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Correlation between field and laboratory indicators of drought tolerance in wheat-barley disomic addition lines

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ABSTRACT

In order to detect quantitative trait loci (QTLs) involved in the genetic of field and laboratory predictors of drought tolerance and to discover association between field and laboratory indicators of drought tolerance, wheat-barley disomic addition lines were used in a randomized complete block design (RCBD) in the field and completely randomized design (CRD) in the laboratory with three replications. The results of analysis of variance exhibited highly significant differences for promptness index (PI), root length (RL), coleoptile length (CL), yield potential (Yp) and stress yield (Ys) indicating the presence of genetic variation and possible chromosomal localization of the genes controlling field and laboratory indices of drought tolerance. Mean comparison indicated that most of the QTLs responsible for drought tolerance are located on chromosomes 4H and 5H. Association between field (stress tolerance index = STI) and laboratory (germination stress index = GSI) indices of drought tolerance showed that GSI can be considered as an early selection criterion for drought tolerance. Three –D plot and cluster analysis confirmed that most of the QTLs controlling field and laboratory criteria of drought tolerance in barley are located on chromosomes 4H and 5H.

Keywords: Disomic addition lines, chromosomes 4H and 5H, GSI and STI.

INTRODUCTION

Evaluation of grain yield performance in areas exposed to frequent stress remains the most widely applied criterion for characterizing cultivar adaptation to stressful conditions. Breeding for drought tolerance by selecting for grain yield is difficult because the heritability of yield under drought condition is low, due to small genotypic variance or to the large genotype – environment interaction variances [1, 2, 3].

In stressful environments, yield per se is not always the most suitable or easiest selection trait and an approach based on the evaluation of some of the physiological traits involved in stress tolerance was proposed [4, 5, 6].

The incorporation of such attributes into a potentially high – yielding genotype may improve its adaptability and thus its response to environmental variability [7, 2].

Screening techniques based on physiological criteria should be rapid, simple and inexpensive, especially for the evaluation of large population [8].

One of the screening techniques based on physiological traits is the use of various osmotica to induce stress in plant tissues. Germination in mannitol and polyethylene glycol (PEG), measurements of root length or rooting depth, and the survival or growth of seedlings subjected to osmotica have been suggested for drought screening [9 ,2, 10]. Sapra et al. [11] and Baalbaaki et al. [12] evaluated the effect of PEG on wheat, Leshem (1996)[13] on pepper and cucumber, Mohammadi et al. [3] in wheat – rye disomi addition lines and Farshadfar et al. [2] on wheat – agrpyron and concluded that PEG was very suitable for the adjustment of osmotic potential.

Identification of the genetic architecture, locating the genes and management of adaptational genes is a perquisite for improvement of drought tolerance [14,15, 3, 16].

Because of the complex nature of drought tolerance, little information is available on the chromosome location of the genes conditioning drought tolerance [1, 3, 17].

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 identify chromosomes carrying the genes controlling drought tolerance predictors and form the starting point for cytogenetic transfer of genetic material (chromosome engineering) into the genotypes under investigation [18 ,19, 20, 17, 21].

The present investigation was therefore carried out (i) to detect the association between field and laboratory predictors of drought tolerance and (ii) to locate QTLs involved in the inheritance of field and laboratory indicators of drought tolerance using wheat – barley disomic addition lines.

MATERIALS AND METHODS

To locate QTLs controlling field and laboratory predictors of drought tolerance and to detect association between agronomic and physiological criteria of drought tolerance, nine genotypes including 7 disomic addition lines (DAL) of barley (*Hordeum vulgare* L., 2n = 2x =14. cv. Betzes) in the genetic background of bread wheat (*Triticum aestivum* L., 2n = 6x =24, AABBDD, cv. Chinese Spring) 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 during 2010-2011 growing season at the experimental farm and laboratory of Dryland Agricultural Research Institude in Sararood Station, Kermanshah, Iran (47°20′ N latitude, 34°20′E longitude and 1351m altitude). Climate in this

region is classified as semi-arid with mean annual rainfall of 478mm and mean annual temperature of 13.8°c.

Stress tolerance Index (STI): In the field, nine genotypes were evaluated using randomized complete block design (RCBD) with three replications under two different environments (irrigated and rainfed). Each plot consisted of 3 rows with 1m in length and spaced by 20cm. Using yield potential (Yp) and stress yield (Ys), the stress tolerance index was calculated with the formula suggested by Fernandez [22]:

$$STI = \left(\frac{Y_P}{\overline{Y}_P}\right)\left(\frac{Y_S}{\overline{Y}_S}\right)\left(\frac{Y_S}{\overline{Y}_P}\right) = \frac{(Y_P)(Y_S)}{(\overline{Y}_P)^2}$$

Where, Ys, Yp and $\overline{Y}p$ represent yield under stress condition, yield under non – stress condition and overall mean of the entries in non – stress condition, respectively.

Germination and seedling characters: Seeds were initially treated with 5% sodium hypochlorite for 5 min. residual chlorine was eliminated through washing of seeds with distilled water. 25 seeds were then germinated on filter paper in Petri – dishes of 25mm diameter in an incubator at 22 ± 2°c. The experiment was conducted under normal (o bar) and stress (-0.8 MPa) created with the help of polyethylene glycol 6000 (PEG – 6000) by the method suggested by Michel and Kauffman (23). The experiment was carried out within completely randomized design (CRD) under two different stress and non – stress (normal) conditions described above. In the stress and normal treatment 6ml of PEG solution and distilled water added to each petridish respectively, in 1day and 4ml added in 6day to compensate the losses due to evaporation. The emergence of 2mm of radical and plumule was taken as the criterion for germination.

Germination stress index: After 10days the number of germinated seeds was recorded and promptness index (PI) and germination stress index (GSI) were calculated using the formula proposed by Sapra et al. (11) and Bouslama and Schapaugh (24):

$$PI = nd_2\,(1.0) + nd_4\,(0.8) + nd_6\,(0.6) + nd_8\,(0.4) + nd_{10}\,(0.2)$$

In which nd_2, nd_4, nd_6, nd_8 and nd_{10} represent the percentage of germinated seeds after 2, 4, 6, 8, and 10 days after sowing, respectively.

$$GSI\ (\%) = \left[\frac{(PIS)}{(PINS)}\right] \times 100$$

Where, PIS is PI under drought stress condition and PINS is PI under normal condition.

Efficiency of added chromosome (EAC): EAC was calculated (25) as:

$$EAC = \frac{Y_{DAL} - Y_{CS}}{Y_{CS}} \times 100$$

Where, Y_{DAL} = yield of disomic addition lines and Y_{CS} = yield of recipient (Chinese spring = CS)

The data for germination percentage, root length (cm) and coleoptiles (cm) were recorded on the 10th day after sowing. Statistical analyses were performed using SPSS and MSTAT – C statistical softwares.

RESULTS AND DISCUSSION

The results of analysis of variance (Table 1) revealed highly significant differences for promptness index (PI), coleoptiles length (CL), root length (RL), yield potential (Yp) and stress yield (Ys), indicating the presence of genetic variation and possibility of locating QTLs involved in the inheritance of field and laboratory predictors of drought tolerance.

Table 1. Mean squares of the characters studied in the field and laboratory

S.O.V	F	PIS	PINS	RL	CL	Y_P	Y_S
Genotypes	10	577.6**	105.6**	7.17**	2.87**	389.11**	151.94**
Error	22	37.17	30.65	0.73	0.62	29.93	9.93
CV (%)	–	10.91	6.70	10.40	21.3	6.15	6.39

***: Significant at 1% level of probability*

Comparison of means grouped the entries in different classes (Table 2). Maximum PIS belonged to addition lines 4H and 5H having significant difference with recipient indicating that most of the QTLs controlling promptness index under stress condition are located on chromosomes 4H and 5H, while chromosomes 4H, 5H and 6H are responsible for promptness index under non – stress condition.

Table 2. Mean comparison between disomic addition lines and parents for the characters investigated.

Genotypes	PIS	PINS	RL	CL	Yp	Ys	GSI	STI	EAC
1H	37.5f	73.73b	7.43d	2.82de	72.74f	38.14f	51.0	34.61	-20.94
2H	38.33f	77.40ab	7.50d	2.30e	77.28ef	42.90ef	49.3	34.39	-35.52
3H	43.77ef	70.50b	5.76e	2.76de	79.47df	43.84df	63.6	35.62	-22.42
4H	71.70ab	85.10a	10.23ab	4.53ac	99.20b	57.61a	84.2	41.59	27.08
5H	70.60ab	86.63a	9.56bc	4096ab	102.6ab	55.19ab	81.4	47.96	39.24
6H	49.70de	86.23a	8.04d	2.88de	83.68de	47.56ce	57.4	36.12	-19.06
7H	36.33ef	84.77a	7.18de	3.16ce	88.71cd	41.35f	54.7	47.36	-11.21
CS(recipient)	58.67cd	85.10a	7.54d	3.56be	95.22bc	49.09cd	68.8	46.13	–
H(donor)	57.73cd	85.53a	7.44d	4.06ad	84.36de	51.27bc	67.4	33.09	–

The longest root length and coleoptile length and maximum yield potential and stress yield were attributed to chromosomes 4H and 5H, hence most of the QTLs involved in the genetic background of these characters are located on chromosomes 4H and 5H.

Using PIS and PINS values, the GSI was calculated for all the genotypes (Table 2). Addition lines 4H and 5H showed the highest values of GSI. Sapra et al. (11), Mohammadi et al. (3) and Farshadfar et al. (26) reported that genotypes with higher GSI exhibited higher drought tolerance, therefore with regard to GSI chromosomes 4H and 5H carry the genes responsible for drought tolerance.

Table 3. Association between laboratory and field predictors of drought tolerance

Characters	PIS	PINS	RL	CL	Y_P	Y_S	GSI	STI
PIS	1							
PINS	0.64**	1						
RL	0.71**	0.34*	1					
CL	0.78**	0.38*	0.60**	1				
Y_P	0.83**	0.61**	0.69**	0.61**	1			
Y_S	0.88**	0.64**	0.66**	0.64**	0.71**	1		
GSI	0.94**	0.34*	0.70**	0.79**	0.80**	0.81**	1	
STI	0.92**	0.57**	0.74**	0.68**	0.92**	0.93**	0.88**	1

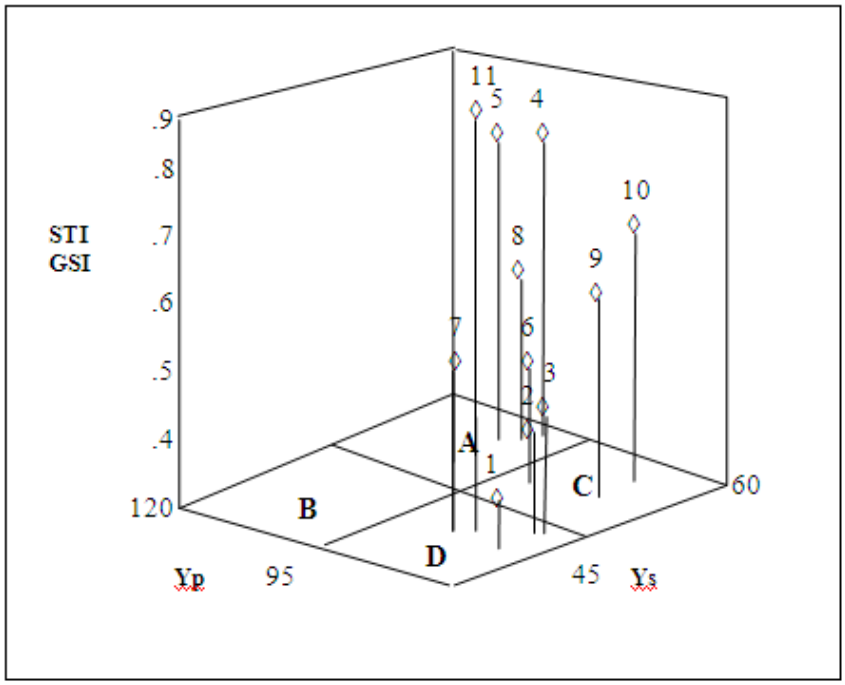

Fig. 1. The genotypes distribution in 3- D plot based on STI and GSI.

The yield of genotypes in stress and non – stress conditions in the field study was used to calculate stress tolerance index (STI). The results indicated that the higher values of STI (Table 2) was related to chromosomes 4H, 5H and 7H but as the efficiency of added chromosome for 7H is negative (-11.21), accordingly most of the QTLs monitoring drought tolerance in the field condition are located on chromosomes 4H and 5H.

The efficiency of added chromosomes (EAC) (Table 2) indicated that QTIs located on chromosomes 4H and 5H displayed the highest amount of efficiency with positive effect and icreasement of drought tolerance in barley and wheat.

Chromosomes 3R and 5R in rye and 3E and 5E in agropyron were also reported to enhance drought tolerance, indicating a close relationship between relatives of wheat and barley which will be useful for comparative mapping (26, 3). Multiple correlation analysis (Table 3) revealed a

highly significant correlation between the laboratory (PIS, PINS, RL and CL) and field (STI) predictors

Rescaled Distance Cluster Combine

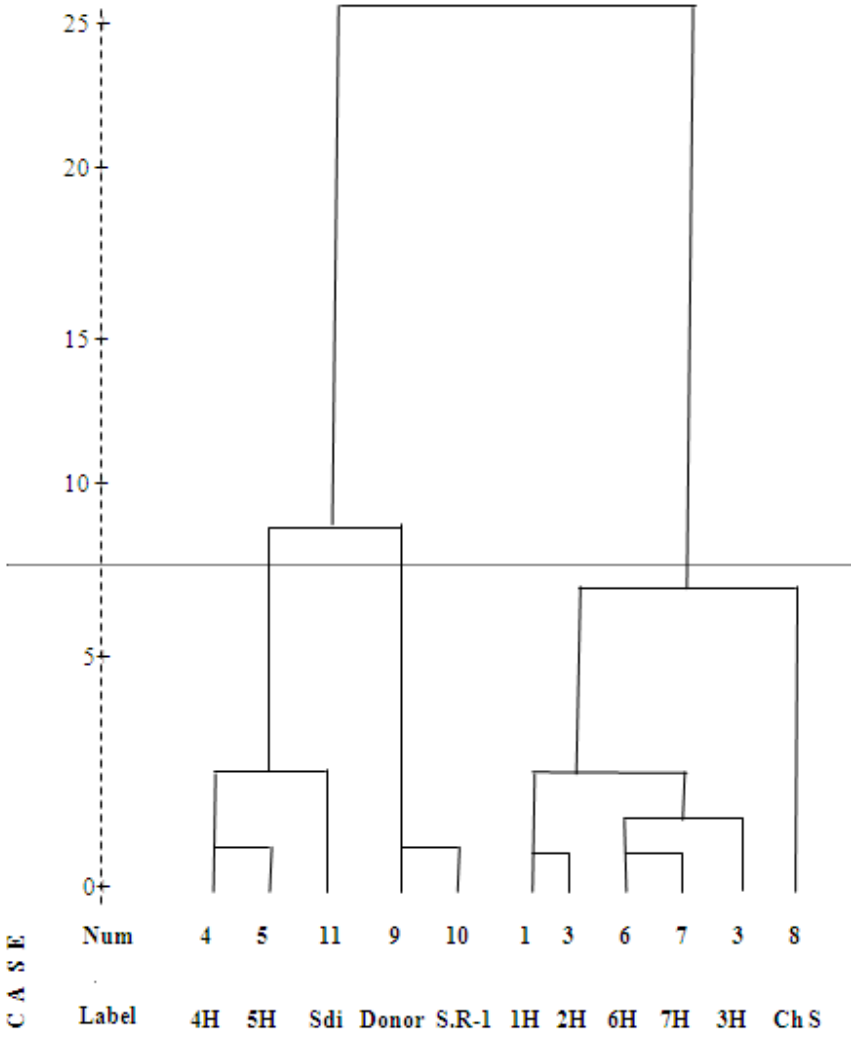

Fig. 2. Cluster analysis of genotypes based on STI and GSI using UPGMA procedure.

**: Significant at 1% level of probability of drought tolerance, indicating that germination stress index (GSI) can be screened as a drought tolerance criterion for the selection of drought – tolerance cultivars. Mohammadi (27, 3) and Farshadfar (26) found high correlation coefficients between PI, STI and GSI, which is in agreement with the results of this experiment. They also mentioned that the growth ability of the roots under stress conditions is an important factor in the survival and promptness index of the plant.

Root length and coleoptile length were also screened as indices of drought tolerance (28, 3, 5). High significant and positive correlation between all the traits and STI showed that these traits confer drought tolerance to a genotype. Thereby, in a breeding program they can be used as a selection index for drought tolerance, under controlled conditions and at earlier stage of the plant growth (29), and hence save money and time (26, 3, 5). A three – dimensional representation of Ys, Yp, STI and GSI is shown in Fig. 1. The area of the 3D plot was divided into 4 regions, A, B, C and D (22). Addition lines 4H and 5H were placed in the A region of the plot, which had the highest GSI, STI, Ys and Yp, this means that chromosomes 4H and 5H carry the QTLs involved in the inheritance of field and laboratory predictors of drought tolerance and accordingly they can be used as the raw material for mapping and QTL analysis of drought tolerance using DNA markers and thereafter for marker assisted selection. They can also be incorporate into a genetic background of a high yielding cultivar for enhancement of drought tolerance through chromosome engineering. Using germination stress index (GSI) and stress tolerance index (STI) for cluster analysis of disomic addition lines by the UPGMA method, it was observed that chromosomes 4H and 5H were classified in one group indicating significant difference with recipient (CS) (Fig. 2).

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