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Chromosomal Localization of the Genes Controlling Agronomic and Physiological Indicators of Drought Tolerance in Barley Using Disomic Addition Lines

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Abstract: In order to locate the genes involved in the inheritance of agronomic and physiological indicators of drought tolerance an experiment was carried out using a wheat-barley disomic addition lines. The results of analysis of variance revealed highly significant differences for most of the traits investigated. Mean comparison exhibited that most of the genes controlling drought tolerance criteria are located on chromosomes 4H and 5H. The overall consideration of the indices using Stress Tolerance Index (STI), Germination Stress Index (GSI) and physiological Multiple Selection Index (MSI) indicated that most of the genes responsible for the inheritance of drought tolerance predictors are located on chromosomes 4H and 5H, hence they can be transferred for the breeding of drought tolerance in barley through chromosome engineering and for mapping QTLs by the molecular breeding procedures. A three dimensional-plot and cluster analysis confirmed the same conclusion. Correlation analysis discarded chlorophyll a and b also proline as an indicator of drought tolerance, but proved that Excised Leaf Water Retention (ELWR), Relative Water Content (RWC), Relative Water Deficit (RWD), Relative Water Loss (RWL), Chlorophyll Fluorescence (CHF), Cell Membrane Stability (CMS) and Leaf Chlorophyll Content (LCC) are physiological indices of drought tolerance and they can be used for the improvement of drought tolerance and grain yield via correlated response. Association between field (STI) and laboratory (GSI) indicators of drought tolerance showed that GSI can be considered as an early selection criterion for drought tolerance.

Key words: Gene location, disomic addition lines, stress tolerance index, germination stress index, multiple selection index

INTRODUCTION

Among the abiotic environmental stresses, drought is one of the most contributors to yield reduction in semiarid regions. Improving drought resistance is therefore a major objective in plant breeding programs for rainfed agriculture in these regions (Andrew *et al*., 2000; Farshadfar *et al*., 2003; Zarei *et al*., 2007). Various quantitative indices have been proposed for selection of genotypes based on their yield performance in stress and non-stress environments (Eric *et al*., 2005; Jiang *et al*., 2006; Mascher *et al*., 2005). Based on these indicators genotypes are compared in irrigated and rainfed conditions. It is worthwhile, therefore to look at the methods that have been used to quantify tolerance. Relative yield performance of genotypes in drought-stressed and more favorable environments seems to be a common starting point in identification of the traits related to drought tolerance and selection of genotypes for use in breeding for dry environment (Mohammadi *et al*., 2003, 2007).

Breeding for drought tolerance by selecting solely for grain yield is difficult, because the heritability of yield under drought conditions is low, due to small genotypic variance or to large genotype-environment interaction variance (Ludlow and Muchow, 1990; Koszegi *et al*., 1996; Farshadfar *et al*., 2008a). Moreover drought tolerance does not exist as a unique and easily quantifiable plant attribute, it is a complex physiological, morphological and molecular character connected with relative water content, relative water loss, chlorophyll fluorescence, stomatal resistance, cell membrane stability, accumulation of free proline in response to osmotic stress, etc. (Zarei *et al*., 2007; Farshadfar *et al*., 2008b, d).

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 (Emmerich and Hardegree, 1990; Kocheva *et al*., 2004; Farshadfar *et al*., 2002). Sapra *et al*. (1991) and

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Baalbaaki *et al.* (1999) evaluated the effect of PEG on wheat, Leshem (1996) on pepper and cucumber, Mohammadi *et al.* (2003) in wheat-rye disomic addition lines and Farshadfar *et al.* (2002) on wheat-agropyron and concluded that PEG was very suitable for the adjustment of osmotic potential.

Identification of the genetic architecture of drought tolerance is a prerequisite for improvement of drought tolerance, but the studies conducted so far offer little information on the genetics of the characters associated with drought tolerance (Koszegi *et al.*, 1996; Farshadfar *et al.*, 1995). Therefore, there is a need for approaches to focus more upon the genetic aspects, identification and management of adaptational genes (Morgan, 1991; Farshadfar *et al.*, 2003).

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 indicators and form the starting point for cytogenetic transfer of genetic material into the genotypes under investigation (Mahmood and Quarrie, 1993; Ellis *et al.*, 2000; Farshadfar *et al.*, 2008c).

Wheat-Barley disomic addition lines have been used to evaluate gene expression and physical mapping of barley (Cho *et al.*, 2006).

The objectives of the present research were screening drought tolerance indicator and locating the genes involved in the inheritance of drought tolerance criteria in barley.

MATERIALS AND METHODS

The plant material consisted of 11 genotypes including 7 Disomic Addition Lines (DAL) of barley (*Hordeum vulgare* L., 2n = 2x = 14, H H, CV Betzes) (H = donor) in the genetic background of bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD, cv, Chinese Spring = CS) along with their parental wheat, barley, Sardary (Sar = wheat) and the control (Sararood-1 = Sar1 = barley). The DALs were named as 1H to 7H indicating addition of chromosome 1H to 7H into the genome of CS, respectively. The seeds were kindly provided by Dr. M. Tahir, ICARDA, Syria. The genotypes were cultivated in a randomized complete block design with three replications under two different environments (irrigated and rainfed) in the Agricultural Research Institute of Sararood, Kermanshah, Iran during the year 2004-2005 (47°20′N latitude, 34°20′E longitude and 1351 m altitude). Climate in this region is classified as semi-arid with mean annual rainfall of 478 mm). Each plot consisted of 3 rows with 1 m in length and spaced by 20 cm. Besides yield potential (Y_p = yield of each plot in the non-stress condition) and stress yield (Y_s = yield of each plot in the stress condition) the following physiological and biochemical attributes were measured.

Excised Leaf Water Retention (ELWR): the youngest leaves were collected and weighed, left for 5 h, then wilted at 30°C and reweighed. ELWR was calculated using the following formula (Farshadfar *et al.*, 2001):

ELWR = [1–(weight of fresh leaves-weight of leaves after 5 h)/weigh of fresh leaves]×100

Relative Water Content (RWC): A sample of 3 leaves were taken randomly from the flag leaves of each genotype and fresh weight (FW) was measured. The samples were rinsed in distilled water for 4 h in the low light density and the turgor weight (TW) was measured. Leaf samples were oven dried and weighed (dried weight = DW) in 70°C for 72 h. RWC was calculated using the following formula (Eric *et al.*, 2005).

$$RWC\ (\%) = \left[\frac{FW\text{-}DW}{TW\text{-}DW}\right]\times 100$$

Relative Water Deficit (RWD): RWD was measured (Tourneux *et al.*, 2003) as:

$$RWD\ (\%) = 100\text{-}RWC$$

Relative Water Loss (RWL): Three leaves were taken randomly from each line and weighed. The leaves were then wilted at 30°C for 2 h and reweighed, transferred to the oven for 48 h and weighed again. RWL was calculated using the formula suggested by Yang *et al.* (1991).

$$RWL = \left(\frac{W_1 - W_2}{W_3}\right)\left(\frac{t_1 - t_2}{60}\right)$$

where, W_1, W_2, W_3 are initial, wilted and dried weights, t_1 and t_2 are the time of measurement for initial and wilted weight (in minutes).

Chlorophyll Fluorescence (CHF): From each line 5 flag leaves were selected and quantum yield (QY) was recorded using a Mini-Pam (Genty *et al.*, 1989) as:

$$QY = \frac{F_v}{F_M}$$

where, F_v and F_m are variable and maximum fluorescence, respectively.

Cell Membrane Stability (CMS): Cell membrane stability of leaf tissues was calculated using the following equation (Zarei *et al.*, 2007):

$$I(\%) = \frac{1-(1-\frac{T_1}{T_2})}{(1-\frac{C_1}{C_2})} \times 100$$

$$CMS(\%) = 1-I(\%)$$

where, T_1 and T_2 are first and second conductivity measurement of desiccation treatment, C_1 and C_2 are first and second conductivity measurement of control and I is injury.

Leaf Chlorophyll Content (LCC): Using a chlorophyll meter (SPAD-502) five flag leaves were selected and LCC was measured during heading date (Yavad, 1986).

Chlorophyll a and b (CHLa, CHLb): Using Ashraf *et al.* (1994) method Spectrophotometer with 663 and 645 nm and the following formulas CHLa and CHLb were determined:

$$CHLa\left(mg\ mL^{-1}\right) = \frac{12.7(a663\ nm)-2.69(a645\ nm)}{(100)(W)} \times V$$

$$CHLb\left(mg\ mL^{-1}\right) = \frac{22.9(a645\ nm)-4.69(a663\ nm)}{(100)(W)} \times V$$

Where:
W = Fresh weight
V = Sample size

Proline Content (PC): Proline of leaves was determined by Bates *et al.* (1973) method. Data were measured on 5 flag leaves at 520 nm by Bausch and Lomb spectrophotometer 70. A standard curve, 12.5, 62.5, 31.25, 15.62, 7.8 and 1.9 mg of proline was prepared. Proline content of treated extracts was calculated using the standard curve and following formula:

$$Proline = \left[\frac{CDV}{DM \times 11.5 \times 10^6}\right] 1\times10^{-7}$$

Where:
C = Content of proline absorption
D = Precision degree
V = Toleon volume
DM = Dry weight of leaf sample

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. Twenty five seeds were then germinated on filter paper in Petri-dishes of 25 mm 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). The experiment was carried out within completely randomized design under two different stress and non-stress (normal) water regimes described above. In the stress and normal treatment 6 mL of PEG solution and distilled water added to each petridish, respectively in 1 day and 4 mL added in 6 day to compensate the losses due to evaporation. The emergence of 2 mm of radical and plumule was taken as the criterion for germination. After 10 days 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.* (1991) and Bouslama and Schapaugh (1984):

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

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, PSI is PI under drought stress condition and PINS is PI under normal condition.

The data for germination percentage, root length (cm) and coleoptiles (cm) were recorded on the 10th day after sowing.

Stress Tolerance Index (STI): (Fernandez, 1992), Multiple Selection Index (MSI) and Efficiency of Added Chromosome (EAC) were calculated (Farshadfar *et al.*, 2004) as:

$$STI = (Y_S)(Y_P)/(\overline{Y}_P)^2$$

$$MSI = ELWR_{std}+RWC_{std}+RWL_{std}+CHF_{std}+LCC_{std}+CMS_{std}$$

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

Where:
Y_{DAL} = Yield of disomic addition lines
Y_{CS} = Yield of recipient (Chinese spring = CS)

Analysis of variance, mean comparison, correlation analysis, discriminant analysis and cluster analysis were done using the softwares, MSTAT-C and SPSS.

RESULTS AND DISCUSSION

The results of analysis of variance showed highly significant differences between the genotypes for ELWR, RWC, RWD, RWL, LCC, CMS, Y_P and Y_S indicating the presence of genetic variation and possibility of locating the genes involved in the inheritance of drought tolerance criteria in barley. No significant difference was found between the genotypes for CHLa, CHLb, CHF and proline, but as F-test in the analysis of variance can only detect large differences between the genotypes, therefore non-significancy in the table of analysis of variance does not mean no differences between the genotypes for the characters investigated, hence using Duncan's multiple range test (DMRT) in the mean comparison, it is possible to discover the fine differences between the lines for the traits studied (Farshadfar, 2001b).

Mean comparison between the genotypes (Table 1) indicted significant difference between control (Sararood-1) and all the addition lines for ELWR except 5H. After the control the highest amount of ELWR belonged to addition line 5H which is greater than recipient (CS), hence it can be concluded that probably the genes responsible for ELWR are located on chromosome 5H.

Significant difference was found between control and additional lines, also between donor and recipient for RWC. The highest amount of RWC between addition lines is related to addition line 4H which is significantly different from that of recipient, indicating that most of the genes involved in the inheritance of osmoregulation (RWC) (Morgan, 1991) are located on chromosome 4H. Kocheva *et al.* (2004) reported that RWC is a suitable criterion for water measurement in barley. Drought tolerant genotypes have also higher amount of RWC than drought sensitive ones which conserve the photosynthesis rate during water stress (Huilian *et al.*, 1996). Teulat *et al.* (2003) showed that chromosome 6H carry the genes responsible for RWC which can be in agreement with our results because 6H chromosome exhibited no significant difference with 4H but 4H is more outstanding. The least amount of RWD was also attributed to chromosome 4H with significant difference

Table 1: Mean comparison between the genotypes for the characters studied

	Genotypes											
Character*	1H	2H	3H	4H	5H	6H	7H	CS	H	S.R-1	Sar	±SD+
ELWR	81.63de	84.13ce	84.13ce	86.90bc	87.87ac	85.97cd	85.67cd	86.03cd	90.57ab	92.20	91.9a	6.40
RWC	82.84f	81.39f	81.39f	90.56bc	79.68cd	88.97cd	85.36e	87.22de	92.28ab	93.83a	93.8a	7.70
RWD	17.16ab	18.61a	18.61a	9.44ef	10.32de	11.02de	14.44bc	12.78cd	7.78fg	6.17g	6.2g	7.70
RWL	0.20a	0.20a	0.20a	0.11d	1.10de	0.16b	0.17b	0.14c	0.09e	0.11de	0.09de	0.07
CMS	0.68f	0.76cd	0.76cd	0.81b	0.81b	0.78c	0.76de	0.74e	0.82ab	0.82a	0.84a	0.08
LCC	3947.00e	37.73e	37.73e	50.22bc	48.51c	43.18d	39.30e	36.69e	47.40c	52.40ab55a		11.40
CHF	0.79c	0.80c	0.80c	0.82ab	0.82a	0.80c	0.81ac	0.80c	0.82ab	0.82a	0.83a	0.03
CHLa	0.21ac	0.18bc	0.18bc	0.20ac	0.18c	0.22ac	0.19ac	0.25a	0.24ab	0.19ac	0.24ab	0.04
CHLb	0.10ad	0.09cd	0.09cd	0.10bd	0.09d	0.11ac	0.09cd	0.12a	0.12a	0.09cd	0.11ab	0.03
Proline	0.49ac	0.61ab	0.61ab	0.46bc	0.49ac	0.83c	0.58ac	0.47bc	0.62ab	0.71a	0.63ab	0.17
PIS	37.50f	38.33f	43.77ef	71.70ab	70.60ab	49.70de	36.33ef	58.67cd	57.73cd	-	-	-
Y_P	72.74f	79.47df	79.47df	99.20b	102.6ab	83.68de	88.71cd	95.22bc	84.36de	86.45ce	109.00a	19.70
Y_S	38.14f	43.84df	43.84df	54.61a	55.19ab	47.56ce	41.35f	49.09cd	51.27bc	57.24a	57.90a	12.20

*Common letter(s) in each row means no significant difference between the genotypes for that specific character in the column. +: SD = Standard error for each character

Table 2: STI, MSI, GSI and EAC* of disomic addition lines

	Genotypes								
Characters	1H	2H	3H	4H	5H	6H	7H	CS	Control
ELWR	-5.11	-5.90	-2.20	1.00	2.10	0.00	-0.34	-	-
RWC	-5.02	-4.92	-6.68	3.83	2.82	2.02	-2.13	-	-
RWD	34.25	33.54	42.59	-29.11	-19.25	-13.77	14.55	-	-
RWL	42.85	14.28	42.58	-21.42	-21.42	14.28	21.42	-	-
CMS	-8.59	0.13	2.94	9.63	9.23	4.81	2.27	-	-
LCC	7.57	2.48	2.83	36.87	32.21	17.68	7.11	-	-
CHF	-0.86	0.24	-0.62	2.35	260.00	-0.12	1.36	-	-
CHLa	-16.00	-22.80	-25.60	-20.00	-28.00	-9.60	-21.20	-	-
CHLb	-14.51	-21.73	-24.19	-18.54	-26.61	-9.67	-20.96	-	-
Proline	18.42	38.99	45.93	11.48	18.42	-7.17	40.19	-	-
Y_P	-23.60	-18.80	-16.50	4.20	7.70	-12.10	-6.80	-	-
Y_S	-22.30	-12.60	-10.70	17.40	12.40	-3.10	15.80	-	-
STI	34.61	34.39	35.62	41.59	47.36	36.12	47.36	46.13	29.21
MSI	4.62	4.78	4.72	5.45	5.47	5.01	4.88	4.91	5.62
GSI	51.00	49.30	63.60	84.20	81.40	57.40	54.70	68.80	71.60

*The data in the first part of the table are the efficiency of the added chromosomes for each character

with recipient and no significant difference with 5H and 6H, hence most of the genes monitoring RWD are located on chromosomes 4H, 5H and 6H with outstanding feature of 4H. The least amount of RWD and RWL is an indicator of drought tolerance (Tourneux *et al*., 2003; Yang *et al*., 1991) which belongs to chromosomes 4H and 5H with no significant difference with recipient, therefore most of the genes responsible for RWL are located on chromosomes 4H and 5H.

Suprunova *et al*. (2004) and Farshadfar *et al*. (2004) displayed that RWL is a quick screening technique for discrimination of drought tolerant genotypes.

The highest amount of CMS was attributed to the addition lines 4H and 5H with significant difference with recipient, hence most of the genes involved in the genetics of CMS are located on chromosomes 4H and 5H. Kocheva *et al*. (2004) reported that barley genotypes with higher CMS reduced less water during the drought period. Zarei *et al*. (2007) described CMS as an indicator of drought tolerance. The highest amount of LCC was related to chromosome 4H with no significant difference with 5H but with significant difference with recipient, accordingly the genes controlling LCC are located on chromosomes 4H and 5H. The highest amount of CHF observed in chromosomes 4H and 5H with significant difference with recipient displaying the importance of the genes located on chromosomes 4H and 5H in the genetics of CHF. Genty *et al*. (1989) and Farshadfar *et al*. (2004) explained a positive correlation between CHF and drought tolerance. No significant difference was found between addition lines and recipient for CHLa, CHLb and Proline, therefore it was not possible to locate the genes controlling these characters. It may be because of interallelic interaction between the genes at addition lines and recipient. Maximum PIS belonged to addition lines 4H and 5H with significant difference with recipient indicating that most of the genes controlling promptness index under stress condition are located on chromosomes 4H and 5H.

GSI was calculated for all the genotypes (Table 2). Addition lines 4H and 5H showed the highest values of GSI. Sapra *et al*. (1991), Mohammadi *et al*. (2003) and Farshadfar *et al*. (2003) 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.

Addition lines 4H and 5H revealed significant difference with recipient for Y_p and Y_s, accordingly the genes involved in the inheritance of Y_p and Y_s, are located on chromosomes 4H and 5H. The efficiency of added chromosomes (EAC) (Table 2) indicated that chromosomes 4H and 5H had higher efficiency with positive effect for improvement of ELWR, RWC, RWD, RWL, CMS, LCC, Y_p and Y_s, while the rest of the addition lines except 6H exhibited negative effect on the characters investigated.

For the overall consideration of disomic addition lines an agronomic quantitative index (STI) (Table 2) and a physiological Multiple Selection Index (MSI) were calculated (Table 2) which confirms that the highest amount of STI and MSI relate to chromosomes 4H and 5H, hence the ultimate Judgement is that according to the results of this experiment most of the genes involved in the inheritance of drought tolerance in barley are located on chromosomes 4H and 5H. Chromosomes 3R and 5R in rye (Farshadfar *et al*., 2004), 3E and 5E in agropyron (Farshadfar *et al*., 2003), 1A, 5A, 7A, 5B, 1D, D, 5D in wheat (Farshadfar *et al*., 1995) and 4H and 5H in barley (Farshadfar *et al*., 2008), were also reported to enhance drought and salt tolerance, indicating a close relationship between relatives of wheat and barley which will be useful for comparative mapping and identification of genes in cereals using bioinformatic techniques (Mohammadi *et al*., 2003).

Generation mean analysis (Farshadfar *et al*., 2001a) and combining ability analysis (Farshadfar *et al*., 2000) indicated overdominance type of gene action in the inheritance of Ys, RWL and ELWR, while RWC, CHF and Yp were controlled by additive type of gene action, hence selection in early segregated generations, pedigree selection and mass selection are suggested. While for characters under the control of non-additive type of gene action, biparental mating offers good prospects for increasing the frequency of genetic recombination, hastening the rate of genetic improvement. The epistatic effects (additive by additive) for Yp, Ys and RWL, additive by dominance for ELWR and dominance by dominance for RWL were also found to be outstanding (Farshadfar *et al*., 2000, 2001a). Triple test cross analysis indicated epistatic effects for proline content in the rainfed condition (Farshadfar *et al*., 2008b). Accordingly selection in segregating generations and hybridization breeding methods are offered.

Correlation analysis (Table 3) can be used for screening of drought tolerance criteria and applying them as direct and indirect (correlated response) selection indices (Farshadfar *et al*., 2002, 2003, 2008c). One criterion for a character to be an index of drought tolerance is to have positive significant association with Y_s, STI and MSI (Zarei *et al*., 2007; Farshadfar *et al*., 2008). Significant correlation coefficient was found between ELWR, RWC, RWD, RWL, CMS, LCC and CHF with Y_s, Y_p, STI GSI and MSI, therefore selection of these characters will improve simultaneously grain yield and drought tolerance of barley genotypes, however selection efficiency is related to magnitude of heritability and genetic advance which is high for Yp, ELWR and RWC hence, effective progress can be made through selection (Farshadfar *et al*., 2001a, 2008b). Farshadfar *et al*. (2001a) reported low genetic

Table 3: Matrix of correlation between drought tolerance indicators investigated

Traits	ELWR	RWC	RWD	RWL	CMS	LCC	CHF	CHLa	CHLb	Proli	Y_p	Y_s	STI	MSI
ELWR	1													
RWC	0.82**	1												
RWD	-0.82**	-1.00**	1											
RWL	-0.76**	-0.78**	-0.79**	1										
CMS	0.75**	0.79**	-0.79**	-0.75	1									
LCC	0.68**	0.82**	-0.82**	-0.67	0.79**	1								
CHF	0.73**	0.76**	-0.76**	-	0.80**	0.79**	1							
CHLa	0.31	0.27	-0.26	-0.24	0.11	0.04	0.03	1						
CHLb	0.19	0.23	-0.23	-0.17	0.04	0.03	-0.05	0.93**	1					
Proline	0.35*	0.13	-0.13	-0.28	0.28	0.24	0.35*	-0.11	-0.24	1				
Y_p	0.55*	0.53**	-0.52**	-065**	0.59**	0.62**	0.12	0.05	0.57**	0.03	1			
Y_s	0.59**	0.75**	-0.75**	-0.74**	0.78**	0.83**	0.70**	0.10	-0.07	0.11	0.77**	1		
STI	0.61**	0.69**	-0.69**	-0.74**	0.74**	0.78**	0.72**	0.13	0.05	0.08	0.91**	0.93**	1	
MSI	0.85**	0.91**	-0.91**	-0.91**	0.88**	0.89**	0.87**	0.19	0.14	0.27	0.65**	0.82**	0.79**	1
GSI	0.55**	0.64**	-0.64**	0.59**	0.69**	0.75**	0.63**	0.015	-0.019	0.063	0.80**	0.81**	0.42*	0.73**

*,**Significant at 5 and 1% level of probability, respectively

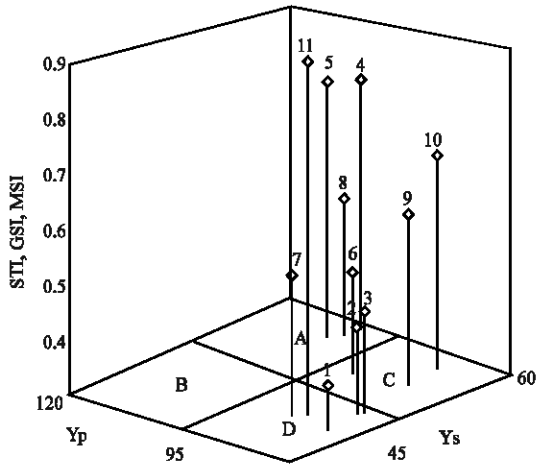

Fig. 1: The genotypes distribution in 3D plot based on Yp, Ys and STI, GSI or MSI

advance for Y_s and RWL indicating the importance of indirect selection through correlated characters with high heritability and genetic advance. The importance of these traits for breeding drought tolerance was reported by Zarei *et al.* (2007), Farshadfar *et al.* (2003, 2004, 2008c) and Mohammadi *et al.* (2003). No significant difference observed between CHLa, CHLb and proline with Y_s, Y_p, STI, GSI and MSI, therefore they can be discarded as drought tolerance criteria, although Proline is genotype dependent (Farshadfar *et al.*, 2008c).

Using a three-D plot (Fig. 1) between Y_p, Y_s, GSI, STI or MSI, addition lines were grouped as: genotypes express uniform superiority in both irrigated and rainfed environments (group A), genotypes perform favorably only in rainfed environments (group B); genotypes yield relatively higher only in rainfed environments (group C) and genotypes perform poorly in both stress and non-stress environments (group D). The optimal selection criterion (STI) should distinguish group A from the other

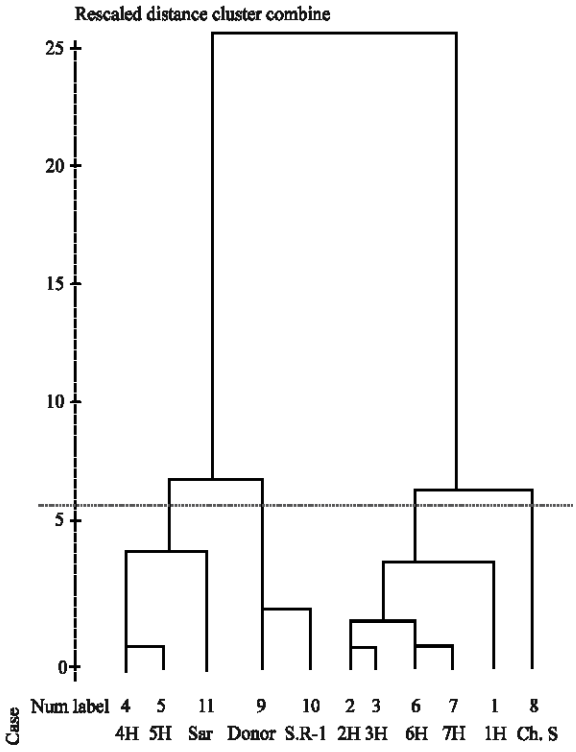

Fig. 2: Cluster analysis of genotypes based on STI, GSI and MSI using UPGMA procedure

three groups (Fernandez, 1992). Three dimensional plot revealed that genotypes 4H, 5 Hand the check are located in group A showing that genes controlling Y_p,Y_s, GSI and STI are located on chromosomes 4H and 5H, accordingly they can be used for improvement of drought tolerance in barley via chromosome engineering or as the raw material for mapping and QTL analysis of drought tolerance using DNA markers and molecular breeding techniques and thereafter for marker assisted selection.

Cluster analysis based on STI, GSI and MSI (Fig. 2) using UPGMA, grouped the genotypes into 4 clusters. 4H and 5H were classified in the first cluster, donor and control in the second cluster, addition lines 1H, 2H, 3H, 6H and 7H in the third cluster and the recipient in the 4th cluster, exhibiting the high performance of 4H and 5H and genetic variation between donor and recipient. Farshadfar *et al.* (2008c) showed that the genes controlling salt tolerance are also located on chromosomes 4H and 5H supporting the hypothesis that there is an association between resistances to stresses (Galiba, 1994).

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