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Annals of Biological Research, 2011, 2 (6):586-598  
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ISSN 0976-1233  
CODEN (USA): ABRNBW

# Non parametric estimation of phenotypic stability in wheat-barley disomic addition lines

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## ABSTRACT

Identification of the genetic architecture of phenotypic stability and management of adaptational genes is a prerequisite for improvement of adaptation. To locate the genes controlling adaptation, disomic addition lines of Barley into the genetic background of Chinese Spring were used in a randomized complete block design with three replications under two different conditions (rainfed and irrigated) for three years. Combined analysis of variance showed significant differences for genotypes (G) and GE interaction indicating variability between genotypes and their effects in the GE interaction and possible chromosomal localization of the genes controlling yield and yield stability in Barley. Nonparametric statistical procedures and rank method indicated that most of the quantitative trait loci (QTLs) involved in controlling phenotypic stability and yield in Barley are located on the chromosomes 3H and 4H. Screening nonparametric estimates using biplot technique classified the stability measures in 3 groups. Group 1 (G1) included  $NPi^{(1)}$ . The PCs axes separated  $Si^{(1)}$ ,  $Si^{(2)}$ ,  $Si^{(3)}$ ,  $Si^{(6)}$ ,  $NPi^{(2)}$ ,  $NPi^{(4)}$  and  $YSi^{(2)}$  in group 2 (G2),  $YSi^{(1)}$ ,  $NPi^{(3)}$ , RS and GSI were classified as Group 3 (G3).

**Key words:** Barley, disomic addition lines, stability, nonparametric methods.

## INTRODUCTION

Cultivated barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop, after wheat, maize, and rice. It is grown over a broader environmental range than any other cereal. The popularity of barley is due to its broad ecological adaptation, utility as a feed and food grain, and superiority of barley malt for use in brewing [1].

Barley provides an excellent system for genome mapping and genetic studies, due to (1) its diploid nature, (2) low chromosome number ( $2n=14$ ), (3) relatively large chromosomes (6-8

μm), (4) high degree of self fertility, and (5) ease of hybridization. Its only drawback is the size of the genome, which is relatively large with  $5.3 \times 10^9$  bp for a haploid [2].

The main breeding objectives of barley are high yield, and resistance to biotic and abiotic stresses. Furthermore, malting cultivars need to have high malting quality, which includes plumpkernels, rapid and uniform germination, and optimal values for protein content and enzymatic activity.

It is apparent that the phenotype of barley is a joint contribution of both genes as well as environment. The genotype-environment interaction reduces association between phenotypic and genotypic values and leads to bias in the estimates of gene effects and combining ability for various characters sensitive to environmental variations. Such traits are less amenable to selection [3]. The existence of genotype-environment interaction (GEI) complicates the identification of superior genotypes for a range of environments and calls for the evaluation of genotypes in many environments to determine their true genetic potential [4]. Measuring GEI helps to determine an optimum breeding strategy, to breed for specific or general adaptation, which depends on the expression of stability under a limited or wide range of environments [5]. The importance of  $G \times E$  interactions in national cultivar evaluation and breeding programs have been demonstrated in almost all major crops [6, 7, 8].

Many statistical methods have been investigated and proposed to study genotype  $\times$  environment interactions [7, 8, 9]. Most of these methods, however, fail to distinguish between significant crossover interaction (change in genotypic rank) and non-crossover interaction (no change in genotypic rank) [10].

The  $G \times E$  interaction is considered as crossover or qualitative if it leads to change in relative ranking of genotypes in different environments. The non-crossover or quantitative  $G \times E$  interaction, on the other hand results in differential change of mean but not of ranking of different genotypes. Crossover interactions are of interest in plant breeding because these affect the genotypes to be selected in a given environment. Such interactions also suggest that genotypes are specifically adapted to environments. The non-crossover interaction, on the other hand, influences the nature and magnitude of components of genetic variances and other related parameters like heritability and genetic advance.

Interpretation of  $G \times E$  interaction can be aided by statistical modeling. Models can be parametric (univariate and multivariate) or nonparametric methods. The mostly used, classical parametric approaches for an analysis of genotype  $\times$  environment interaction are based on several assumptions: normality of the distribution, homogeneity of variances, additivity. If some of mentioned assumptions are not fulfilled, the validity of these methods may be questionable. By use of nonparametric methods which relates environments and phenotypes relative to biotic and abiotic environmental factors without making specific modeling assumptions, all of the mentioned assumptions are avoided. The nonparametric procedures have the following advantages over the parametric stability methods: they reduce the bias caused by outliers, 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 do not cause much variation of results [11, 12, 13].

Nassar and Huehn [14] proposed four nonparametric statistics of phenotypic stability ( $S_i^{(1)}$ ,  $S_i^{(2)}$ ,  $S_i^{(3)}$  and  $S_i^{(6)}$ ) based on the classification 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. Fox et al. [15] suggest a nonparametric superiority measure for general adaptability. They used stratified ranking of the cultivars in each environment to determine the proportion of sites in which each cultivar occurred in the top, middle, and bottom third of the ranks, forming the nonparametric measures TOP, MID and LOW, respectively. Rank-sum [16] and simultaneous selection for yield and stability ( $Ys_i$ ) [17] are other nonparametric stability statistics where both yield and Shukla's [18] stability variance are used as selection criteria. This statistics assigns a weight of one to both yield and stability and enables the identification of high-yielding and stable genotypes.

Thenarasu [19] proposed non-parametric statistics  $NP_i^{(1)}$ ,  $NP_i^{(2)}$ ,  $NP_i^{(3)}$  and  $NP_i^{(4)}$  based on ranks of adjusted means of the genotypes in each environment and defined stable genotypes using Nassar and Huehn [14]'s definition. The level of association among stability estimates of different models is an indicative of whether one or more estimates should be obtained for reliable predictions of cultivar behaviors and also helps the breeder choose the best adjusted and most informative stability parameter (s) to fit the static and dynamic concepts of stability [20].

Irrespective of how a stability parameter is measured, one of the most critical question is whether it is genetic? If the characteristic measured by the parameter is non- genetic, it is not heritable and thus selection for such a parameter is fruitless [21, 22]. Various authors have proved that stability indices are genetic and hence heritable [23, 24, 25].

If stability is heritable, the next step in the genetic analysis is identification of the chromosomal location of the genes controlling adaptation [26]. To understand the genetics of continuous variation, it is necessary to identify the chromosomal location of the genes controlling quantitative attributes such as yield and yield stability [27].

Various techniques (biometrical, cytogenetic and molecular) have been used to locate the genes monitoring quantitative traits among which cytogenetic methods (monosomic, disomic, substitution and disomic addition analysis) have been widely used. Because of the complex nature of phenotypic stability, very little information is available on the chromosomal location of the genes conditioning adaptation [28, 29, 30].

Disomic addition lines in which a single pair of chromosomes from related species is added to the full chromosome complement of the recipient, can be used to indentify chromosomes carrying the genes controlling adaptation and phenotypic stability and form the starting point for gene transfer and genetic improvement of genotypic stability [31, 32]. Wheat-barley disomic addition lines have been used to evaluate gene expression and physical mapping of barley [33]. In this paper we report the results of phenotypic stability experiments on chromosome addition lines of (*Hordeum vulgare* L.,  $2n = 2x = 14$ , cv. Betzes) into the genetic background of bread wheat (*Triticum aestivum* L.,  $2n = 6x = 24$ , AABBDD, cv. Chinese Spring) along with their parental barley and wheat lines.

## MATERIALS AND METHODS

To locate the genes controlling yield and yield stability, disomic addition lines of nine genotypes including 7 disomic addition lines (DAL) of barley (*Hordeum vulgare* L.,  $2n = 2x = 14$ . cv. Betzes) (Donor) in the genetic background of bread wheat (*Triticum aestivum* L.,  $2n = 6x = 24$ , AABBDD, cv. Chinese Spring) (Recipient) along with their parental barley and wheat lines were used. The DALs were named as 1H to 7H indicating addition of chromosome 1H to 7H into the genome of Chinese Spring (CS), respectively. The seeds were kindly provided by Dr. M. Tahir, ICARDA, Syria.

The experiment was conducted in a randomized complete block design with three replications under two different environments (irrigated and rainfed) at the experimental farm of College of Agriculture, Razi University, Kermanshah, Iran ( $47^{\circ}20'$  N latitude,  $34^{\circ}20'E$  longitude and 1351m altitude) during 2007-2009. Climate in this region is classified as semi-arid with mean annual rainfall of 478mm and mean annual temperature of  $13.8^{\circ}c$ . The plots consisted of 3m rows and at  $15 \times 25$  cm inter-plant and inter-row distances, respectively. The environments were considered as random factors, while genotypes as fixed factors. At the time of harvesting grain yield was measured in 5 environments (stress and non-stress).

### Statistical Analysis

#### 1-Test of significance GE interaction

Combined analysis of variance (ANOVA) (F-test) was used to test the significance of GE interaction.

#### 2- Non parametric stability approaches

Huehn [34] and Nassar and Huehn [14] proposed four non-parametric stability statistics that combine mean yield and stability. Four parameters based on yield ranks of genotypes in each environment are derived 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  $S_i^1$  = mean of the absolute differences among the classification l-th cultivar in j-th environment,  $S_i^2$  = variance of classification l-th cultivar in j-th environment,  $S_i^3$  = sum square of classification l-th cultivar in all environment divide to mean classification of cultivar in all environment and  $S_i^6$  = sum of mean absolute deviations in yield units of each classification relatives to mean classification, l = number of genotypes, m = number of environments,  $r_{ij}$  = the

rank of the  $i$ th genotype in the  $j$ th environment and  $\bar{r}_i =$  the mean rank across all environments for the  $i$ th genotype.

Significant test to  $S_i^1$  and  $S_i^2$  was calculated with  $\chi^2 = \sum Z_i^m$ ,  $m=1, 2$  which  $E(S_i^m)$  and  $V(S_i^m)$  are mean and variance of  $Z$  respectively.

Kang [35] suggested 3 selection criteria: 1- rank sum (Rsm), 2- altered rank sum and 3- yield stability statistic ( $Y_i$ ) ( $Y_{si}^1$  and  $Y_{si}^2$  are two rank sum statistics that are resulted from sum rank of both yield and  $S_i^1$  and  $S_i^2$ ). In this method both yield and Shukla's [18] stability variance are used as selection criteria. The method attributes equal weight for yield and stability statistics although breeder should assign a weight to yield more than stability statistic's weight.

A ranking method presented by Ketata [36] that genotypes are ranked in all environments based on grain yield separately. Then the mean and standard deviation of the rank of each genotype considering its yield are calculated. In this method a genotype with maximum performance gains rank 1 and if a genotype exhibited mean rank closer to 1 and less standard deviation of the rank was known as the most stable variety.

The stratified ranking technique of Fox et al. [15] consists of scoring the number of environments in which each genotype ranked in the top, middle, and bottom third of trial entries. A genotype that occurred mostly in the top third (high TOP value) was considered as a widely adapted cultivar.

Thennarasu [19] proposed the four following nonparametric stability measures based on justified rank ( $r_{ij}^*$ ) of each genotype mean in any environment. Genotype with fixed rank compare to others in all environments is the most stable.

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

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

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

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

In the above formulas,  $r_{ij}^*$  is the rank of  $X_{ij}^* = X_{ij} - X_i$ ,  $\bar{r}_i$  and  $M_{di}^*$  are the mean and median ranks for adjusted values, where  $\bar{r}_i$  and  $M_{di}$  are the same parameters computed from the original (unadjusted) data. Standard deviation of rank (SDR) and rank mean (R) [36] were measured as:

$$S_i^2 = \frac{\sum_{j=1}^m (R_{ij} - \bar{R}_i)^2}{l-1}$$

where  $R_{ij}$  is the rank of  $X_{ij}$  within the  $j$ th environment,  $\bar{R}_i$  ( $R$ ) is the mean rank across all environments for the  $i$ th genotype and  $SDR = (S^2_i)^{0.5}$ . Genotypes with minimum  $R$  and  $SDR$  are the most stable.

Spearman's coefficient of rank correlation ( $r_s$ ) was employed [37] to statistically compare the stability indices used in this study. All the genotypes evaluated were respectively assigned stability values according to the procedure and definitions used, and were then ranked in order to determine Spearman's rank correlation coefficient between the different procedures. Assume  $n$  genotypes are arranged in the same following order to two stability parameters  $X_i$  indicates the ranking order (or ranking number) of the  $i$ th genotype for the first parameter,  $Y_i$ , indicates the ranking number of the  $i$ th genotype of the second parameter, then  $d_i = X_i - Y_i$  ( $i = 1, 2, \dots, n$ ) and Spearman's rank correlation coefficient ( $r_s$ ) can be described as:

$$r_s = 1 - \frac{6 \sum d_i^2}{(n-1)n(n+1)}$$

Ranking numbers are whole numbers and when two or more equal ranking numbers occur, the average of the ranking numbers that they otherwise would have received, are ascribed to each genotype. The correlation of the parameters and its significance level was determined using the software package STATISTICA. To understand better relationships among stability methods, principal component analysis (PCA), was performed.

### 3- AMMI stability value (ASV)

The ASV is the distance from the coordinate point to the origin in a two-dimensional scattergram of IPCA1 scores against IPCA2 scores in the AMMI model [38]. Because the IPCA1 score contributes more to the GE interaction sum of squares, a weighted value is needed. This weight is calculated for each genotype and each environment according to the relative contribution of IPCA1 to IPCA2 to the interaction SS as follows:

$$ASV = \sqrt{\left[ \frac{IPCA1_{sumofsquare}}{IPCA2_{sumofsquare}} (IPCA1_{score}) \right]^2 + (IPCA2_{score})^2}$$

Where  $\frac{SS_{IPCA1}}{SS_{IPCA2}}$  is the weight given to the IPCA1-value by dividing the IPCA1 sum of squares by the IPCA2 sum of squares. The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller IPCA scores indicate a more stable genotype across environments.

### 4- Yield stability index (YSI)

A new approach known as yield stability index (YSI) is recommended, calculated by ranking the mean grain yield of genotypes ( $RY$ ) across environments and rank of AMMI stability value ( $RASV$ ). YSI incorporate both mean yield and stability in a single criterion as:

$$YSI = RASV + RY$$



A low value of this parameter shows desirable genotypes with high mean yield and stability.

## RESULTS AND DISCUSSION

The results of combined analysis of variance (Table 1) showed significant differences for genotypes and genotype  $\times$  environment interaction indicating variability between genotypes and their effects in the GE interaction and possible localization of the genes monitoring yield and yield stability. As GE interaction was significant, it was possible to proceed and calculate phenotypic stability [30, 39].

**Table 1. Combined analysis of variance over irrigated and rainfed conditions**

S.O.V	df	MS
Environment (E)	4	489.6
Error 1	15	1919.9
Genotype (G)	8	2152.2*
G $\times$ E	22	2615.5*
Error 2	80	1078.2

\* Significant at 5% probability level

### Non- parametric phenotypic stability measure

The  $S_i^{(1)}$  and  $S_i^{(2)}$  [14] statistics are two rank stability measures, the  $S_i^{(1)}$  statistic measuring the mean absolute rank difference of a genotype over environments, with  $S_i^{(1)} = 0$  for a genotype with maximum stability, while  $S_i^{(2)}$  gives the variance between the ranks over environments, with zero variance being an indication of maximum stability. The exact variance and expectation of  $S_i^{(1)}$  and  $S_i^{(2)}$  were given by Huehn [40]. The nonparametric  $S_i^{(1)}$  and  $S_i^{(2)}$  statistics are measures of stability alone and are strongly correlation with each other even when using the uncorrected yield data, being nearly perfectly correlated with each other if the uncorrected yield data is adjusted for genotypic effects using the corrected values. However, the values of the  $S_i^{(1)}$  and  $S_i^{(2)}$  statistics obtained using the uncorrected yield data and the corrected data are often considerably different and show only medium or low correlation [34]. The  $S_i^{(1)}$  statistic is preferred for practical applications because it is very easy to calculate and allows a clear and objective interpretation it represents the mean absolute rank difference between the environments. Furthermore, an efficient test of significance is available for this statistic [40].

The statistics  $S_i^{(1)}$ ,  $S_i^{(2)}$ ,  $Z_i^{(1)}$  and  $Z_i^{(2)}$  were calculated for 9 genotypes over 5 different rainfed and irrigated conditions (Table 2). The significant tests for  $S_i^{(1)}$  and  $S_i^{(2)}$  were developed by Nassar and Huehn [14]. For each genotype  $Z_i^{(1)}$  and  $Z_i^{(2)}$  values were calculated based on the ranks of adjusted data and summed over genotypes to obtain Z values (Table 2 ). As  $Z_i^{(1)}$  sum = 7.96 was greater than critical value of  $\chi^2_{1}=7.95$ , therefore significant differences was found in rank stability among the nine genotypes grown in the five environments and  $Z_i^{(2)}$  sum = 9.56 less than the critical value of  $\chi^2_{2} = 6.6$  thus indicating no significant differences in rank stability among the nine genotypes grown in the eleven environments. No genotype was significantly unstable relative any of the other genotypes because they all showed small Z values compared with the critical  $\chi^2$  values. These two statistics ranked genotypes similarly for stability. For example, according to both  $S_i^{(1)}$  and  $S_i^{(2)}$ , disomic addition line 4H had the smallest changes in ranks and is thus regarded as the most stable genotype, unlike disomic addition line 5H, which

was unstable. It is therefore concluded that chromosome 4H carry the QTLs controlling stability in barley.

Two other nonparametric statistics, described by Huehn [41],  $S_i^{(3)}$  and  $S_i^{(6)}$  combine yield and stability based on the yield ranks of genotypes in each environment. These statistics measure stability in units of the mean rank of each genotype, described in more detail in the original paper by Huehn [41], with the lowest value for each of these statistics indicating maximum stability for a certain genotype. For example, the  $S_i^{(1)}$  and  $S_i^{(2)}$  statistics showed that disomic addition line 4H was the most stable genotype, and this was supported by the  $S_i^{(3)}$  and  $S_i^{(6)}$  statistic indicated that disomic addition line 4H was the most stable genotype with high grain yield, hence most of the QTLs monitoring yield and yield stability in barley are located on chromosome 4H (Table 2).

**Table 2. Mean and Nassau and Huehn stability measures**

Lines	Mean	$S_i^1$	$Z_i^1$	$S_i^2$	$Z_i^2$	$S_i^3$	$S_i^6$
1H	50.8	3.3	0.33	7.29	0.15	12.4	4.2
2H	49.9	2.1	0.75	3.62	0.63	6.33	3.0
3H	65.3	3.7	1.1	9.75	1.24	6.1	3.46
4H	63.3	1.6	2.1	3.14	0.9	3.21	1.85
5H	56.2	3.8	1.4	10.29	1.8	13.5	4.25
6H	55.5	2.1	0.75	3.8	0.54	5.51	2.28
7H	56.7	3.3	0.33	7.57	0.22	8.59	2.86
H(donor)	57.1	2.1	0.75	2.91	1.05	3.3	1.83
Ch.S.	86.5	3.4	0.48	6.62	3.03	5.25	1.62

$$\chi^2_1=7.95, \chi^2_2=6.6, E(S_i^2)=0.95, E(S_i^1)=2.8, V(S_i^1)=0.7, V(S_i^2)=9.71$$

Results of Thennarasu's nonparametric stability statistics, which are calculated from ranks of adjusted yield means, are shown in Table 3, and the ranks of genotypes according to these parameters are given in Table 4. According the methods  $NP_i^{(1)}$ ,  $NP_i^{(2)}$ ,  $NP_i^{(3)}$  and  $NP_i^{(4)}$  genotypes H2, H6, H4 and H4 were stable in comparison with other disomic addition lines, respectively. But the grain yield of H2 and H6 was less than H4.

The highest value of  $NP_i^{(1)}$ ,  $NP_i^{(2)}$ ,  $NP_i^{(3)}$  and  $NP_i^{(4)}$  belonged to addition lines 3H, 3H, 2H, 1H and 5H, respectively indicating their instability, although 3H exhibited high grain yield. Therefore,  $NP_i^{(1)}$  and  $NP_i^{(2)}$  had a negative relationship with yield.  $NP_i^{(3)}$  and  $NP_i^{(4)}$  Because of having negative correlation with high yield should not be used to selection high performance varieties and it is better to be replaced with parametric statistics. The results of two NPs ( $NP_i^{(3)}$ , and  $NP_i^{(4)}$ ) were very similar to each other and identified 4H as stable, with high mean yield performance. Therefore, according to the methods of Nassar and Huehn [14] and Thennarasu nonparametric stability statistics most of the QTLs involved in controlling adaptation are located on chromosome 4H. Kaya and Tanner [13] reported that  $S_i^1$  and  $S_i^2$  are more effective for stability scrutiny than  $NP_i^1$  and  $NP_i^6$ .

Another set of nonparametric stability statistic was Kang's [42] rank-sum (RS), where both yield and Shukla's stability variance are the selection criteria, that assigns a weight of one to both yield and stability, which allows identification of high-yielding and stable varieties. In this method, both the highest yielding genotype and the genotype with the lowest stability variance are ranked 1 and after ranking all the genotypes the ranks by yield and by stability variance are added for each genotype and the genotype with the lowest RS (Rank Sum) value is considered the



most desirable. According to the RS the most stable genotype with high grain yield was identified addition line 4H. Kang and Pham [16] and Sabaghnia [20] reported that RS statistics study dynamic aspect of stability because it is related to high grain yield. Because of integrating yield and stability, RS is probably one of the most important criteria to select varieties in compare to other methods under low intensity humidity stresses. But Baker [43] stated that this method had not been succeeded in distinction between qualitative (crossover) and quantitative (non-crossover) effects. The least amount of yield stability indices 1 and 2 ( $YSi^{(1)}$  and  $YSi^{(2)}$ ) also discriminated addition line 4H as the most stable genotype with least  $YSi^{(1)}$  and  $YSi^{(2)}$  in comparison with other genotypes.

### Genotype selection index (GSI)

A new approach known as genotype selection index (GSI) [26, 44] was calculated by ranking the mean grain yield of genotypes (RY) across environments and rank of AMMI stability value (RASV). GSI incorporates both mean yield and stability in a single criterion as:

$$YSI = RASV + RY$$

Low value of this parameter shows stable genotypes with high mean yield. Based on GSI the most desirable genotype was addition line 4H (Table 3).

**Table 3. Nonparametric stability measures of barley genotype across 5 environments**

Lines	Mean	R.M*	R.D	$NPI^1$	$NPI^2$	$NPI^3$	$NPI^4$	RS	$YSi^1$	$YSi^2$	GSI
1H	50.8	3.57	2.69	2.29	0.57	0.26	1.8	12	11	14	11
2H	49.9	3.43	1.9	0.14	0.52	0.39	1.4	14	10	12	10
3H	65.3	5.29	0.09	2.9	0.71	0.22	1.5	8	7	10	10
4H	63.3	5.85	1.77	2.85	0.33	0.13	0.87	4	5	5	5
5H	56.2	4.57	3.2	2.7	0.45	0.26	1.8	13	11	14	10
6H	55.5	4.14	1.95	1.1	0.29	0.14	0.98	9	8	1	12
7H	56.7	5.29	2.75	2	0.31	0.18	1.3	11	9	12	12
H(donor)	57.1	5.29	1.7	1.4	0.24	0.11	0.74	7	5	5	10
Ch.s.	86.5	7.57	2.57	2.9	0.36	0.19	1.21	10	5	6	10

\*:R.M. =Rank mean and R.D. = Rank standard deviation

### Screening non-parametric stability indicators

#### 1- Biplot analysis

To better understand the relationships, similarities and dissimilarities among the 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 [45].

The  $PCA_1$  and  $PCA_2$  axes which justify 79.06% 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. Nevertheless, the angles are informative enough to allow a whole picture about the interrelationships among the stability estimates [46]. Biplot

clustered the stability measures in 3 groups. Group 1 (G1) included  $NPi^{(1)}$ . The PCs axes separated  $Si^{(1)}$ ,  $Si^{(2)}$ ,  $Si^{(3)}$ ,  $Si^{(6)}$ ,  $NPi^{(2)}$ ,  $NPi^{(4)}$  and  $YSi^{(2)}$  in group 2 (G2),  $YSi^{(1)}$ ,  $NPi^{(3)}$ , RS and GSI were classified as Group 3 (G3). G1 introduced addition line 2E as stable which showed low mean yield. All of the stability indices in G2 discriminated addition line 4H as stable except  $NPi^{(2)}$  which introduced genotype 6H as stable with low mean yield. Non-parametric stability measures in G3 identified 4H as the most stable addition line.

The correlation coefficients among the five test locations are presented in Table 3. 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 G3 indices (an obtuse angle) and independence or very weak correlation (almost right angle) between G1 and G2 stability measures.

This procedure was also employed in chickpea [8] for clustering stability statistics and in durum wheat [47] for screening selection criteria of different climate and water regime conditions.

## 2- Ranking method

The estimates of stability indicators (Table 4) exhibited that the identification of stable genotypes based on a single criterion was contradictory. For example, according to the methods  $NPi^{(1)}$  disomic addition lines 2H, with regard to  $NPi^{(2)}$  disomic addition lines 6H, while  $NPi^{(3)}$  and  $NPi^{(4)}$  discriminated 4H as phenotypically stable genotypes.

To determine the most desirable stable genotype according to the all indices mean rank and standard deviation of ranks of all stability criteria were calculated and based on these two criteria the most desirable stable disomic addition lines were identified.

Total rank mean (TRM) of all the genotypes investigated distinguished addition lines 3H and 4H as the most stable genotypes with high grain yield, therefore most of the QTLs involved in controlling phenotypic stability in barley are located on chromosomes 3H and 4H, hence they can be used for improvement of adaptation in barley and related species through chromosome engineering.

**Table 4. Total rank mean (TRM) of various nonparametric stability statistics**

Lines	Mean	$S_i^1$	$S_i^2$	$S_i^3$	$S_i^6$	R.M*	RD	$Np_i^1$	$Np_i^2$	$Np_i^3$	$Np_i^4$	RS	$YS_i^1$	$YS_i^2$	GSI	TRM
1H	8	4	6	8	9	2	6	6	2	2	1	7	5	6	3	5
2H	9	5	3	5	6	1	4	8	3	1	3	8	1	5	2	4.3
3H	2	2	8	6	7	7	1	1	1	3	2	2	2	4	2	3.3
4H	3	6	2	1	3	8	3	2	6	7	7	1	3	2	1	3.4
5H	6	1	9	9	8	4	9	3	4	2	1	7	8	6	2	5.3
6H	7	5	4	4	4	3	5	7	8	6	6	4	7	1	4	5.0
7H	5	4	7	7	5	6	8	5	7	5	8	6	8	5	4	6.0
H(donor)	4	5	1	2	2	5	2	6	6	8	4	2	6	2	2	3.9
Ch.s.	1	3	5	3	1	9	7	1	5	4	5	7	4	3	2	3.8

\*:R.M. =Rank mean and R.D. = Rank standard deviation

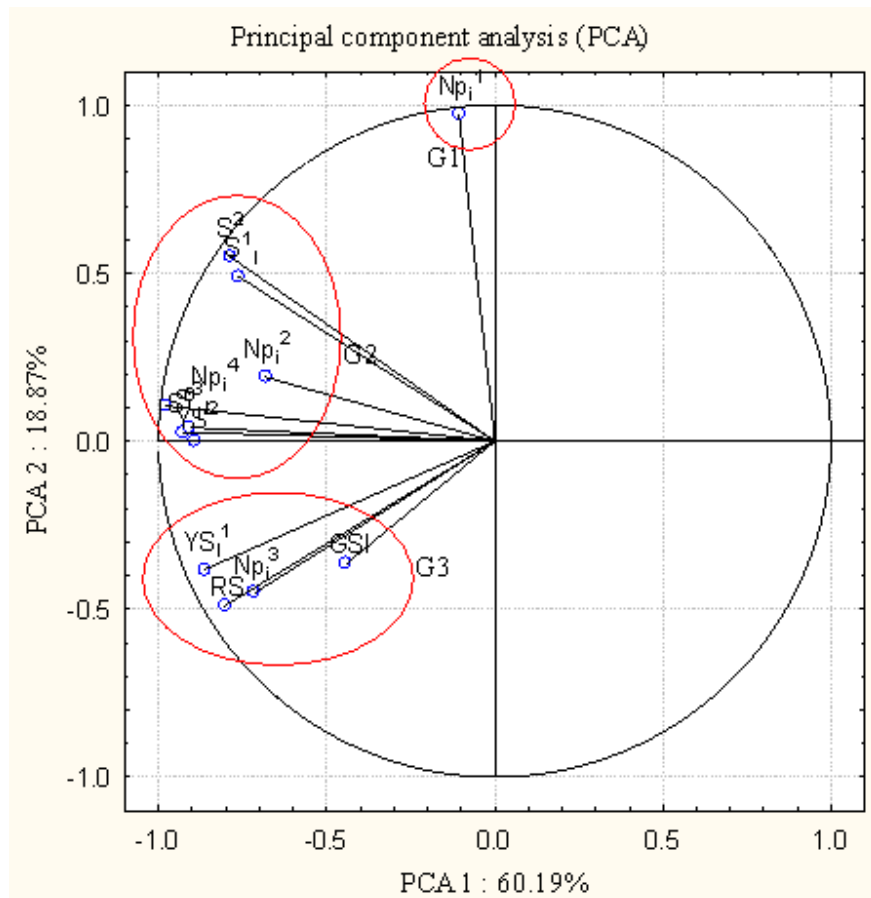


Fig. 1. Biplot analysis of non-parametric indicators of phenotypic stability in wheat-barley disomic addition lines

The same procedures have been used for screening quantitative indicators of drought tolerance in wheat [47], in maize [48], and in rye [30].

Using cytogenetic techniques, AMMI analysis, ASV and GSI, Farshadfar and Sutka (2003) reported that most of the QTLs controlling adaptation in wheat are located on chromosomes 4A and 5A in A genome, 4B in B genome and 2D and 7D in D genome. They also showed that chromosomes 3A, 4A, 3D and 7D carry the genes controlling specific adaptation to rainfed condition and QTLs responsible for adaptation to irrigated condition were located on chromosomes 1A, 3D, 1D and 7D. Using wheat-barley disomic addition lines, Farshadfar et al. [32] exhibited that genes controlling agronomic and physiological indicators of drought tolerance were located on chromosomes 4H and 5H. Farshadfar [44] and Farshadfar et al. [26] revealed that most of the quantitative trait loci (QTLs) involved in controlling phenotypic stability and yield in *Agropyron* are located on the chromosome 7E and most of the genes controlling yield and yield stability in Rye are located on chromosome 5, respectively.

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