***Crop Breeding Journal*, 2011, 1(1)**

Non-parametric analysis of phenotypic stability in chickpea (*Cicer arietinum* L.) genotypes in Iran

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Received: June 2010 Accepted: December 2010

Abstract

Zali, H., E. Farshadfar, and S. H. Sabaghpour. 2011. Non-parametric analysis of phenotypic Stability in chickpea (*Cicer arietinum* L.) genotypes in Iran. **Crop Breeding Journal 1(1):** 89-100

The objective of this study was to compare non-parametric stability procedures, and to apply different non-parametric tests for genotype × environment interaction (G×E) on seed yield data of 17 chickpea genotypes grown during 2004-05 growing seasons in 10 rainfed environments in Iran. The non-parametric measures used for G × E interaction were highly significant ($P<0.01$), suggesting differential responses of chickpea genotypes to the test environments. Spearman's rank correlation was used to measure the relationship between the stability statistics. To understand better relationships among the non-parametric methods, principal component analysis was performed. The results of this analysis and correlation analysis of non-parametric stability statistics and grain yield indicated that only seed yield-stability statistic (Ys_i) would be useful for simultaneous selection for high grain yield and stability. According to Ys_i statistic, genotype no. 13 was identified as the most stable genotype. It was observed that this non-parametric statistics were associated with high seed yield. $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $NP_i^{(1)}$ were positively correlated to mean seed yield, however, $S_i^{(6)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ were negatively correlated to mean seed yield.

Key words: Chickpea, Non-parametric methods, Seed yield, Stability and Genotype × Environment interaction.

Introduction

Food legumes are important source of good quality protein in the diets of people and are valuable as animal feed. They also increase and sustain the productivity of the soil by reducing chance of build-up of diseases, insect pests and obnoxious weeds in rotation with cereals (Anonymous, 2001). Chickpea (*Cicer arietinum* L.) is a crop that provides cash income, from its grain, for farmers. It requires no N fertilizers owing to its ability to fix atmospheric N and in rotation can improve the N nutrition and yield of subsequent cereal crop (Fatima *et al*., 2008).

Chickpea is the most important food legume crop in Iran, and occupies about 64% of the areas grown to food legumes in the country, which is 5.1% chickpea growing area in the world and produces 2.75% of global production (Sabaghpour *et al*., 2003). Chickpea productivity in Iran is less than half of the world average yield (Sabaghpour, 2000). Chickpea with 17-24% protein and 41-50.8% carbohydrates is one of the most important food crops (Kay, 1979; Witcombe and Erskine, 1984).

Non-parametric measures have been used for evaluation of G × E interactions and phenotypic stability in chickpea (Ebadi Segherloo *et al*., 2008; Huehn and Leon, 1995; Huehn, 1990). Huehn (1990) has stated that the non-parametric procedures have the following advantages over the 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, and iv) additions or deletions of one or few genotypes do not cause much variation in results. Several non-parametric methods have been developed to describe and interpret the responses of genotypes to environmental variation (Thennarasu, 1995; Fox *et al*., 1990; Kang, 1993; Nassar and Huehn, 1987).

Huehn (1990) and Nassar and Huehn (1987) have proposed four non-parametric stability statistics ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$) that combined mean grain yield and stability. The $S_i^{(1)}$ statistic measures the mean absolute rank difference of a genotype over environments. $S_i^{(2)}$ represents the variance among the ranks over environments, while $S_i^{(3)}$ is the sum of square deviations in yield units of each classification relative to the mean classification, and that $S_i^{(6)}$ is the sum of absolute deviations in yield units of each classification relative to the mean classification. Thennarasu (1995) has also proposed the non-parametric statistics; NP_1, NP_2, NP_3, and NP_4 as stability measures, based on ranks of adjusted mean of the genotypes as those whose position relative to the others remained unaltered in the set of test environments.

Most of these procedures, however, failed to distinguish between crossover and non-crossover interaction (Baker, 1990). Many non-parametric statistical procedures have been proposed to study crossover and non-crossover G × E interactions. These procedures include; the Brdenkamp method (Bredenkamp, 1974), the Hildebrand method (Hildebrand, 1980), the Kubinger method (Kubinger, 1986), and the Van der Laan and De Kroon method (De Kroon and Van der Laan, 1981). These methods provide useful alternative to parametric methods such as the ANOVA currently used, based on the original data values, for evaluation of G × E interaction.

The objectives of this study were to: (i) identify chickpea genotypes that have both high mean grain yield and stability across different environments, (ii) study the relationship among non-parametric stability statistics.

Material and Methods

This study was carried in 2004 and 2005 growing seasons in five different research stations in Iran. The locations included: Ghachsaran, Gorgan, Ilam, Kermanshah, and Lorestan. These genotypes were developed at different research institutes/ stations of Iran and the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. The names, origin and genotypic codes of these genotypes are given in Table 2. Experimental layout was a randomized complete block design with four replications in each environment. Each plot consisted of four rows of 4 meter length. Row spacing and hill-to-hill distances were 30 cm and 10 cm, respectively. Data on seed yield were taken from the middle two rows of each plot. At harvest seed yield was determined for each genotype at each test environments.

Statistical analysis procedures

Three non-parametric statistical procedures of Kubinger (1986), Hildebrand (1980) and Van der Laan and De Kroon (1981) were used to test the significance of G × E interaction. The Kubinger (1986) and Hildebrand (1980) methods are based on the usual linear model for interaction (deviation from additively of main effects for genotypes and environments). De Kroon and Van der Laan (1981) method defines G × E interaction using crossover interaction model. The test statistics of above mentioned methods follow approximately χ^2 distribution with (n-1) (m-1) degrees of freedom, where n is the number of genotypes and m is the number of environments. These statistical methods have been described in detail by Huehn and Leon (1995).

The four non-parametric stability statistics ($S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $S_i^{(6)}$) that combine mean yield and stability were calculated (Huehn, 1979; Nassar and Huehn,1987). The non-parametric stability measures ($NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$) (Thennarasu, 1995), and Ys_i statistic were also calculated (Kang,1993).

The stability statistics were compared using Spearman's rank correlation. Spearman's coefficient of rank correlation was calculated on the ranks to measure the relationship between the statistics using STATISTICA software. To understand relationships among the non-parametric methods, principal component analysis (PCA) was performed.

Results

Analysis of G × E interaction

The summary of different statistical procedures used for determining the effect of G × E interaction on seed yield of chickpea genotypes is presented in Table 1. G × E interaction effects in the three statistical methods were of the same significance level (P<0.01). These results were in agreement with analysis of variance method.

Table 1. The test of significance for G × E interaction for chickpea seed yield

Statistics	**d. f.**	**χ^2-statistic**
ANOVA(F)	144	396837**
Kubinger	144	7020**
Hildebrand	144	6627**
Kroon/ Van der Laam	144	4241**

**: Significant at the 0.01 probability level.

Stability analysis procedures

Evaluation of the genotypes based on the nine different non-parametric measurements and genotypes mean seed yield are presented in Table 2. for each genotype, $Z_i^{(1)}$ and $Z_i^{(2)}$ values were calculated based on the rank of the corrected data and summed over genotypes to obtain Z values (Table 2). The $Z_i^{(1)}$ with sum of 19.34 and the $Z_i^{(2)}$ with sum of 26.30 both less than the critical value of $\chi^2_{0.05,\ df=16} = 26.30$, indicated no significant differences in the rank stability among the 17 chickpea genotypes grown in ten environments. Inspecting the individual Z values, it was found that some genotypes were significantly unstable relative to others, because they showed large Z values, in comparison with the critical value $X^2_{0.05,\ df=1} = 3.84$ (Table 2). 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 (Becker and Leo, 1988).

Considering both $S_i^{(1)}$ and $S_i^{(2)}$, genotype no. 6 had the smallest change in its ranks and was regarded as the most stable genotype followed by genotypes no. 8, 9, and 13. Two other non-parametric statistics, $S_i^{(3)}$ and $S_i^{(6)}$, combine yield and stability based on yield ranks of genotypes in each environment (Huehn, 1979). These statistics measure stability in units of the mean rank of each genotype (Huehn, 1979). The lowest value for each of these statistics indicates maximum stability for a certain genotype. Genotype no. 8 was the most stable genotype considering the $S_i^{(3)}$ and $S_i^{(6)}$ statistics, however, it had the lowest mean yield performance. The highest mean yield observed for genotype no. 4 followed by genotypes no. 7, 15 and 17, respectively (Table 2).

Results for Thennarasu's (1995) non-parametric stability statistics, calculated from ranks of adjusted yield means, are presented in Table 2. The ranks of genotypes based on these statistics are given in Table 3. Considering $NP_i^{(1)}$, genotypes no. 1, 9, 8 and 13 were stable in comparison with the other genotypes. Genotypes no. 4 and 13 had the lowest value of $NP_i^{(2)}$ and were considered stable. $NP_i^{(3)}$ and $NP_i^{(6)}$ also identified genotypes no. 4 and 13 as the most stable genotypes that had also high mean yield.

Kang's (1993) Ys_i statistic identified genotypes no. 13, 4 and 7 as stable genotypes with high yield in comparison with other genotypes (Table 2).

Table 2. Mean values and non-parametric stability statistics for seed yield and tests of non-parametric stability measures ($Z_i^{(1)}$ and $Z_i^{(2)}$) for 17 chickpea genotypes across 10 environments.

Genotypes name	Code	mean	$S_i^{(1)}$	$Z_i^{(1)}$	$S_i^{(2)}$	$Z_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP_1	NP_2	NP_3	NP_4	Ys_i
FLIP 97-211	G1	1774	5.76	0.01	23.79	0.00	28.53	4.69	3.70	0.32	0.472	0.59	6
FLIP 97-113	G2	1610	5.27	0.16	23.21	0.01	20.77	4.05	3.50	0.44	0.568	0.65	-10
FLIP 97-85	G3	1647	5.80	0.03	25.21	0.03	22.21	4.33	3.90	0.65	0.652	0.79	1
FLIP 97-78	G4	1884	5.89	0.06	24.32	0.00	26.20	3.67	3.90	0.27	0.383	0.48	11
FLIP 97-41	G5	1734	6.71	1.24	34	1.71	24.70	4.08	4.20	0.41	0.570	0.69	5
FLIP 97-30	G6	1631	3.71	4.11*	10.72	3.02	17.40	3.73	2.30	0.29	0.414	0.49	-4
FLIP 97-102	G7	1838	6.62	1.04	35.51	2.27	19.85	3.38	4.20	0.38	0.544	0.64	9
FLIP 97-79	G8	1579	4.18	2.36	12.71	2.19	12.15	3.17	2.80	0.33	0.412	0.51	1
X95TH1	G9	1579	3.84	3.58	12.06	2.44	19.58	5.91	2.70	0.54	0.563	0.66	-1
X95TH154	G10	1688	6.00	0.14	26.40	0.10	33.17	5.91	4.20	0.76	0.706	0.87	1
FLIP 97-43	G11	1654	5.96	0.11	25.96	0.07	23.62	4.73	4.60	0.54	0.531	0.65	-2
FLIP 97-95	G12	1672	6.67	1.15	32.04	1.11	24.47	4.60	4.60	0.48	0.568	0.71	-1
FLIP 97-114	G13	1718	4.40	1.71	14.18	1.65	18.31	4.02	3.00	0.27	0.368	0.45	12
X94TH45K10	G14	1683	5.09	0.34	31.43	0.95	29.42	4.88	4.50	0.41	0.554	0.53	0
X95TH5K10	G15	1835	7.38	3.29	40.84	4.86*	34.00	4.79	5.40	0.39	0.523	0.64	8
X45TH150K10	G16	1612	5.76	0.01	23.21	0.01	27.00	5.00	3.90	0.46	0.571	0.72	-5
Arman	G17	1796	5.73	0.01	25.96	0.07	25.77	4.80	3.80	0.40	0.546	0.65	7
				19.34		20.46							

Test statistics

$E(S_i^{(1)}) = 6.65$ $E(S_i^{(2)}) = 24$

$V(S_i^{(1)}) = 0.912$ $V(S_i^{(2)}) = 58.4$

χ^2 Sum = 26.30 $\chi^2 Z_1Z_2 = 3.84$

Yield mean: 1702 kg ha^{-1}

$S_i^{(1)}$: the statistic measures the mean absolute rank difference of a genotype over environments; $S_i^{(2)}$: the common variance of the rank; Z-statistics: measures of stability; χ^2 Z_1, Z_2: Chi-square for $Z_i^{(1)}$ and $Z_i^{(2)}$; X^2: sum Chi-square for sum of $Z_i^{(1)}$, $Z_i^{(2)}$; NP: non-parametric stability statistics; Ys: the statistic of simultaneous selection for high and stability

Table 3. Ranks of 17 chickpea genotypes using mean seed yield for 10 environments for analysis of G × E interaction and nine different non-parametric stability statistics.

Code	Mean Seed Yield	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP_1	NP_2	NP_3	NP_4	Ys_i
G1	5	8	7	14	10	6	4	5	6	6
G2	15	6	5	6	6	5	11	12	10	17
G3	12	10	9	7	8	8	15	16	16	8
G4	1	11	8	12	4	8	1	2	2	2
G5	6	16	15	10	7	11	10	14	13	7
G6	13	1	1	2	2	1	3	4	3	15
G7	2	14	17	5	3	11	6	8	8	3
G8	16	3	3	1	1	3	5	3	4	8
G9	16	2	2	4	17	2	13	11	12	12
G10	8	13	12	16	16	11	16	17	17	8
G11	11	12	10	8	11	14	14	7	11	14
G12	10	15	14	9	9	14	13	13	14	12
G13	7	4	4	3	5	4	2	1	1	1
G14	9	5	13	15	14	13	9	10	5	11
G15	3	17	16	17	12	17	7	6	7	4
G16	14	8	5	13	15	8	12	15	15	16
G17	4	7	10	11	13	7	8	9	9	5

Si, Huehn's (1979) nonparametric stability statistics.
NP, Thennarasu's (1995) nonparametric stability statistics.
Ysi, Kang's (1988) stability statistics.

Relationship between mean yield and stability statistics:

Spearman's coefficient of rank correlation between mean yield and the nine non-parametric stability measures are presented in Table 4. Mean yield was significantly and positively correlated with $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and Ys_i statistics, but it was not correlated with $S_i^{(6)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ (Table 4). The high correlation between mean yield and stability statistics is expected as the values of these statistics were higher for high yielding genotypes. The non-significant correlation and negative significant correlation between yield and stability parameters suggest that stability statistics provide information that cannot be gleaned from average yield (Mekbib, 2003).

The stability statistics $S_i^{(1)}$ and $S_i^{(2)}$ were positively correlated with each other and also with $S_i^{(3)}$ (Table 4). Scapim *et al*. (2000), Ebadi *et al*. (2008)

and Mohammadi *et al.,* (2007) also reported positive and significant correlations between $S_i^{(1)}$ and $S_i^{(2)}$. The correlations were also significant (P<0.05) between $S_i^{(3)}$ and $S_i^{(6)}$ (Table 4). Other researchers also found positive and significant correlations between $S_i^{(3)}$ and $S_i^{(6)}$ non-parametric statistics (Kang and Pham, 1991; Ebadi *et al*., 2007; Mohammadi *et al*., 2007.

The spearman's rank correlation between $S_i^{(1)}$, $S_i^{(2)}$ and $S_i^{(3)}$ with NP_i $^{(1)}$ was positive and significant (Table 4). The correlation between Ys_i and NP_i $^{(2)}$, however was negative and significant. The positive correlation was also observed between NP_i $^{(2)}$, NP_i $^{(3)}$, NP_i $^{(4)}$ and $S_i^{(6)}$ (Table 4).

Table 4. Spearman's coefficient of rank correlation for mean seed yield and 9 non-parametric stability measures of 17 chickpea genotypes evaluated in 10 environments of Iran

	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP_1	NP_2	NP_3	NP_4	Ys_i
$S_i^{(2)}$	0.87^{**}								
$S_i^{(3)}$	0.55^{*}	0.59^{*}							
$S_i^{(6)}$	0.06^{ns}	0.14^{ns}	0.59^{*}						
NP_1	0.84^{**}	0.90^{**}	0.64^{**}	0.27^{ns}					
NP_2	0.27^{ns}	0.23^{ns}	$0.19n^{s}$	0.63^{**}	0.36^{ns}				
NP_3	0.34^{ns}	0.32^{ns}	0.32^{ns}	0.55^{*}	0.33^{ns}	0.86^{**}			
NP_4	0.43^{ns}	0.30^{ns}	0.26^{ns}	0.55^{*}	0.36^{ns}	0.92^{**}	0.94^{**}		
Ys_i	0.30^{ns}	0.34^{ns}	0.15^{ns}	-0.30^{ns}	0.11^{ns}	-0.54^{*}	-0.45^{ns}	-0.43^{ns}	
Mean seed yield	0.58^{*}	0.64^{**}	0.50^{*}	-0.13^{ns}	0.45^{ns}	-0.42^{ns}	-0.27^{ns}	-0.27^{ns}	0.81^{**}

*and **: Significantly rank corrected at the 0.05 and 0.01 probability levels, respectively.
ns: Non-Significant.
Si, Huehn's (1979) non-parametric stability statistics.
NP, Thennarasu's (1995) non-parametric stability statistics.
Ysi, Kang's (1988) stability statistics.

To understand the relationship among the non-parametric statistics, principal component analysis (PCA), based on the rank correlation matrix (Table 4), was performed. The first two PCAs explained 80.91% (48.19 and 32.72% by PCA_1 and PCA_2, respectively) of the variances in the original variables. The relationships among different stability statistics are graphically displayed in a biplot of PCA_1 and PCA_2 (Fig. 1). The PCA_1 and PCA_2 axes mainly distinguish the non-parametric measures in different groups. We refer to mean seed yield groups with Ys_i as Class 1 = C_1 stability measures. The PCs axes separated $S_i^{(1)}$, $S_i^{(2)}$, $S_i^{(3)}$ and $NP_i^{(1)}$ (We refer to as Class 2 =C_2) from the statistics $Si^{(6)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ (We refer to as Class 3 = C3) (Fig. 1).

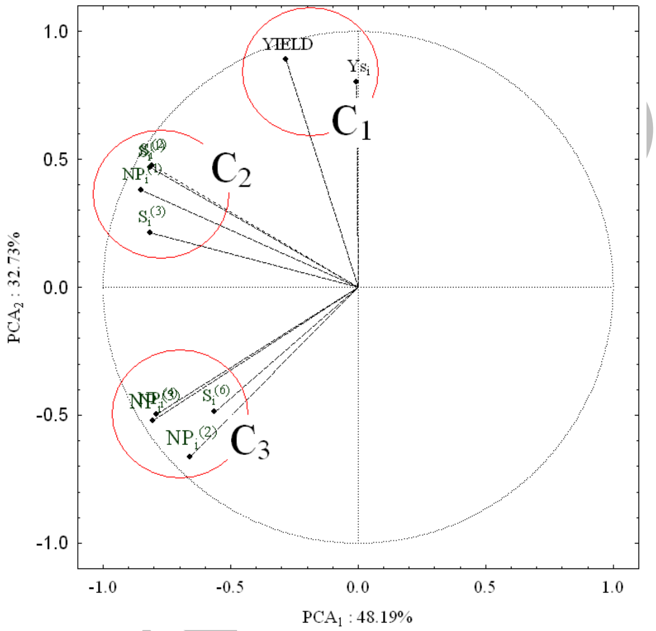

Fig. 1. Principal component analysis (PCA_1 and PCA_2) plot of ranks of yield stability, estimated by nine non-parametric statistics using mean seed yield of 17 genotypes grown in 10 environments.

Discussion

Genotype × environment interactions are important sources of variation in crops and the term stability is sometimes used to characterize a genotype, shows a relatively constant yield, independent of changing environmental conditions (Ebadi *et al.*, 2008). On the basis of this assumption, genotypes with a minimal variance for yield across different environments are considered stable.

Considering biplot of principal component analysis, the PCAs axes separated Ys_i and mean seed yield from the other statistics. These PCAs distinguish between measures based on two different concepts of stability: the static (biological) and dynamic (agronomic) concepts (Sabaghnia *et al*. 2006). The statistic Ys_i is related to dynamic stability and other remaining measures are associated with static stability. Kang and Pham (1991), Mohammadi *et al*. (2007) and Sabaghnia *et al*. (2006) found statistics that were related to high seed yield performance; therefore, they concluded that these stability statistics define stability with dynamic concept. We found that three non-parametric statistics of Huehn ($S_i^{(1)}$, $S_i^{(2)}$ and $S_i^{(3)}$) and the $NP_i^{(1)}$ statistic of Thennarasu (1995) clustered together as C_2 stability measure. These methods classify genotypes as stable or unstable in a similar manner. Consequently, only one of these statistics would be sufficient for selecting stable genotypes in a breeding program. Among Huehn's three rank-based stability statistics $S_i^{(1)}$, $S_i^{(2)}$ and $S_i^{(3)}$, the first two ($S_i^{(1)}$ and $S_i^{(2)}$) were highly and positively associated among themselves. Overall, $S_i^{(1)}$, $S_i^{(2)}$ and $S_i^{(3)}$ could be highly satisfactory measures for stability, whereas $S_i^{(1)}$ was better than others (Huehn, 1990). According to C2 measures, genotypes no. 6, 8 and 9 had the smallest ranks and regarded as the most stable genotypes.

The stability statistics $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ and $S_i^{(6)}$ were positively and significantly correlated, and separated in the same group (C_3), indicating that these four measures were similar under different environmental conditions. Consequently, only one of these statistics in each class of C_2 and C_3 would be sufficient for selecting the stable genotypes in a breeding program (Sabaghnia *et al*., 2006; Mohammadi *et al*., 2007). In spite of the fact that the C_3 stability statistics were non-significant and negatively correlated with mean seed yield, but considering C_3 measures, genotypes no. 13 and 4 had the smallest ranks and regarded as the most stable genotypes with high seed yield. Ys_i statistic (C_1) was also strongly and positively correlated with high seed yield ($p<0.01$). Therefore, it could be recommended as useful measure for cultivar selection. To recommend these statistics as selection measures, it is always essential to investigate the relationship among these statistics and compare their powers for different stability models. In the present study the significant and positive correlation ($P < 0.01$) between Ys_i and mean seed yield indicated that Ys_i was the best statistic for identifying high-yielding genotypes. Considering Ys_i statistic, genotypes no. 13 (FLIP 97-114), 4 (FLIP 97-78) and 7 (FLIP 97-102) were the superior genotypes with high and stable seed yield.

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