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Non-Parametric Estimation and Component Analysis of Phenotypic Stability in Chickpea (*Cicer arietinum* L.)

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Abstract: In order to determine phenotypic stability and contribution of yield components in the phenotypic stability of grain yield 21 genotypes of chickpea were evaluated in a randomized complete block design with three replications under rainfed and irrigated conditions in college of Agriculture, Razi University of Kermanshah, Iran, across 4 years. Non-parametric combined analysis of variance showed high significant differences for genotypes and genotype-environment interaction indicating the presence of genetic variation and possibility of selection for stable genotypes. The genotype number 8 (Filip92-9c) with minimum $S_i^{(2)}$ and $S_i^{(2)}$ of yield stability and grain yield in one parameter also revealed that genotype Filip92-9c was the most desirable variety for both yield and yield stability. Component analysis using Ci-value displayed that number of shrub per unit area has the most contribution on the grain yield phenotypic stability.

Key words: Chickpea, non-parametric, phenotypic stability, component analysis

INTRODUCTION

Chickpea is the second most cultivated grain legume in the world after phaseolus bean (Rubio *et al.*, 1998, 2004).

It is cultivated mostly in the Mediterranean basin, the near east, central and south Asia, east Africa, south America, north America and, more recently, in Australia. Iranian chickpea (*Cicer arietinum*) is cultivated in 33 countries of the world and 650 thousand hectors of Iran's area is under the cultivation of chickpea (Imamjomah, 1999; Yaghotipoor, 2002). It is apparent that the phenotype of chickpea is joint contribution of both genes as well as environment. The GE interaction reduces association between phenotypic and genotypic value. The existence of genotype-environment interaction call for the evaluation of genotypes in many environments to determine their true genetic potential (Roy, 2000; Chahal and Gosal, 2002; Farshadfsr and Sutka, 2003).

In many practical situations, the experimenter is not interested in a knowledge of the numerical amount of GE interaction *per se*, but he is only interested in the presence of GE that leads to different ordering of genotypes in different environments. This concept of GE interaction is closely related to the concept of selection in plant breeding. The breeder is mainly interested in rank orders of genotypes in different environments and in changes of these rank orders. Therefore it is an obvious idea to use rank information for a quantitative description

of these relationships (Kang, 1990; Huehn, 1990a; Yaghotipoor, 2002). The detection and quantification of GE interaction has been attempted through parametric and non-parametric methods. Sabaghnia *et al.* (2006) studied four statistical methods for the analysis of G×E interaction and suggested that for analysis of noncrossover interactions, the methods of Brdenkamp, Hildebrand and Kubinger are closely connected with the ANOVA. If some of the necessary assumptions are violated, the validity of the interferences 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).

Also parametric measures are relatively more sensitive to errors of measurements and addition or deletion of one or few observations causes great variation in the parametric stability measures. Thus it is worthwhile to go for a non-parametric measure for stability. Some essential advantages of non-parametric statistics compared to parametric ones are: reduction or even avoidance of the bias caused by outliers, no assumptions are needed about the distribution of the analyzed values, homogeneity of variances and additivity (linearity) of effects are not necessary requirements, statistics based on ranks and rank-orders are often easy to use and to interpret (Kang, 1990; Huehn, 1990b, c; Huehn and Nassar, 1987, 1989; Farshadfar, 1998; Roy, 2000; Chahal and Gosal, 2002).

The objectives of the present investigation were (i) evaluation of phenotypic stability of chickpea genotypes under rainfed and irrigated conditions and (ii) determination of the contribution of yield components in the phenotypic stability.

MATERIALS AND METHODS

In order to evaluate phenotypic stability of 19 breeding lines and two local varieties of Iranian chickpeas, a randomized complete block design with three replications was carried out under two different conditions (rainfed and irrigated) across 4 years from 1997 to 2000 in the research station of the College of Agriculture, Razi University, Kermanshah, Iran.

Twenty one plots were projected for each replication, so that each plot had 1.5 m length and 0.5 m wide. The distance between two plots was 50 cm. Single seeds were sown in three rows with 10 cm distance. Each row consisted of 12 seeds.

Maximum and minimum temperature in the research station was 44 and -27°C and the average rain fall was 378 mm. Maximum and minimum rainfall distribution was in March and April, respectively and the region was semiarid.

After separation of border effects from each three-row plot, Number of Shrub Per Unit Area (NSPA), Number of Capsules in Shrub (NCIS), Number of Seeds Per Shrub (NSPS), Thousand Seed Weight (TSW) and Grain Yield (GY) were measured.

Statistical analysis

Non-parametric stability measures: The following concept of phenotypic stability were calculated (Huehn, 1990a; Kang, 1990).

$$S_i^{(1)} = \frac{\sum_{j \neq i} |r_{ij} - r_{ij}|}{N(N-1)/2} = \frac{2 \sum_{j=1}^{N-1} \sum_{i=j+1}^N |r_{ij} - r_{ij}|}{N(N-1)}$$

Where, $S_i^{(1)}$ is the mean of the absolute rank differences of a genotype I over the N environments.

$$S_i^{(2)} = \frac{\sum_{i=1}^N (r_{ij} - \bar{r}_0)^2}{N-1} \text{ and } \bar{r}_0 = \frac{r_{ij}}{N}$$

\bar{r}_0 can be interpreted to be the expectation of each r_{ij} under the hypothesis of maximum stability (= equal ranks). Where $S_i^{(2)}$ is the common variance of the ranks.

$$S_i^{(3)} = \sum_{j=1}^N \frac{|r_{ij} - \bar{r}_0|}{\bar{r}_0}$$

(= sum of the absolute deviations of the r_{ij} 's from maximum stability expressed in \bar{r}_0 -units).

Tests of significance for stability of a single genotype and stability comparisons between certain genotypes was done using the following formula:

$$Z_i^{(m)} = \left[S_i^{(m)} - E(S_i^{(m)}) \right]^2 / V(S_i^{(m)}), \quad m = 1, 2$$

where $Z_i^{(m)}$ have an approximate chi-squared distribution with one degree of freedom and, similarly, the statistic

$$S^{(m)} = \sum_{i=1}^k Z_i^{(m)}, \quad m = 1, 2$$

may be approximated by a chi-squared distribution with k degrees of freedom with $E(S_i^{(m)}) = \text{Expectation (= mean) of } S_i^{(m)}$ and $V(S_i^{(m)}) = \text{variance of } S_i^{(m)}$.

Under the null hypothesis that all genotypes are equally stable. The means $E(S_i^{(m)})$ and variance $V(S_i^{(m)})$ may be computed from the discrete uniform distribution (1, 2, ..., k). The following formula are used:

$$E(S_i^{(1)}) = (K^2 - 1)/3K$$

$$E(S_i^{(2)}) = (K^2 - 1)/12$$

$$V(S_i^{(1)}) = (K^2 - 1) [(K^2 - 4)(N + 3) + 30] / 45K^2 N(N - 1)$$

$$V(S_i^{(2)}) = (K^2 - 1) [2(K^2 - 4)(N - 1) + 5(K^2 - 1)] / 360 N(N - 1)$$

The contribution of yield components in the phenotypic stability was calculated as:

$$Y = X_1 \cdot X_2 \cdot X_3 \dots X_n$$

Where, Y is the grain yield and $X_1, X_2, X_3, \dots, X_n$ are the yield components.

$$\text{Log}(Y) = \text{log}(X_1) + \text{log}(X_2) + \dots + \text{log}(X_n)$$

Where $\text{log}(Y)$ denotes the natural logarithm of Y.

$$C_i = \text{cov}(\text{log}(Y), \text{log}(X_i))$$

Where, C_i (coefficient of variation) is the measure for the contribution of the I-th yield components to the phenotypic stability of yield (Piepho, 1995).

Combined analysis of variance, mean comparison using Duncan's Multiple Rang Test (DMRT) were carried out using MSTATC and SPSS softwares. Nonparametric analysis of phenotypic stability and component analysis were done by the biometrical genetic in plant breeding software provided by Farshadfar (1998).

RESULTS AND DISCUSSION

Genotypes of annual crops evaluated for grain yield on a multi-locational, multi-year basis frequently show genotype-environment interactions that complicate the selection or recommendation of materials. Coping with Genotype-Year (GY) and Genotype-Location-Year (GLY) interaction effects is possible only by selection for yield stability across environment defined as location-year combinations (Annicchiarico, 1997).

There are two possible strategies for developing genotypes with low G×E interactions. The first is sub-division or stratification of a heterogeneous area into smaller, more homogeneous sub-regions, with breeding programs aimed at developing genotypes for specific sub-regions. However, even with this refinement, the level of interaction can remain high, because breeding area does not reduce the interaction of genotypes with locations on years (Eberhart and Russell, 1966; Tai, 1971). The second strategy for reducing G×E interaction involves selecting genotypes with a better stability across a wide range of environments in order to better predict behavior (Eberhart and Russell, 1966; Tai, 1971). Various methods use the G×E interaction to facilitate genotype characterization and as a selection index together with the mean yield of the genotypes. Accordingly, 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; therefore, they prefer a dynamic concept of stability (Becker and Leon, 1988).

According to Huehn (1990a) the nonparametric procedures have some advantages over the parametric stability methods. 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. Even, we can use nonparametric methods for balance data

with normal distributions because they are relatively simple. Stability estimates from nonparametric models based on the relative classification of the genotypes in a given set of environments do not require previous assumptions and a good alternative for parametric measurements (Nassar and Huehn, 1987; Huehn and Nassar, 1989). The interaction concepts of the classification they represent are strongly related to that of selection in which breeders are interested i.e., whether the best genotype in one environment is also the best in other environment and they can define static and dynamic concepts of stability.

Combined analysis of variance: The results of combined analysis of variance for yield and yield components over 7 different environments (Table 1) showed high significant differences for genotypes and genotype-environment interaction indicating the presence of genetic variation and possibility of selection for stable genotypes. As G×E interaction was significant, therefore we can further proceed and calculate the phenotypic stability of varieties (Farshadfar and Sutka, 2003). Chandra *et al.* (1974) observed that G×E interaction with location is more important than G×E interaction with year.

Mean comparison: Mean performance (Table 2) of Grain Yield (GY) and yield components over 7 different irrigated and rainfed conditions ranged from 55.59 g for genotype number 2 to 100.3 g for genotype number 12. The same performance was observed for genotypes number 2 for Number of Shrub per Unit Area (NSPA), Number of Capsules in Shrub (NCIS) and Number of Seed per Shrub (NSPS) but maximum NSPA was attributed to genotype number 14 and NCIS to genotype number 20, NSPS to genotype number 15 and TSW to genotype number 12.

Chi-Square values with l-1 (for genotype), m-1 (for environment) and (l-1)(m-1) (for G×E interaction) degrees of freedom for the methods of Brdenkamp and Van der Leen-de Kroon at the levels probability were tested. The null hypothesis for Bredekamp is no noncrossover G×E interactions and for van der Laan-de Kroon is no crossover G×E interaction. The results indicated that both significant noncrossover and crossover interaction [G×(E) and E×(G)] were found according to Brdenkamp

Table 1: Combined analysis of variance using non-parametric method
Scaled squares

Source of variation	Degree of freedom	Grain yield
Environment (E)	6	187.6250**
Environment * Replication	14	12.9600**
Genotypes (G)	20	21.7256**
G×E	120	114.4490**

**Significant at the 0.01 probability level

Table 2: Mean comparisons of yield and yield components using Duncan's multiple range test

Characters					
Genotypes	No. of shrub per unit area	No. of capsules in shrub	No. of seeds per shrub	Thousand seed weight	Grain yield
1	14.47cd	20.47bcd	20.04bcd	27.35b	66.22ef
2	12.8d	19.36d	18.65d	295.00ab	55.59f
3	12.93d	19.73cd	19.05cd	289.7ab	57.82f
4	14.87bcd	23.81abcd	23.4abc	278.9ab	79.4bcde
5	15.2bcd	23.93abcd	23.71ab	297.5ab	74.25de
6	14.93bcd	24.6ab	24.67ab	280.6ab	82.3abcde
7	15.67abc	25.72a	25.1a	287.3ab	86.05abcd
8	15.53abc	25.91a	25.09a	277.3ab	91.22abcd
9	17.2ab	22.69abcd	21.71abcd	293.9ab	89.84abcd
10	16.4abc	24.53abc	24.28ab	287.6ab	87.55abcd
11	17.33ab	23.63abcd	23.85ab	296.5ab	93.65abc
12	15.93abc	23.77abcd	22.76abcd	299.9a	100.3a
13	14.87bcd	26.36a	25.04a	278.9ab	83.08abcde
14	17.8a	25.57a	25.16a	296.6ab	97.01ab
15	15.53abc	26.49a	25.41a	288.8ab	90.19abcd
16	15.07bcd	24.8ab	25.07a	293.6ab	85.89abcd
17	15.00bcd	24.65ab	25.16a	290.5ab	91.41abcd
18	13.93cd	26.52a	24.71ab	287.8ab	81.74abcde
19	14.87bcd	22.92abcd	22.14abcd	294.7ab	74.88cde
20	13.87cd	27.72a	25.81a	275.9ab	83.09abcde
21	13.93cd	26.02a	26.35a	277.8ab	83.85abcde

Mean values with different letters are not statistically significant

(for non crossover) and the van der Laan-de Kroon (for crossover) methods. In comparison the result of ANOVA with non-parametric analysis procedures, We found that both methods were in agreement with each other, but non-parametric analysis provide more specific information about the presence of crossover and non-crossover G×E interaction (Truberg and Huehn, 2000).

The overall consideration of yield and yield components introduced line 12 as the most outstanding genotype for grain yield with no significant difference with bivanich a landrace of kermanshah but better performance of genotype 12.

Non-parametric phenotypic stability measures: The statistics $S_i^{(1)}$, $S_i^{(2)}$, $Z_i^{(1)}$ and $Z_i^{(2)}$ were calculated for 21 genotypes over 7 different environments (Table 3). The significant tests for $S_i^{(1)}$ and $S_i^{(2)}$ were developed by Nassar and Huehn (1987). For each genotype, $Z_i^{(1)}$ and $Z_i^{(2)}$ values were calculated based on the ranks of adjusted data and summed over genotype to obtain Z values (Table 3). It is seen that $Z_i^{(1)}$ sum = 68.61 and $Z_i^{(2)}$ sum = 881.5. Since both of these statistics were less than the critical value $\chi^2_{0.05, df = 20} = 37.7$, no significant differences in rank stability were found among the 21 genotypes grown in 7 environment. In the original data the null hypothesis that all the genotypes have equal genotypic stability is rejected at 5% level of probability because

$$\chi^2_{n.s.n} = 37.7 < \begin{cases} \sum Z_i^{(1)} = 68.61 \\ \sum Z_i^{(2)} = 881.5 \end{cases}$$

This statistic indicates that genotypes have different adaptability for irrigated and rainfed conditions. On

Table 3: Non-parametric measure of phenotypic stability over rainfed and irrigated conditions

Genotypes	$S_i^{(1)}$	$Z_i^{(1)}$	$S_i^{(2)}$	$Z_i^{(2)}$
1	6.00	26.57	9.05	56.33
2	5.90	29.24	8.57	51.24
3	9.05	62.67	9.52	65.90
4	3.62	10.62	4.86	16.00
5	6.19	26.90	8.67	50.81
6	6.67	31.57	7.05	34.33
7	7.14	40.24	5.71	30.57
8	3.62	9.57	4.29	13.62
9	6.67	31.33	6.29	29.95
10	3.71	10.24	4.48	114.24
11	6.57	30.33	8.29	45.90
12	5.43	31.95	7.05	34.48
13	7.43	37.81	7.71	40.57
14	6.95	33.24	7.90	45.90
15	8.86	52.24	9.62	64.95
16	6.95	35.14	8.10	43.57
17	7.24	36.24	8.38	48.95
18	5.33	20.81	7.14	35.57
19	5.05	18.48	6.76	35.24
20	9.14	59.95	9.24	61.81
21	8.67	51.00	9.43	61.57
	$S_i^{(1)} = 136.19$		$S_i^{(2)} = 686.14$	

$S_i^{(1)}$ is the mean of the absolute rank differences of a genotype I over the N environments. $S_i^{(2)}$ is the common variance of the ranks. $Z_i^{(m)}$ have an approximate chi-squared distribution with one degree of freedom

inspecting the individual Z values, it was found that the genotype were significantly unstable relative to others, because they showed large Z values, in comparison with the critical value $\chi^2_{0.05, df = 1} = 3.84$.

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 (Becker and Leon, 1988). The $S_i^{(1)}$ estimates based on all possible pair-wise rank differences across environments

for each genotype, whereas $S_i^{(2)}$ is based on variance of ranks for each genotype across environments (Nassar and Huehn, 1987). These two statistics ranked genotypes similarity for stability. Nassar and Huehn (1987) reported that the $S_i^{(1)}$ and $S_i^{(2)}$ are associated with the static (biological) concept of stability, as they define stability in the sense of homeostasis. The stability statistics of $S_i^{(1)}$ and $S_i^{(2)}$ represent a static concept of stability and were correlated neither positively nor negatively with mean yield. Therefore, these stability statistics could be used as compromise methods that select genotypes with moderate yield and high stability.

The most adaptable genotype is that with the least $S_i^{(1)}$ and $S_i^{(2)}$ over environments. As $S_i^{(1)} = S_i^{(2)} = 0$ means maximum stability therefore, the variety filip92-9c (number 8) showed the highest stability for rainfed and irrigated conditions followed by genotypes 10, 12, 14, 17 and 19. It is to be mentioned that stability of chickpea is highly associated with location (Dani and murty, 1982) and stability of grain yield is correlated with number of capsule and seed weight (Yaghotipoor, 2002).

Component analysis: Component analysis is a simple method of analysing yield components. It allows quantification of the contribution of each component to the variability of final yield (Sparuaaij and Bos, 1993; Piepho, 1995). C_i assesses the joint effect of the variability of the i th yield components, quantified by σ_i^2 as well as the compensating (or sometimes mutually enhancing) relation with other components, quantified by σ_{ij} . This meets with empirical finding that high variability in yield may be associated with high variability in the yield components if there is little compensation among the components. If, on the contrary, the crop under investigation shows a high plasticity in its yield structure, increments in one component may be offset by decreases in other components, which implies a negative covariance between the components. Thus, a component that is highly variable (large variance), but is well compensated for by other components (negative covariance), will exert a small effect on the variability of yield. This will be reflected by a low C_i -value of the component. In summary it can be stated that C_i is an aggregate measure of the i th component, contribution to the variability in yield, which assess the variability of that component as well as its interdependence with other yield components. The results of components analysis (Table 4) revealed that for most of the genotypes seed weight followed by number of seed per shrub exhibited more C_i values indicating that instability is mainly caused by these components, while the influence of number of shrub per unit area and number of capsule per shrub was low. As number of shrub per unit area showed negative C_i this implies that number of shrub can highly be compensated by other components

Table 4: Component analysis of yield and yield components

Genotypes	$C_1 = \text{NSPA}$	$C_2 = \text{NCIS}$	$C_3 = \text{NSPS}$	$C_4 = \text{TSW}$
1	-0.02220	0.00954	0.01500	-0.02687
2	0.02160	0.01517	0.01300	0.13310
3	0.02640	0.00269	0.01140	0.10390
4	-0.01010	0.03216	0.02510	0.02990
5	-0.00991	0.01170	0.01790	0.00565
6	-0.00313	0.02150	0.02630	0.00506
7	0.00747	0.03660	0.05220	0.02300
8	0.00593	0.00563	0.01130	0.03630
9	-0.00184	0.00874	0.01520	0.03040
10	-0.00139	0.01880	0.02210	0.05200
11	0.00194	0.01690	0.02010	0.02860
12	0.01080	0.00705	-0.00191	0.05430
13	-0.00074	0.00276	0.00632	0.02550
14	-0.00146	-0.00306	0.000595	0.02610
15	0.01430	0.02120	0.02270	0.00720
16	-0.01070	0.00605	0.01020	0.04080
17	-0.00766	0.00508	0.00632	0.00454
18	-0.01200	0.03620	0.04160	-0.000815
19	-0.00343	0.01750	0.02100	0.02930
20	-0.00802	0.03910	0.04540	0.00754
21	0.05180	0.07130	-0.03460	-0.00715

(negative covariation) and has a little effect on the instability of grain yield. The overall judgement of the components analysis displayed that selection for improvement of phenotypic stability must be apparently through the selection of number of shrub per unit area followed by number of capsule per shrub (Sparuaaij and Bos, 1993; Piepho, 1995).

Simultaneous consideration of yield and yield stability: Simultaneous consideration of yield and yield stability in only one parameter is of particular interest and importance.

The parameter

$$\sum_{j=1}^m |r_{ij} - \bar{r}_{i0}| / \bar{r}_{i0} = \sum_{j=1}^m |1 - r_{ij} / \bar{r}_{i0}|$$

has been proposed by Huehn (1979) and applied by Leon (1986) and Huehn (1990).

This parameter realizes a confounding and simultaneous evaluation of yield stability and yield since the numerator measures the stability (= variability of the ranks r_{ij}), while the denominator reflects the yield level (= mean of the ranks r_{ij}). Another method based on ranks for combining yield and stability has been proposed by Schuster and Zchoche (1981) and by Kang (1990) : Ranks were assigned for mean yield (highest yield = lowest rank of one) as well as for the stability variance (Kang, 1990) or ecovalence (Schuster and Zchoche, 1981) (lowest variance or ecovalence = lowest rank of one) and both ranks were summed (Kang, 1990) or multiplied (Schuster and Zchoche, 1981). The lowest rank-sum or rank-product would be the most desirable. Using the above mentioned parameters (Table 5) genotype number 8 was the most desirable for both yield and yield stability.

Table 5: Simultaneous consideration of yield and yield stability in one parameter using Kang and Schuster methods

Genotypes	S _i ⁽¹⁾		S _i ⁽²⁾	
	Kang	Schuster	Kang	Schuster
1	27	152	25	114
2	28	147	29	168
3	40	400	41	420
4	17	30	18	45
5	27	162	25	126
6	25	156	24	143
7	20	75	22	85
8	4	3	4	3
9	18	77	17	70
10	9	18	8	12
11	14	40	13	36
12	7	6	13	12
13	29	204	28	192
14	16	28	15	26
15	29	190	29	190
16	22	117	23	126
17	24	128	23	120
18	19	70	19	70
19	21	68	21	68
20	37	336	36	320
21	29	198	29	198

Consequently, nonparametric stability measurements to be useful alternative to parametric measurements (Yue *et al.*, 1997). Many parametric and nonparametric measures of stability have been presented and compared with Lin *et al.* (1986) and Flores *et al.* (1998). For making recommendations, it is essential to investigate the relationship among these parametric and compare their powers for different stability models. This topic will be considered in details a subsequent study.

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