stability in units of the mean rank of each genotype [17]. The lowest value for each of these statistics indicates maximum stability for a certain genotype. Like $S_i^{(1)}$ and $S_i^{(2)}$, G11 followed by G3 were the most stable according to the $S_i^{(3)}$ and $S_i^{(6)}$ parameters. The mean yield of G6 followed by G9 was the lowest among the genotypes tested. Most yield mean observed for G4 followed 'Sardari' and G10 which ranked first, second and third for mean yield, respectively. These genotypes are relatively stable (Table 3).

Results of Thennarasu's [6] nonparametric stability statistics, which are calculated from ranks of adjusted yield means, are shown in Table 2 and the ranks of genotypes according to these parameters are given in Table 3. According to the first method $NP_i^{(1)}$, genotypes G11 followed by G1 and G3 were stable in comparison with the other genotypes. These three genotypes (G11, G1 and G3) had the lowest value of $NP_i^{(2)}$ and were stable. Because of the high values for $NP_i^{(2)}$, the stabilities of G8 followed by 'Sardari', G5 and G4 were low (Table 2). $NP_i^{(3)}$, identified G9 as the most stable genotype, although had the lowest mean yield. The next most stable genotypes were G3 and G6 which had low mean yield performance. The unstable genotypes based on $NP_i^{(3)}$ were G4 followed by 'Sardari' and G10, which had the highest

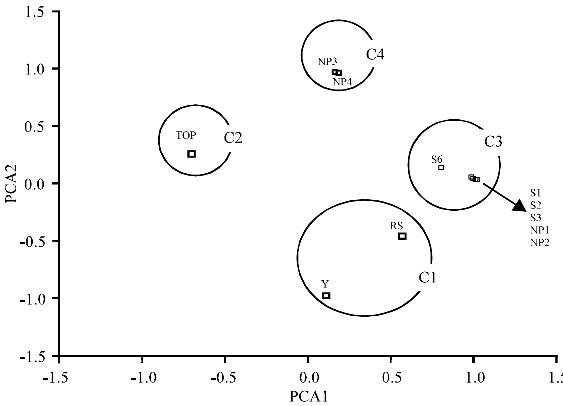

Fig. 1: Principal component analysis (PC1 and PC2) plot of ranks of yield stability, estimated by 10 methods using yield data from 20 genotypes grown in 18 environments and showing interrelationship among these parameters

mean yield, respectively. Therefore, $NP_i^{(3)}$ had a negative relationship with yield ($p<0.01$). Stability parameter $NP_i^{(4)}$ identified G9 as a stable genotype, followed by G3 and G6; but like $Np_i^{(3)}$, identified G4, 'Sardari' followed by G10 as unstable. The results of two NPs ($NP_i^{(3)}$ and $NP_i^{(4)}$) were very similar to each other and identified G4, 'Sardari', G10, G18 and G1 unstable, although they had highest mean yield performance. According to $NP_i^{(4)}$, G9 followed by G3 and G6 were stable genotypes, although they had the lowest minimum mean yield performance.

Kang's [3] rank-sum uses both yield and Shukla's stability variance. The genotypes with the lowest rank-sum are the most favorable one. According to the rank-sum statistic, G18 followed by G1, G17 and G4 had the minimum value for rank-sum and therefore were stable genotypes with high yield (Table 2).

The nonparametric superiority parameter of Fox *et al*. [5] consists of scoring the percentage of environments in which each genotype ranked in the top, middle and bottom third of trial entries. A genotype usually found in the top third of entries across environments can be considered relatively well adapted and stable. In this study the TOP parameter was not able clearly separated the adapted genotypes relative to other. According to TOP parameter the genotypes G8 followed by G2, G4, G13 and G20 were relatively adapted.

Interrelationship among the nonparametric methods: The Spearman's rank correlations between each pair of nonparametric stability methods were calculated (Table 4) and demonstrated a high positive significant rank correlation among $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(1)}$ and $NP_i^{(2)}$ ($p<0.01$). These parameters had significantly negatively correlation with the percentage of environments in which it ranked in the top third of genotypes (Top, $p<0.01$). The parameters, $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $NP_i^{(2)}$ were positive correlated with rank-sum($p<0.05$). The positive correlation was observed also between rank-sum and mean yield of genotypes ($p<0.05$). Mean yield was significantly negatively correlated with the parameters $NP_i^{(3)}$ and $Np_i^{(4)}$ ($p<0.01$).

To better understand the relationships among the nonparametric methods, principal component (PC) analysis based on the rank correlation matrix (Table 4) was performed. The first two PCs explained 87.72% (58.48% and 29.24% by PC1 and PC2, respectively) of the variance of original variables. The relationships among the different stability statistics are graphically displayed in a biplot of PC1 and PC2 (Fig. 1). In this biplot, the PC1 and PC2 axes mainly distinguish the nonparametric measures in different groups. Mean yield (Y) groups with rank-sum and we refer to these as Class 1 (C1) stability measures. The PCs axes separated TOP (which we will refer to as class2, C2) from the parameters $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$, $S_i^{(6)}$, $NP_i^{(1)}$, $NP_i^{(2)}$ (Class 3, C3) and the stability measurements $NP_i^{(3)}$ and $NP_i^{(4)}$ (Class 4, C4) (Fig. 1).

DISCUSSION

According to biplot method, the PCs axes separated rank-sum and mean yield (Y) from the other methods. These PCs distinguish methods based on two different concepts of stability: the static (biological) and dynamic (agronomic) concepts [18]. The parameter rank-sum is related with dynamic stability and other remaining methods are associated with static stability. Kang and Pham [20] and Sabaghnia *et al*. [18] found the rank-sum is related with high yield performance and therefore this stability parameter defines stability with dynamic concept. We found that four nonparametric statistics of Huehn ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$) and the $NP_i^{(1)}$ and $NP_i^{(2)}$ parameters of Thennarasu [6] clustered together as class 3 (C3) statistics. These methods classify genotypes as stable or unstable in a similar fashion. The stability parameters $NP_i^{(3)}$ and $NP^{(4)}_i$ were positively and significantly correlated ($p<0.01$) and separated in same group (C4), indicating that the two measures were similar under different environmental conditions (Table 4 and Fig. 1). Consequently, only one of these parameters in each class of C3 and C4 would be sufficient to select the stable genotypes in a breeding program. Similarly, Sabaghnia *et al*. [18] also found significantly positive correlation among these parameters in Lentil (*Lens culinaris*, Medic). Scapim *et al*. [21] also found significantly positive correlation between $S_i^{(1)}$ and $S_i^{(2)}$ in Maize (*Zea mays* L.). The C3 stability statistics represent a static concept of stability and were correlated neither positively nor negatively with mean yield or the C1 statistics. Therefore, the stability statistics could be used as compromise methods that select genotypes with moderate yield and high stability. Also in C4, the $NP_i^{(3)}$ and $NP_i^{(4)}$ nonparametric methods were strongly negatively correlated with high yield ($p<0.01$). Therefore, we don't recommend use of these statistics for cultivar selection. Consequently, these nonparametric stability measurements to be useful alternative to parametric measurements [22]. For making recommendations, it is essential to investigate the relationship among these parameters and compare their powers for different stability models. This topic will be considered in details a subsequent paper.

ACKNOWLEDGEMENTS

The data information of this study was taken from the database maintained at the Drayland Agricultural Research Institute (DARI) trials of Iran is gratefully acknowledged.

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