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Locating QTLs Controlling Salt Tolerance in Barley Using Wheat-Barley Disomic Addition Lines

[1]E. Farshadfar, [2]S.A. Safavi and [3]M. Aghaee-Sarbarzeh
[1]Agricultural Faculty, Razi University, Kermanshah, Iran
[2]Science and Research Branch of Islamic Azad University, Tehran, Iran
[3]Seed and Plant Improvement Institute, Karaj, Iran

Abstract: In order to investigate the chromosome(s) involved in salt tolerance based on criteria such as Na^+, K^+ and K^+/Na^+ discrimination, Stress Tolerance Index (STI) and Multiple Selection Index (MSI), an experiment was carried out under hydroponics culture using a wheat-barley chromosome addition lines. The results of this investigation based on the morphological, physiological and statistical analysis proved the hypothesis that most of the genes controlling traits affecting salt tolerance are located on chromosomes 4H and 5H. Though, chromosome 6H was also ranked among the genotypes with high STI and MSI, this may show that this chromosome carry QTLs affect on salt tolerance, as well. However, higher Na^+ and lower K^+/Na^+ values of this line compared to 4H and 5H show the less effectiveness of this line on salt tolerance. Carrying higher positive traits on chromosome 4H compared to the 5H and 6H may indicate that the 4H chromosome has proportion of positive genes controlling salinity tolerance. The addition line carrying 4H chromosome of barley can be used in wide hybridization programs to transfer useful salt tolerance genes into wheat germplasm. This line may also be used in QTL mapping in the basic research programs.

Key words: Barley (*Hordeum vulgare*), Salt tolerance, K^+/Na^+ discrimination, STI, MSI, gene location

INTRODUCTION

Salinity is considered as a major limiting factor for crop plants in many parts of the world, especially in arid and semi-arid regions. Abut 7% of the world total land areas is affected by salt, as is a similar percentage of its arable land. The area is still increasing as a result of irrigation or land clearing (Forster *et al*., 1990; Munns *et al*., 2002). The majorities of crop species are extremely susceptible to salt and most are unable to tolerate concentrations higher than 100 mol m^{-3} NaCl. Among the crop plants, barley (*Hordeum vulgare*) is one of the most salt tolerant (Munns *et al*., 2002).

Several methods have been applied to reduce the salinity level of soil, including large scale irrigation and drainage schemes. Though, these methods are frequently applied, however, development of salt tolerant varieties is considered as an important alternative to these costly schemes for crops grown in areas at risk of salinisation. This requires new genetic sources for salt tolerance and more efficient techniques to identify tolerant germplasm, so that new genes for tolerance can be introduced into the crop cultivars.

Various tests and selection methods have been used to identify salt tolerant genotypes such as biomass or yield of plants under saline condition. To avoid the necessity of growing plants for long periods of time to measure biomass or yield, practical selection techniques can be based on physiological traits (Shannon and Nobel, 1990; Flowers and Yeo, 1995; Munns *et al*., 2002). Hollington (1998) described screening techniques for use in the development of salt tolerant wheat genotypes. Gorham (1990) provided a hydroponics system in which NaCl levels from 50 mol m^{-3} were applied as discrimination levels. Growth parameter assessments were made and the procedure was strengthened by K:Na discrimination analysis. Traits for salt tolerance that have been used to screen germplasm collection have included rates of Na^+ or Cl^- accumulation in leaves, degree of leaf injury, seedling root length and germination percentage (Gorham, 1990; Munns *et al*., 2002; Yeo and Flowers, 1986; Schachtman *et al*., 1991).

The K^+/Na^+ discrimination is a trait affecting salt tolerance which has been reported in many crops (Gorham *et al*., 1985, 1997; Gorham, 1990; Deal *et al*., 1999; Munns *et al*., 2002). Salt tolerance in the *Triticeae* is associated whit enhanced ability to discriminate between Na^+ and K^+ in the soil solution and to preferentially accumulate K^+ and exclude Na^+ (Gorham *et al*., 1985; Omielan and Dvorak, 1991). In wheat,

Corresponding Author: E. Farshadfar, Agricultural Faculty, Razi University, Kermanshah, Iran

salt tolerance is associated whit low rates of transport of Na^+ to shoots with high selectivity for K^+ over Na^+ (Gorham, 1990; Dvorak *et al*., 1994). Accumulation of potassium ions (K^+) in expanding or most recently expanded leaves and exclusion of sodium ions (Na^+) from them (K^+/Na^+) have been shown to be associated whit salt stress tolerance in wheat (Schachtman and Munns, 1992; Deal *et al*., 1999).

Barley (*Hordeum vulgare*) is one of the most salt tolerant crop species (Rawson *et al*., 1988; Munns *et al*., 2002). Using disomic wheat/barley addition lines Forster *et al.* (1990) reported that the genes with positive effects on salt tolerance, measured by morphological traits, were located on chromosome 4H and 5H of *H. vulgare*. The present study, therefore, was undertaken first to investigate the chromosome(s) involved in salt tolerance in barley based on physiological traits such as Na^+, K^+ and K^+/Na^+ discrimination, stress tolerance and multiple selection indices. Secondly, to study the traits affecting salt tolerance based on correlation and regression analysis whit the use of wheat/barley chromosome addition lines. These lines carry the wheat chromosome complement and an additional pair of chromosome from barley. The addition lines, substitution lines and ditelosomic lines have provided useful information on contribution of each chromosome to the tolerance of several abiotic stresses such as drought stress (Forster *et al*., 1990; Farshadfar and Sutka, 2003).

MATERIALS AND METHODS

The plant material consisted of nine genotypes including 7 disomic addition lines (DAL) of barley (*Hordeum vulgare* L., 2n = 2x = 14, HH, cv. Betzes) in the genetic background of bread wheat (*Triticum aestivun* L., 2n = 6x = 42, AABBDD, cv. Chinese Spring) along with their parental barley and wheat lines. The DALs were named as 1H to 7H indicating addition of chromosome 1H to 7H to the genome of Chinese Spring, respectively. The seeds were kindly provided by Dr. M. Tahir, ICARDA, Syria.

The experiment was carried out under hydroponics condition in a growth chamber at Sararood station, Kermanshah, Iran in the year 2002. The lines were evaluated under five different salinity levels, i.e. 0, 50, 100, 150, 200 mol m^{-3} of NaCl in tanks. The 45 treatments were evaluated in a factorial experiment based on the Randomized Completely Block Design (RCBD).

From each genotype 70 seeds with similar size were selected, surface sterilized with 5% calcium hypochlorite for 3 min, rinsed in distilled water and placed on sterile blotting paper in Petri plates. They were germinated at 27°C and then transferred into growth chamber. At two leaves stage, 45 seedlings with roots approximately of 5 cm long from each genotype were selected and transferred to tanks.

Hogland solution was the basic nutrient stock solution used in hydroponic culture system (Gorham, 1990). Saline solution in each treatment was made using NaCl and $CaCl_2$ salts with pH of 5.45-5.55. The solution was changed once a week.

The plantlets were hanged in the solution in such a way that roots were immersed in the aerated solution. The tanks were kept in a growth chamber at 16-24°C, 300 μmol m^{-3} sec^{-1} light intensity, 18 h/6 h (light/darkness) and 45-50% relative humidity (Forster *et al*.,1990). Plants were grown in tanks for two to three weeks (2-3 leaf stage of seedling), the saline solution was gradually added to the container in such a way that the final concentration was reached after 4 day

The youngest and fully expanded leaf (the 5th leaf) was collected and used for data recording as the emergence of this leaf starts when the solution reaches its final salt concentration. At this stage, 5 plants from each genotype were selected and traits such as Root Length (RL), Shoot Length (SL), 5th Leaf Weight (LW) and biomass (Bio) were measured. The fully expanded leaf blade samples from each genotype were collected, rinsed in distilled water, for 5 second and dried at 70°C. The samples were ground with a mortar and pestle, dissolved in acetic acid 10% and nitric acid 0.1% and kept at room temperature for 24 h for complete digestion. Each sample was diluted 1: 6 with acid solution and analysed by flame photometer apparatus, to determine the concentration of Na^+, K^+ and K^+/Na^+ ratio.

The data was analyzed based on the Randomized Complete Block Design (RCBD) model. Further analysis was performed to group the materials into different classes by cluster analysis using UPGMA method. Classes were tested by discriminate analysis. Tri-plot demonstration was used to demonstrate the statues of the genotypes for biomass and, K^+, Na^+ and K^+/Na^+ ion concentration. Principal Component Analysis (PCA) and bi-plot demonstration were also performed to compare the lines for salt tolerance, keeping all the traits into consideration. Simple correlation and regression analysis were performed to estimate the relationship between the traits. Several indices were calculated to identify the most salt tolerant lines as follow: Multiple Stress Index (MSI): sum of the standardized RL, SL, LW, Biomass, Na^+, K^+ and K^+/Na^+ all in the 5th leaf. Stress Tolerance Index (STI):$[(Y_S)(Y_P)]/(\overline{Y}_P)^2$, where, Y_S, Y_P and $\overline{Y}_P$ = Yield in stress condition, yield in normal condition and overall mean, respectively (Fernandez, 1992).

Tolerance (TOL): Y_P-Y_S where Y_P and Y_S are yield in normal condition and yield in stress condition, respectively for a given genotype (Fernandez, 1992).

Added Chromosome Efficiency (ACE)%: $(Y_a$-$Y_{CS}).100/(Y_{CS})$, where Y_a and Y_{CS}, are the value for a trait for a given addition line and that of Chinese Spring, respectively.

RESULTS AND DISCUSSION

The analysis of variance and mean comparison of the treatment (based on the 4th level of salinity) are given in Table 2 and 3, respectively. Low coefficient of variation for all the traits reflects the accuracy of the experiment. The results show the presence of significant differences between salinity levels. The difference among the genotypes was also highly significant for all the characters (Table 1, 2) indicating the presence of genetic variability among them for salinity response.

No significant genotype × salt concentration interaction was observed for all the traits except for the shoot length displaying the similar performance of the genotypes in different levels of salinity. This was expected as the lines with tolerance to salinity, are also expected to be tolerant in the higher salinity level compared to the susceptible ones (Table 1, 2).

This may show that specific chromosome(s) conferring salt-tolerance, has also significant effects on the traits. Significant genotype × salt concentration for SL may indicate specific chromosome other than that conferring salt tolerance genes carry QTLs affecting shoot length.

Mean comparison of lines for different traits (Table 2) revealed that the lines 7H, 6H, barley, 2H, 4H and 3H had the highest root length and are located in the same group. On the other hand, the lines 7H, 6H, barley and 4H produced the longest shoot. No significant difference was observed among the genotypes for LW. All the lines were placed in the same group for the biomass, except the 1H and CS lines. These lines placed in a group with higher leaf Na^+ concentration. This character was the least in the 4H and 5H group (Table 2). The K^+ ion accumulation in leaf, which is considered as a positive character in salt tolerance, was highest in the addition lines 4H, 5H and barley. These lines indicated low Na^+ concentration in their leaves. In wheat, salt tolerance is associated with low rates of Na^+ transport to shoots with high selectivity for K^+ over Na^+ (Gorham, 1990). Munns *et al.* (2002) also used low Na^+ accumulation for screening salt tolerance genotypes in durum wheat.

The K^+/Na^+ discrimination a criteria for salt tolerance was found to be high in 5H, 4H, 6H and Barley lines. The hexaploid line CS exhibit low values of K^+ and K^+/Na^+. Increase of K^+ and K^+/Na^+ ion accumulation in addition lines 4H and 5H was the same as that observed in barley lines, indicating these two chromosomes do enhance K^+/Na^+ and therefore salt tolerance in wheat (Table 2). The hexaploid wheat line, CS, had high value of Na^+ but lowest value of K^+ and K^+/Na^+, but the CS harboring 4H and 5H of barley displayed salt resistance.

Salt tolerance criteria defined as: TOL for Na^+, STI for K^+/Na^+ and MSI considering all the traits, were calculated (Table 3). The level of TOL is the index of interest, while shows the less difference between the trait recorded under the stress and normal conditions

Table 1: Analysis of variance and mean square of the traits investigated

		Mean square						
Source	df	RL	SL	LW	Biomass	Na^+	K^+	K^+/Na^+
Replication	1.00	0.142**	0.0002	0.000094*	0.00003	0.111	0.407**	0.122
Salinity level (A)	4.00	0.253**	0.225**	0.00056**	0.002**	8.606**	2.301**	4.157**
Genotype (B)	8.00	0.052**	0.033**	0.000008*	0.00026**	1.050**	0.400**	0.234*
AB	32.00	0.014	0.006*	0.00003	0.00009	0.131	0.053	0.038
Error	24.00	0.012	0.003	0.00002	0.00007	0.115	0.042	0.048
CV%	8.36	3.530	0.510	1.160	11.760	7.070	15.730	

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

Table 2: Mean comparison between the genotypes with Duncan's Multiple Range test for the traits studied

Genotype	Root length	Shoot length	Leaf weight	Biomass	Na^+	K^+	K^+/Na^+
1H	18.84^{BC}*	32.51^{C}	0.01^{a}	0.03^{C}	10.01^{A}	6.88^{CD}	1.10^{BC}
2H	22.54^{ABC}	35.40^{BC}	0.01^{a}	0.04^{ABC}	8.82^{AB}	7.31^{BCD}	1.25^{BC}
3H	20.42^{ABC}	34.83^{BC}	0.01^{a}	0.04^{ABC}	9.31^{AB}	7.30^{BCD}	1.11^{BC}
4H	20.89^{ABC}	38.82^{AB}	0.02^{a}	0.05^{AB}	5.05^{AB}	8.75^{CD}	1.88^{AB}
5H	17.22^{C}	30.83^{C}	0.01^{a}	0.05^{AB}	4.72^{D}	9.97^{A}	2.32^{A}
6H	25.70^{AB}	42.95^{A}	0.02^{a}	0.05^{AB}	7.24^{BC}	8.17^{BC}	1.63^{ABC}
7H	26.98^{A}	43.95^{A}	0.02^{a}	0.06^{A}	8.87^{AB}	8.28^{BC}	1.23^{BC}
CS	16.67^{C}	31.84^{C}	0.01^{a}	0.03^{BC}	9.12^{AB}	6.06^{D}	0.96^{C}
Barley	22.59^{ABC}	40.83^{AB}	0.02^{a}	0.05^{A}	6.93^{BC}	8.51^{AB}	1.52^{ABC}
LSD	0.13	0.066		0.001	0.408	0.247	0.264

* = Different superscripts capital letter significant at 1% level of significant. Different superscripts small letter significant at 1% level of significant

Table 3: Mean comparison of the DAL's, CS and barley genotypes for salinity tolerance indices

	Tol			STI		
DALs	Na^+	K^+	K^+/Na^+	Biomass	K^+/Na^+	MSI
1H	-11.13	5.39	-4.479	0.233	0.548	-5.42
2H	-9.89	3.39	-4.211	0.384	0.579	-2.15
3H	-8.65	5.24	-3.759	0.307	0.529	-4.25
4H	-3.35	4.28	-3.147	0.553	0.673	5.70
5H	-3.19	3.03	-2.762	0.408	0.690	4.94
6H	-7.18	4.69	-5.549	0.618	0.663	4.10
7H	-8.75	6.14	-3.795	0.675	0.550	2.44
CS	-9.30	6.22	-3.097	0.216	0.466	-8.16
Barley	-5.71	4.45	-3.043	0.706	0.596	3.43

Table 4: Comparison of chromosome efficiency (%) of individual added barley chromosomes for its effects on the traits of investigated at the forth level of salinity

DALs	Biomass	Na^+	K^+	K^+/Na^+	Root length	Shoot length	Leaf weight	STI	MSI*
1H	-14.89	10.60	14.31	3.09	10.85	13.76	5.71	17.60	148.91
2H	29.91	-0.78	18.79	7.32	32.45	24.98	18.86	24.25	326.63
3H	16.62	1.77	18.96	4.07	16.21	18.55	7.13	13.52	212.53
4H	40.48	-37.37	42.84	21.12	27.46	32.57	56.14	44.42	753.26
5H	36.89	-49.81	60.51	29.68	5.80	4.88	9.42	48.07	711.96
6H	37.64	-20.14	32.84	15.61	52.73	50.62	40.25	42.27	666.30
7H	57.34	-3.64	34.92	7.26	60.56	51.28	57.15	18.03	576.09
CS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Barley	52.84	-24.07	37.29	14.22	30.93	37.90	53.79	27.90	629.89

* = The ACE of MSI for each genotype was calculated after adding 10 to MSI of corresponding MSI i.e. MSI (ACE)=(MSI of DAL+10)-(MSI of CS+10)×100/(MSI of CS+ 10)

Table 5: Simple correlation coefficient between the characters under investigation

	(X1)	(X2)	(X3)	(X4)	(X5)	(X6)	(X7)	(X8)
Root L. (X1)								
Shoot L. (X2)	0.791*							
Leaf W. (X3)	0.573	0.607						
Biomass (X4)	0.722*	0.806**	0.852**					
Na^+ (X5)	-0.434	-0.511	-0.745*	-0.880**				
K^+ (X6)	0.419	0.584	0.771*	0.837**	-0.893**			
K^+/Na^+ (X7)	0.315	0.399	0.766*	0.811**	-0.977**	0.914**		
MSI (X8)	0.757*	0.822**	0.880**	0.983**	-0.864**	0.875**	0.812**	
STI (X9)	0.887**	0.947**	0.588	0.819**	-0.502	0.541	0.380	0.820**

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

(irrespective to its sign) and therefore, higher stress tolerance (Fernandez, 1992). The lines 4H and 5H had low differences between the two conditions for Na^+, K^+ and K^+/Na^+ (Table 3). This may be due to ability of these chromosomes not to allow Na^+ ions to accumulate in the plant tissues and also ability of them to maintain high level of K^+ and K^+/Na^+ uptake even in the normal condition. It has been reported that Na^+ exclusion from the tissue to be one of the mechanism of salt tolerance (Forster *et al*., 1990; Gorham, 1990; Gorham *et al*., 1997; Munns *et al*., 2002).

The STI values for biomass and K^+/Na^+ was highest for the 4H and 5H chromosomes, but it was moderate for biomass (Table 3). Fernandez (1992) suggested STI as selection criteria for yield under stress and non-stress condition. This value for K^+/Na^+ was high for 4H and 5H lines, whose TOL values were also the least among the other disomic addition lines. Therefore, the STI criteria may also be used as a valuable criteria to select salt tolerance genotypes. The MSI as a combined value of traits recorded in stress condition was also highest for 4H and 5H lines (Table 3). The wheat lines showed the lowest value of STI for K^+/Na^+ and MSI, had the highest susceptibility to salinity condition.

The effect of individual barley chromosome in the CS background was studied by calculating the efficiency of DALs (ACE %) for all the traits at the 4th level of salinity (Table 4). Comparison of ACE for different trait indicated that for Na^+, K^+, K^+/Na^+, MSI and STI, the wheat line carrying 4H and 5H chromosomes of barley were superior over the Chinese Spring (Table 4). These two lines added the highest efficiency to the hexaploid wheat for K^+, K^+/Na^+, STI, MSI and the lowest for N^+. The line 4H had also high ACE% for biomass, RL, SL and LW (Table 4). These results clearly shows that the 4H and 5H lines have desirable values of TOL, STI, MSI and ACE, indicating the efficiency of these chromosomes for salt tolerance and probable location of the gene affecting salt tolerance on these two chromosomes.

Correlation study showed positive and significant correlation between MSI and other traits (Table 5), except for Na^+ concentration in the leaves, which was negative and highly significant. Accumulation of Na^+ in the plant tissues has been reported as negative criteria for salt

Table 6: Multiple linear regression analysis using stepwise method

Source	df	Sum of square	Mean square	F-value
Regression	2	0.00232	0.0016	24.627**
Residual	15.000707	0.000047		
Total	17	0.00303	-	

R^2adj. = 0.735

tolerance (Munns *et al*., 2002). On the other hand, K^+/Na^+ discrimination as a valuable criteria revealed highly significant correlation with MSI (Table 5). Significant correlation between growth traits (root and shoot length, leaf weight and biomass) with K^+, Na^+ and K^+/Na^+ concentration and together with salt tolerances criteria, MSI and STI suggest that each one of the traits with positive correlation with MSI, could be used as an indicator to select for salt tolerance. However, the simplest way may be used according to availability of facilities. Here, we also recommend K^+/Na^+ ratio as criteria for salt tolerant genotypes. Similar result was reported by Omielan and Dvorak (1991). They showed that K^+/Na^+ ratio was highly correlated with salinity tolerance, as measured by grain yield and biomass production in the field.

Regression analysis using stepwise method considering biomass as a dependent variable was performed (Table 6). The results showed that the following model in which K^+ concentration and root length were inserted. They described about 73.5% of biomass variability under stress condition.

$$\text{Biomass} = -0.0318 + 0.00496K^+ + 0.00141 \text{ Root Length}$$
$$R^2_{adj.} = 73.5\%$$

In order to classify the genotypes for salt tolerance, cluster analysis using UPGMA method, MSI and STI indicators grouped the genotypes into four clusters (Fig. 1). The classification was verified by discriminant analysis. The lines 4H and 5H grouped in a single class with similar performance for MSI and STI. The lines H6, H7 showed similar performance to saline condition as the donor parent (barley line). The classes could be named as highly tolerant (4H and 5H), moderately tolerant (6H, 7H and donor parent, barley), susceptible (2H) and highly susceptible (1H, 3H and recipient wheat parent, CS). This analysis further approved the results of the previous analysis as shown by placing 4H and 5H lines in one group.

Because of importance of K^+/Na^+ discrimination in salt tolerance, 3-D plot demonstration of genotypes using K^+/Na^+ under normal and stress conditions and the STI index of K^+/Na^+ was used to exhibit their graphical distribution (Fig. 2). The 3D plot displayed that the genotypes were distributed almost in two areas, C and D, except the 6H line. Genotypes placed in area C showed high K^+/Na^+ proportion under stress condition and lower

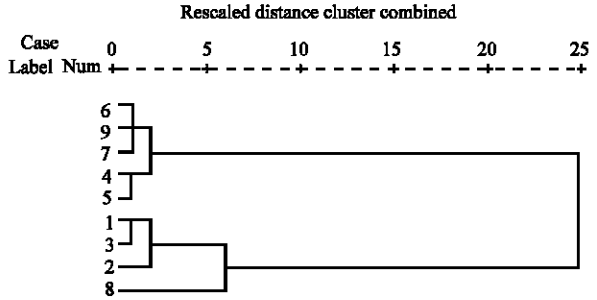

Fig. 1: Different groups identified by cluster analysis for MSI and biomass-STI

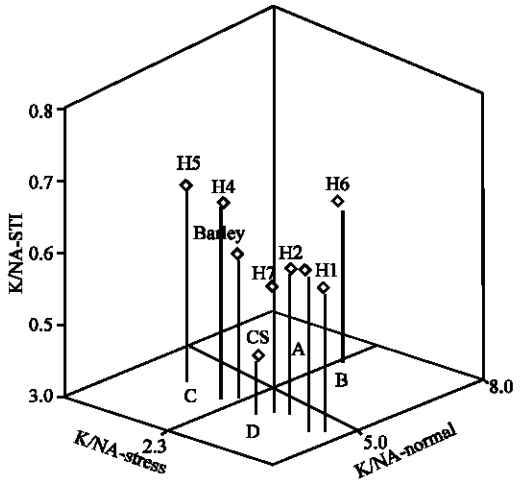

Fig. 2: The genotype distribution in 3D plot based on the K^+, Na^+ and K^+/Na^+ discrimination

K^+/Na^+ under normal condition. In area A, the 5H and 4H lines were placed. The barley donor genotype also placed adjacent to this area near to 4H line. On the other hand, other genotypes, including the recipient parent (CS), placed in area of the plot (area D) with lower value of K^+/Na^+ under both the conditions and low STI for K^+/Na^+ (Fig. 2), indicating their susceptibility to salt stress. Although the 6H line exhibited higher STI, but its K^+/Na^+ index under stress condition was lower than the 4H and 5H lines, almost similar to barley line, indicating the presence of extra genes on this line to have high K^+/Na^+ value under normal condition.

Considering all the traits, Principal Component Analysis (PCA), was performed (Table 7). It showed that the first two principal components (PCA_1 and PCA_2) accounted for 92.6% of the variability in the data matrix. The first PCA revealed positive correlation with the traits enhancing salt tolerance (i.e. K^+, Na^+, K^+/NA^+ discrimination and MSI). Therefore, this component selects the line with high K^+ uptake, high Na^+ exclusion from their tissues and high K^+/NA^+ discrimination, while the second component (PCA_2), with negative correlation

Table 7: Total variance explained in Principal Component Analysis (PCA),considering all the traits

	Initial eigenvalues			Extraction sums of squared loadings		
Component	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)
1	6.848	76.090	76.090	6.848	76.090	76.090
2	1.485	16.467	92.586	1.485	16.497	92.586
3	0.294	3.264	95.851	0.294	3.264	95.851
4	0.218	2.427	98.278	0.218	2.427	98.278
5	0.106	1.183	99.461	0.106	1.183	99.461
6	0.036	0.395	99.856	0.036	0.395	99.856
7	0.013	0.144	100.000	0.013	0.144	100.000
8	1.594E-05	0.000	100.000	1.594E-05	0.000	100.000
9	1.302E-16	1.447E-15	100.000	1.302E-16	1.447E-15	100.000

Table 8: Component matrix and correlation of the PCA's with the recorded traits

	Component								
	1	2	3	4	5	6	7	8	9
MSI	0.999								
Biomass	0.986				-0.131				
K	0.879	-0.363	-0.147	0.121	0.242				
Na	-0.876	0.420	0.119	0.171	105.00				
Leaf W	0.870	-0.149	0.432	0.183					
K/Na	0.823	-0.556							
STI	0.822	0.541	-0.118			0.121			
Shoot l	0.821	0.480	-0.178	0.220		-0.112			
Root l	0.744	0.567	0.154	-0.290	0.122				

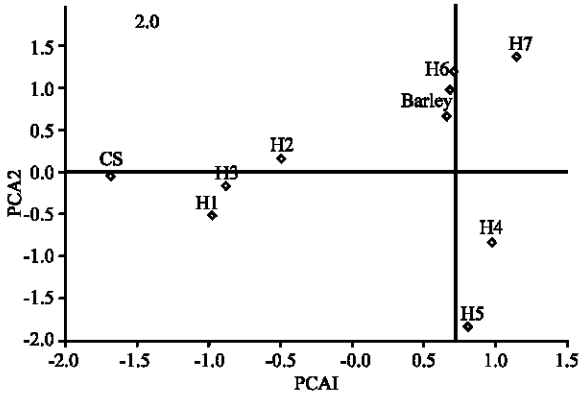

Fig. 3: Biplot illustration of the genotype based on PCA_1 and PCA_2

with K^+ and K^+/Na^+ discrimination and positive correlation with Na^+ (Table 8) is an indicator of salt susceptibility. Biplot representation between PCA_1 and PCA_2 (Fig. 3) demonstrated the 4H and 5H genotypes placed in area of the plot with high values of PCA1 and low values of PCA_2. The barley genotype and 6H located in area with moderate tolerance. The results of this analysis again showed the superiority of the 4H and 5H lines compared to the others with respect to all the traits under investigation.

The results of this study indicated that the efficiency of chromosomes 4H and 5H for the under salinity condition especially elevated accumulation of K^+ and reduced accumulation of Na^+ were high, which clearly indicates that the genes controlling the traits affecting salt tolerance might be presented on these two chromosomes. Similar results have been reported by Forster *et al.* (1990) for morphological traits such as, grains/plant, spikelets, plant, height, days to flowering, stem number, fresh weight/plant. It may also conclude that, either the gene(s) reported by Forster *et al.* (1990) are also located on 4H and 5H chromosomes of barley, or they are the effect of the gene(s) controlling K^+, Na^+, K^+/Na^+ on these chromosomes of barley. The efficiency of chromosome 4H on adaptation to environment particularly water availability and temperature (Chalmers *et al.*, 1992) in salt tolerance (Forster *et al.*, 1993) and potential water use efficiency (Handley *et al.*, 1994) have already been reported.

This study also proves the hypothesis that most of the genes controlling morphological and physiological indicators of salt tolerance are located on these two chromosomes. Though, chromosome 6H also ranked among the genotypes with high STI and MSI, this may show that this chromosome carry genes affects on salt tolerance, as well. However, higher Na^+ and lower K^+/Na^+ values of this line compared to 4H and 5H show the less effectiveness of this line on salt tolerance. Carrying higher positive traits on chromosome 4H compared to the 5H and 6H may indicate that the 4H chromosome carry higher proportion of positive genes controlling salinity tolerance. The addition line carrying 4H chromosome of barley can be used in wide hybridization programs to transfer useful salt tolerance genes into wheat germplasm. This line may also be used in QTL mapping in the basic research programs.

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