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Locating QTLs controlling adaptation in Agropyron using nonparametric stability statistics

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Abstract

In order to locate QTLs controlling adaptation in *Agropyron elongatum* and to study the relationship among non-parametric stability statistics, disomic addition lines of wheat-agropyron were used in a randomized completely block design with three replications under three different environments.The results of combined analysis of variance for grain yield data indicated that main effects of environment (E), genotype (G) and GE interaction were found to be significant indicating variability between genotypes and their effects in the GE interaction and possible localization of the genes monitoring yield and yield stability. The $Si^{(1)}$ and $Si^{(2)}$ statistics showed that genotypes E2 and E6 were the most stable genotypes, and this was supported by the $Si^{(3)}$ and $Si^{(6)}$ statistic indicated that the QTLs controlling yield and stability are distributed on chromosomes E2 and E6 of *Agropyron elongatum*. The methods $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$ also introduced genotypes E6 and E2 as the most stable. Biplot analysis and ranking procedure also revealed that most of the QTLs controlling yield and yield stability are located on chromosomes 2E and 6E of Agropyron. Therefore, they can be transferred to wheat through chromosome engineering for enhancement of yield and yield stability.

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Introduction

Understanding the relationship between crop performance and environment has long been a key issue for plant breeders and geneticists. Crop performance, the observed phenotype, is a function of genotype variety or cultivar, environment, and GEI. GEI is said to occur when different cultivars or genotypes respond differently to diverse environments. Researchers agree that GEI is important only when it is significant and causes significant change in genotype ranks in different environments, i.e., different genotypes are superior in different environments (Yan and Kang, 2003).

One of the reasons for growing genotypes in a range of environments is to estimate their phenotypic stability, because of increasing grower demands for stable varieties, especially in areas where climatic conditions are highly Unpredictable (Ceccarelli, 1994; Adugna and Labuschagne, 2003).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 (Farshadfar *et al*., 2000; Hasheminasab *et al*., 2012). Interpretation of G×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 × 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. Huehn (1990) has stated that the nonparametric 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).

One of the most critical questions 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 (Lin and Binns, 1994; Farshadfar *et al*., 2012; Farshadfar *et al*., 2013). Various authors have proved that stability indices are genetic and hence heritable (Lin and Binns, 1988a; Lin and Binns, 1988b; Lin and Binns, 1991).

If stability is heritable, the next step in the genetic analysis is identification of the chromosomal location of the genes controlling the character (Farshadfar *et al*., 2011). Therefore 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 (Eskridge *et al*., 2000). 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 (Morgan, 1991; Koszegi *et al*., 1996; Farshadfar and Sutka, 2003; Szakacs & Molnar-Lang, 2010).

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 (Ellis *et al*.,

2000). Disomic addition lines have been used to evaluate gene expression and physical mapping of barley (Cho *et al*., 2006), rye (Farshadfar *et al*., 2011) and agropyron (Farshadfar *et al*., 2014). Using wheat-barley chromosome addition lines, isozymes and DNA markers have been physically mapped to chromosomes and chromosome arms (Islam and Shepherd, 1990). Thus, the main objectives of the present investigation were to (i) evaluate the stability performance of wheat-agropyron disomic addition lines (DALs) under different growing conditions using non-parametric methodology, (ii) evaluate the interrelationship between various non-parametric stability statistics.

Materials and methods

Plant genetic materials and experimental design

In order to study chromosomal location of QTLs monitoring adaptation disomic addition lines (DALS) of *Agropyron elongatum* (2n=2x=14) (1E to 7E) into the genetic background of Chinese Spring (CS) wheat (2n=6x=42) were used in a randomized completely block design with three replications under three rainfed (pre-anthesis and pos-anthesis) and irrigated conditions. From each genotype, 40 seeds were selected and placed on filter paper in petridishes (10 cm in diameter) and watered. After two days the germinated seeds were transplanted into Bergman tubes and transferred to vernalization growth chambers due to grouping them according to their vearnlization requirements. The vernalized and non-vernalized seedlings were transplanted into 30/30/30 cm pots each containing 4 kg soil. The soil was a 3:1:1 heat-sterilized (24h, 82˚C) mixture of garden soil, peat and sand. Three seedlings were transplanted into each pot, after which the pots were transferred to phytotron growth chambers. In the phytotron, the pots were arranged in a completely randomized blocks design with three replications so that each pot was considered as an experimental unit. The length of the growing season for the plants arranged in the phytotron was 17 weeks, starting in December and terminating in March. Irrigation was manipulated to create three different drought stress environments: (i) a fully irrigated control treatment. According to the water requirement of the control treatment, each pot was watered every day, (ii) a mid-season water stress treatment where the crop was under progressive stress form approximately floral initiation (pre-anthesis) to flowering (post-anthesis) and rewatered thereafter until maturity and (iii) terminal stress, where irrigation was terminated at grain filling, starting on 8th February and continuing until maturity.

The grain yield data were recorded for each genotype at each environment. Combined analysis of variance for grain yield data was performed to determine the effects of environment (E), genotype (G), and GEI. The mean values of genotypes at each experiment were used to analyze yield stability using the following non-parametric approaches.

Non parametric stability approaches

The following non-parametric stability estimates were used for statistical analysis of phenotypic stability:

Nassar and Huhn (1987) Method

Four nonparametric measures of phenotypic stability of Nassar and Huhn (1987) as: $Si^{(1)}$ the mean of the absolute rank differences of a genotype over the n environments (2) $Si^{(2)}$ the variance among the ranks over the n environments $Si^{(3)}$ and $Si^{(4)}$ the sum of the absolute deviations and sum of squares of rank for each genotype relative to the mean of ranks, respectively.

Thennarasu (1995) Method

Consisted of four nonparametric stability statistics $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$ based on ranks of adjustedmeans of the genotypes in each environment and defined stable genotypes using Nassar and Huehn (1987)'sdefinition.

Kang Method

The third set were Kang's (1993) rank-sum (RS).In RS 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 value is considered the most desirable. Genotype ranks were calculated for grain yield and different nonparametric stability statistics and spearman's rank correlation between each pair of nonparametric statistic ranks were measured to determine relationships between statistics.To understand better relationships among stability methods, principal component analysis (PCA), was performed. The software's MSTAT-C, SPSS and STATISTICA were used for statistical analysis.

Results and discussion

Combined analysis of variance

The results of combined analysis of variance for grain yield data are given in Table 1. The main effects of environment (E), genotype (G) and GE interaction were found to be significant. The variance components for the E, G and GE interaction gave an overall picture of the relative magnitudes of the genotype, environment and GE interaction variance terms. The E effect was the most important source of yield variation, accounted for71.93% of total sum of squares (TSS) followed by GE interaction and genotype effects which accounted for 22.10 and 5.97% of TSS, respectively (Table 1). The environment portion in MET data has been known to be the largest among all sources of variation, but it is regarded as irrelevant for genotype evaluation (Yan and Kang, 2003). This is the reason that the environment effect is removed from the observed phenotypic data, which helps to concentrate on genotype and GE that are relevant for genotype evaluation (Fan *et al*., 2007). The large GE interaction, relative to G effect, suggests the possible existence of different mega-environments with different top-yielding genotypes (Yan and Kang, 2003). The results of combined analysis of variance (Table 1) showed significant differences for genotypes and genotype × 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 (Farshadfar and Sutka, 2003).

Table 1. Combined analysis of variance.

S.O.V	Df	Sum of squares	SS%	Mean square
Treatments	23	568.3		24.71**
Genotypes	7	33.9	5.97	4.84**
Environments	2	408.8	71.93	204.4**
Interactions	14	125.6	22.10	8.97**
Pooled error	48	52.3		1.09

**: significant at 1% probability level.

Non- parametric phenotypic stability measure

The $Si^{(1)}$and $Si^{(2)}$ (Nassar and Huhn, 1987) statistics are two rank stability measures, the $Si^{(1)}$ statistic measuring the mean absolute rank difference of a genotype over environments. $Si^{(1)}$ = 0 is for a genotype with maximum stability, while $Si^{(2)}$ gives the variance between the ranks over environments, with zero variance being an indication of maximum stability. The exact variance and expectation of $Si^{(1)}$and $Si^{(2)}$ were given by Huehn(1990a). The nonparametric $Si^{(1)}$and $Si^{(2)}$ 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 $Si^{(1)}$and $Si^{(2)}$ statistics obtained using the uncorrected yield data and the corrected data are often considerably different and show only medium or low correlation (Huehn, 1990b). The $Si^{(1)}$ 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 (Huehn, 1990a).

The statistics $Si^{(1)}$, $Si^{(2)}$, $Zi^{(1)}$and $Zi^{(2)}$ were calculated for 8 genotypes over 3 different environments (Table 1). The significant tests for $Si^{(1)}$and $Si^{(2)}$ were developed by Nassar and Huehn(1987). For each genotype $Zi^{(1)}$and $Zi^{(2)}$ values were calculated based on the ranks of adjusted data and summed over genotypes to obtain Z values (Table 2). As $Zi^{(1)}$sum = 5.6 was greater than critical value of $X^2_{(1)}$=3.84, therefore significant differences was found inrank stability among disomic addition lines grown in the 3 environments and $Zi^{(2)}$ sum = 4.71greater than critical value of $X^2_{(2)}$=3.84, therefore significant differences was found in rank stability among the 8 genotypes grown in the 3 environments. No genotype was significantlyunstable relative any of the other genotypes because they all showed small *Z* values compared with the critical chi-square (χ^2) values. These two statistics ranked genotypes similarly for stability.

Table 2. Mean values and nonparametric stability statistics for grain yield over different environments.

Genotypes	Yield	$S_i^{(1)}$	$Z_i^{(1)}$	$S_i^{(2)}$	$Z_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP_1	NP_2	NP_3	NP_4	R	SDR
E_1	4.98	3.33	0.35	6.33	0.07	2.38	1.00	2.33	0.47	0.58	0.88	3.67	2.52
E_2	4.85	2.00	0.27	2.33	0.53	0.82	0.59	1.33	0.22	0.33	0.47	3.33	1.53
E_3	3.87	4.00	1.33	9.33	1.03	4.00	1.43	1.00	0.25	0.30	0.43	4.33	3.06
E_4	4.87	4.00	1.33	9.33	0.87	3.60	1.20	2.33	0.47	0.59	0.93	4.00	3.00
E_5	4.10	3.33	0.35	6.33	0.07	2.71	1.14	2.00	0.40	0.53	0.86	4.33	2.52
E_6	3.01	2.00	0.27	2.33	0.53	1.75	1.25	0.67	0.22	0.31	0.50	6.33	1.53
E_7	3.23	4.00	1.33	9.33	1.03	5.09	1.82	2.00	0.67	0.66	1.09	5.33	3.06
CS	4.03	3.33	0.35	8.33	0.59	3.85	1.54	1.33	0.22	0.44	0.62	4.67	2.89
Test statistics													
$\sum Z_i^{(1)}$ =5.60		$\sum Z_i^{(2)}$ = 4.71											
$E(S_i^{(1)})$ = 2.625		$E(S_i^{(2)})$= 5.25											
$V(S_i^{(1)})$ = 1.422		$V(S_i^{(2)})$= 16.178											
X^2 Sum = 30.1		X^2 Z_1Z_2 = 3.84											

Two other nonparametric statistics, $Si^{(3)}$and $Si^{(6)}$ 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, with the lowest value for each of these statistics indicating maximum stability for a certain genotype. For example, the $Si^{(1)}$and $Si^{(2)}$ statistics showed that genotypes 2E and 6E were the most stable genotypes, and this was supported by the $Si^{(3)}$ and $Si^{(6)}$ statistic indicated that the QTLs controlling yield and stability are distributed on chromosomes 2E and 6E of *Agropyron elongatum* (Table 2).

Table 3. Ranks of yield and stability parameters over different environments.

Code	Yield	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP_1	NP_2	NP_3	NP_4	R	SDR
E_1	8	3	3	3	2	7	6	6	6	2	3
E_2	6	1	2	1	1	3	1	3	2	1	1
E_3	3	6	8	7	6	2	4	1	1	4	7
E_4	7	6	6	5	4	7	6	7	7	3	6
E_5	5	3	3	4	3	5	5	5	5	4	3
E_6	1	1	1	2	5	1	1	2	3	8	2
E_7	2	6	7	8	8	5	8	8	8	7	8
CS	4	3	5	6	7	3	1	4	4	6	5

Results of Thennarasu's 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 the methods $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$and $NPi^{(4)}$ genotypes 6E, 2E, 8E and 3E were stable in comparison with other genotypes, respectively. But the grain yield of 6E and 8E was less

than 2E. Therefore, these non-parametric procedures also support the results of Nassar and Huhn (1987) methods. Farshadfar (2011) reported that QTLs controlling adaptation in Agropyron are located on chromosome 7E which is different from our results in this study.

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 (Khodadadi *et al.*, 2011).

Table 4. Rank correlation coefficients of yield and stability statistics.

	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(3)}$	$S_i^{(6)}$	NP_1	NP_2	NP_3	NP_4
$S_i^{(2)}$	095**							
$S_i^{(3)}$	0.87**	0.92**						
$S_i^{(6)}$	0.53	0.61	0.83**					
NP_1	0.40	0.21	0.11	-0.28				
NP_2	0.71*	0.52	0.49	0.15	0.76*			
NP_3	0.44	0.25	0.31	0.10	0.86**	0.82*		
NP_4	0.44	0.23	0.33	0.19	0.80*	0.83*	0.98**	
Yield	0.01	-0.08	-0.33	-0.71*	0.75*	0.26	0.36	0.24

*and **: Significant at 0.05 and 0.01 probability levels respectively.

The PCA1 and PCA2 axes which justify 85.49% 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 (Yan and Kang, 2003). Biplot clustered the stability measures in 4 groups. Group 1 (G1) included Ys and R. The PCs axes separated $Si^{(1)}$, $Si^{(2)}$, $Si^{(3)}$, $Si^{(4)}$ and SDR in group 2 (G2), $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$ were classified as Group 3 (G3) and RSM and Yi were classified as group4 (G4). G1 introduced genotype 2E as stable which showed high mean yield. All of the stability indices in G2 and G3 discriminated genotypes 2E and 6Eas stable (Fig. 1), hence QTLs monitoring simultaneously yield and yield stability are distributed on chromosomes 2E and 6E of Agropyron and they can be transferred to wheat through chromosome engineering for enhancement of yield and yield stability.

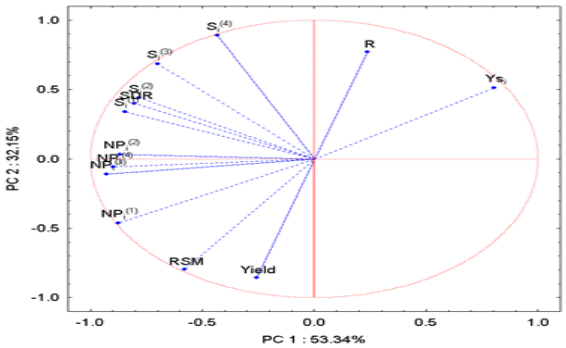

Fig. 1. Biplot analysis of stability statistics over different environments.

Ranking method and association

The estimates of stability indicators (Table 3) exhibited that the identification of stable genotypes based on a single criterion was contradictory. For example, according to the methods $NPi^{(1)}$ disomoc addition lines 1E and 4E, while $NPi^{(2)}$,$NPi^{(3)}$ and $NPi^{(4)}$ discriminated E7 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 caculated and based on these two criteria the most desirable stable disomic addition lines were identified. Minimum mean rank (R) and standard deviation of ranks (SDR) was attributed to chromosome 2E which is in agreement of our previous results.

Spearman's coefficient of rank correlation between mean yield and the non-parametric stability measures are presented in Table 4. Mean yield was significantly and negatively correlated with $Si^{(6)}$, but positively correlated with $NP^{(1)}$.Significant positive correlations were found between stability statistics $Si^{(1)}$, $Si^{(2)}$, $Si^{(3)}$ and $NP^{(2)}$. $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$ also showed positive significant correlations. Significant association between these stability statistics exhibited that they discriminate stable entries at the same manner.

The non-significant correlation and negative significant correlation between yield and stability parameters suggest that stability statistics provide information that cannot be found from average yield (Mekbib, 2003).

Non-parametric statistical procedures indicated that most of the QTLs involved in controlling phenotypic stability in barley are located on the chromosomes 3H and 4H (Farshadfar *et al.*, 2011a), and most of the genes controlling yield stability in *Agropyron* (Farshadfar, 2011) and Rye (Farshadfar *et al.*, 2011b) are located on chromosome 7E and 5R, respectively.

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