



Mapping QTLs of physiological traits associated with salt tolerance in 'Step toe' × 'Morex' doubled haploid lines of barley at seedling stage

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Abstract

Salt stress as one of the most important abiotic stress plays an important role in the yield reduction of crop plants worldwide. It is now recognized that tolerance to salinity is genetically and physiologically complex and also inherited quantitatively. In order to map the Genes/QTLs for salt tolerance, and to determine the portion of each QTL in the phenotypic variation of the physiological traits in barley, 72 doubled-haploid lines derived from a cross between 'Step toe' and 'Morex' were investigated. This experiment was carried out under hydroponic system in Research Lab of Zabol University in 2009. It was arranged as a completely randomized factorial experiment, with 3 replicates and 4 salinity levels (0, 5, 10, 15 dS/m). Seven physiological traits including chlorophyll content, chlorophyll fluorescence (Fo, Fv, Fm/Fv), proline, water soluble carbohydrate (WSC) and relative water content (RWC) were studied. There were significant differences among the lines and different salinity levels for all studied traits. Maximum correlation was observed between Fo and Fm/Fv ($r = 0.92^{**}$). QTL analysis was carried out using genetic linkage map derived from 327 molecular marker of RFLP and QTL cartographer software with composite interval mapping method. In general we found 29 QTLs for the traits (13 QTLs in free salinity condition, 17, 18 and 22 QTLs, respectively, in first, second and third salinity levels and 23 QTLs in the mean of these four conditions). Phenotypic variations that were explained by these QTLs changed from 8.63 to 44.69. The highest and the lowest phenotypic variations were related to Fv and chlorophyll content QTLs (*QFv2H* and *QCh7Ha*) in mean of four condition and third salinity level, respectively. LOD scores ranged from 3.01 to 15.97. The lowest and the highest LOD scores were attained for the QTLs of chlorophyll content in mean of four conditions (*Qch2Ha*) and WSC in second salinity level (*QWSC2H*). Several QTL co-locations confirm the observed correlations among the traits. Some of detected QTLs appear to be quite stable between four salinity levels. Therefore, gain through marker-assisted selection (MAS) in this population would be unlimited.

Key words: QTL, salinity, tolerance, barley, physiological traits.

Introduction

Growth and development of plants is greatly affected by abiotic stresses, such as cold, drought, heat, soil salinity and toxic metal pollution, limiting growth and resulting in yield loss of cultivated plants³⁸. Agricultural productivity is severely affected by soil salinity because salt levels that are harmful to plant growth affect large terrestrial areas of the world. Currently 40% of the world's food production is realized on 17% of the cropland which is under irrigation, but 1-2% of this area is lost every year by secondary salinization. The „White death“ in the form of desertification of agricultural arable land due to salt is the most serious threat for the nutrition of mankind. There is a demand to breed salt tolerant crops, because most of them are sensitive. The damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations⁴⁵. It is estimated that approximately 20% of agricultural land in the world suffers from soil salinity⁹.

Barley (*Hordeum vulgare* L.) is an important food and fodder, short-season, early maturing, diploid and self pollinating crop, thus it is an ideal model plant for genetic and physiological studies of salinity tolerance. Barley is widely cultivated in saline areas as one of the most salt tolerant field crops^{25,31}. However, its growth and production is also greatly affected by salt stress. Improving the salinity tolerance of barley and increasing its productivity has been an important objective in many barley breeding programs⁷.

It is now recognized that tolerance of salinity by higher plants, in common with other environmental stresses, is genetically and physiologically complex, controlled by quantitative trait loci²⁴. Plants respond to environmental stresses, challenges through a number of defense mechanisms to maintain the optimal conditions for growth and development. These mechanisms include several regulatory processes that activate the differential expression of genes responsible for tolerance. Salinity affects numerous growth processes at the levels of sub-cell, cell, tissue and whole plant, and several physiological processes including ionic balance (especially Na^+/K^+ ratio) and distribution⁴³. Final manifestation of several components, such as Na^+ and K^+ uptake, ion balance and ion compartmentation etc have the main role in salt tolerance. For example, ion transport, selectivity, excretion, nutrition and compartmentation are involved together with growth, water use and water use efficiency²⁴.

At the genetic level, salt tolerance is a complex character controlled by a number or groups of genes, and involves a number of component traits which are likely to be quantitative in nature⁹, and significantly modulated by environments⁴². The direct selection of superior salt-tolerant genotypes under field conditions is hindered by the significant influence that environmental factors have on the response of plants to salinity³⁷. There is also evidence

supporting the notion that salt tolerance is a complex trait involving the function of many genes^{8,10}. Salt tolerance in plants appears to be a developmentally regulated process, and the tolerance of the plants at one stage of development is not always correlated with tolerance at other stages^{8,10,13,39}. For example, in barley salt tolerance tends to increase with the age of the plant¹⁰. QTLs associated with salt tolerance at the germination stage were different from those of QTLs associated with salt tolerance at the early stage of growth³⁵. The plants selected by their ability to germinate at high salinity did not display similar salt tolerance during vegetative growth²⁸.

In the last decade, the development of molecular markers has made it possible to investigate the inheritance of complex traits and to tag and manipulate individual QTLs involved⁴⁶. The development of molecular biology techniques has enabled the development of DNA markers that can be used to identify QTLs. The use of QTLs has improved the efficiency of selection, in particular for those traits that are controlled by several genes and highly influenced by environmental factors⁸. QTLs and marker-assisted selection provide several advantages over direct phenotypic screening, particularly because the methodologies used to detect the markers reduce the time needed to screen individuals and reduce the impact of environmental effects on the trait under study. The development of high-density DNA maps and advances in marker-assisted selection techniques will facilitate pyramiding traits of interest to attain substantial improvement in crop salt tolerance⁴⁵.

There is considerable evidence to support the view that salt tolerance and its sub-traits are determined by multiple QTLs and both additive and dominance effects are important in the inheritance of many of the traits associated with salt tolerance^{8,10,14}. In the last decade, QTLs associated with salt tolerance have been mapped in rice^{12,24,28}, wheat^{30,36}, tomato¹⁰ and soybean²⁷. In barley, several authors estimated the loci associated with salt tolerance at the germination, seedling and late growth stage of maturity by using a composite cross population²⁰, isogenic lines^{32,33} or doubled haploid lines^{34,35,43}. Although identification of the QTLs controlling salt tolerance at seedling stage is particularly important, little relevant study has been done to date. In the present study to acquire basic information about the genes controlling salt tolerance at seedling stage we analyzed the relationship between salt tolerance and physiological traits using 'Steptoe' × 'Morex' doubled haploid (DH) lines of barley.

Materials and Methods

Seventy-two doubled haploid lines from the cross of 'Steptoe' (CI15229) × 'Morex' (CI15773) together with both parents, were utilized to determine salt tolerance. The DHs were developed through a modified '*Hordeum bulbosum*' technique, as described by Chen and Hayes⁴, by the Oregon State University Barley Breeding Program and were kindly provided by Hayes (Department of Crop and Soil Science, Oregon State University, Corvallis, USA). The experiment was carried out in the Research Lab of the Zabol University, in 2009. The 72 DHs together with their parents were arranged in 4 salinity levels (0, 5, 10 and 15 dS/m) under hydroponic system in a completely randomized factorial experiment with 3 replications. Each doubled haploid or parental lines were germinated at 25°C in dark conditions in germinator. Seedlings were hydroponically grown with Hoagland nutrient solution¹⁸ in

a growth chamber. For differential display, 2-week old plants were treated with Hoagland solution containing NaCl. The treatments lasted 30 days after which the following parameters were determined: chlorophyll content (Ch), chlorophyll fluorescence (Fo, Fv, Fm/Fv), proline, water soluble carbohydrate (WSC) and relative water content (RWC). SPAD-502 chlorophyll-photometer was used to measure chlorophyll content. Chlorophyll content measured in fresh leaves in the first, medium and last part of the leaf as an average of three leaves. Fo, Fv and Fm/Fv parameters were recorded for chlorophyll fluorescence by means of Handy PEA chlorophyll fluorometer. Proline and water-soluble carbohydrate content were measured according to Bates *et al.*² and Irigoyen *et al.*¹⁹, respectively. Relative water content (RWC) was calculated as:

$$RWC = (fw - dw) / (tw - dw) \times 100$$

where *fw* is leaf fresh weight, *dw* is leaf dry weight and *tw* is leaf turgid weight. Analysis of variance (ANOVA) was performed using proc ANOVA procedure in SAS (SAS Inst. Inc., Cary, NC). Heritability of entry was estimated as narrow-sense heritability by:

$$h^2 = 1 - (MS_{G \times S} / MS_G)$$

where $MS_{G \times S}$ is the variance of genotype and salt interaction and MS_G is the variance of genotype⁴⁰. Simple correlation analysis was carried out for all studied characteristics using entry means and proc CORR procedure in SAS⁴⁹.

A molecular marker linkage map^{16,23} (Current at <http://barleygenomics.wsu.edu/>) was developed by the North American Barley Genome Mapping Project from doubled haploid line population derived from 'Steptoe/Morex' F₁s, and used for mapping of forage quality traits. This map comprises 327 RFLP markers with an average density of 3.75 cM^{16,23}. QTL analysis was conducted separately for each trait in each environment and for combining environments. Analyses were performed using WinQTL cartographer 2.5⁴¹. A series of 1000 permutations were run to determine the experiment-wise significance level at P = 0.05 of logarithm of the odds ratio (LOD) for the traits⁵. Composite interval mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects^{21,47} using model 6 of the Zmapqtl program module. The genome was scanned at 2 cM intervals and the window size was set at 10 cM. Cofactors were chosen using the forward-backward method of stepwise regression. The percentage of phenotypic variance explained by a specific QTL value (R²) was taken as the peak QTL position as determined by WinQTL cartographer 2.5. The LOD peaks were considered to indicate the most likely position of QTL effects. Confidence intervals of 95% were calculated by 1000-bootstrap re-sampling²⁶ as proposed in the WinQTL cartographer 2.5 package.

Results and Discussion

Analysis of variance of 72 doubled haploid lines and their parents ('Steptoe' and 'Morex') showed a highly significant (p ≤ 0.01) genotype effect for chlorophyll content, Fo, Fm/Fv, proline, WSC and RWC (Table 1). Genotype effect on Fv was significant (p ≤ 0.05). Effect of salt was also highly significant (p ≤ 0.01) for all the studied

Table 1. Analysis of variance of 7 physiological traits for 72 Steptoe/Morex doubled haploid lines of barley and their two parents in salt stress and non-stress conditions.

S.O.V	D.F	Mean Squares						
		Ch	Fo	Fv	Fm/Fv	Pr	WSC	RWC
Salt	3	10.37**	4267.64**	194194.16**	0.03**	205.01**	10.82**	3270.92**
Genotype	73	1.51**	1707.69**	14555.46*	0.01**	2.29**	0.45**	143.01**
Salt×Genotype	219	0.80**	649.77 ^{ns}	11316.77 ^{ns}	0.001 ^{ns}	1.42 ^{ns}	0.26**	69.19 ^{ns}
Error	592	0.48	644.72	10621.33	0.001	1.30	0.18	92.85
C.V		19.90	8.23	7.34	3.43	0.13	6.11	14.75
R ²		52.39	42.29	39.60	36.23	58.59	52.53	39.18

* and **, significant at 0.05 and 0.01 probability level, respectively; ns, non-significant.

Ch: Chlorophyll content; Fv, Fo and Fm/Fv: Chlorophyll fluorescence parameters; Pr: Proline; WSC: Water-soluble carbohydrate; RWC: Relative water content.

traits (Table 1). Salt×genotype interaction was highly significant just for chlorophyll content and WSC (Table 1). Effect of salt×genotype interaction on Fo, Fv, Fm/Fv, proline and RWC were not significant ($p \leq 0.05$). Previous studies have also reported significant genotype and genotype×environment interaction effect for different traits in this population¹⁷. Gibson *et al.*¹¹ reported similar variation within this population for grain quality traits.

‘Morex’ showed higher values for traits such as chlorophyll content, Fm/Fv, WSC and RWC, when compared with ‘Steptoe’. Fo, Fv and proline had higher values in ‘Steptoe’ when compared with ‘Morex’ (Table 2). The differences between the means of parents were not significant for all the studied traits, indicating that variation between the parents for these traits were narrow (Table 2). There are three major difficulties in mapping QTLs for physiological traits in an agronomically relevant genetic background: (1) phenotyping the mapping population is an investment of a completely different order from phenotyping a qualitative or visible trait; (2) difficulties in phenotyping constrain the size of any mapping population with corresponding decreases in confidence over QTL location and (3) the use of agronomically relevant parents may mean relatively low levels of polymorphism.

The difference between the means of doubled haploids (\bar{x}_{DHS}) and their midparent (\bar{x}_p) were not significant for all the studied traits (Table 2), indicating that the 72 DHLs in this study are representative of the total possible DHLs from the cross of ‘Steptoe/Morex’ and that the studied traits are mostly controlled by additive gene effect. Phenotypic distribution of DH population was normal. Phenotypic means of parents showed variation for

most traits and fell within the ranges exhibited by progeny individuals (Table 1), providing evidence for transgressive segregation in both directions (positive and negative). Positive transgressive means that a majority of DH lines were superior to the parental lines and negative transgressive means that a majority of DH lines was inferior to the parental lines. The best DH when compared with the best parent showed higher values for all studied traits, except Fm/Fv, indicating positive transgressive for these traits. The worst DH when compared with the worst parent showed lower values for all studied traits, indicating negative transgressive for all studied traits. Positive and negative transgressive genotypes over each parent indicated the wide spectrum variation of studied traits in offspring. The phenomenon of transgressive variation could be interpreted as favorable alleles being dispersed between the two parental lines. Bregitzer and Campell³, in a study to determine the QTLs associated with plant regeneration in this population also reported that transgressive segregation occurred. Narrow-sense heritabilities (h^2) presented in Table 2 showed medium values ranging from 22.20 to 61.90%. Therefore, all studied traits were moderately heritable. Heritability provides an estimate of how much variability is due to genetic factors. Simple correlation analysis indicated that there was a positive highly significant relationship between Fo and Fm/Fv. This may be due to QTLs co-location with positive or negative allelic effects. Moreover, variation of one trait may explain variation of others. Correlations between other studied traits were medium (Table 3).

Table 2. Simple statistics, genetic gain and heritability of 7 physiological traits in a population of 72 Steptoe/Morex doubled haploid lines of barley and their two parents.

Item	Ch	Fo	Fv	Fm/Fv	Pr	WSC	RWC
Steptoe(P ₁)	3.647	318.917	1413.000	0.775	871.410	7.060	59.583
Morex(P ₂)	4.023	291.500	1388.000	0.790	871.110	7.470	67.667
P ₁ -P ₂	-0.376 ^{ns}	27.417 ^{ns}	25.000 ^{ns}	-0.015 ^{ns}	0.297 ^{ns}	-0.414 ^{ns}	-8.083 ^{ns}
\bar{x}_p	3.835	305.208	1400.500	0.782	871.262	7.262	63.625
Worst DH	2.728	282.000	1294.830	0.750	870.040	6.630	57.833
Best DH	4.470	351.583	1480.920	0.755	872.080	7.560	72.750
\bar{x}_{DHS}	1.742	69.583	186.084	0.045	2.040	0.930	14.917
$\bar{x}_{DHS} - \bar{x}_p$	-0.343 ^{ns}	3.434 ^{ns}	3.540 ^{ns}	-0.004 ^{ns}	-0.338 ^{ns}	-0.218 ^{ns}	1.728 ^{ns}
[†] GG _N	-0.92 ^{ns}	-9.500 ^{ns}	-93.170 ^{ns}	-0.030 ^{ns}	-1.07 ^{ns}	-0.43 ^{ns}	-1.750 ^{ns}
^{††} GG _P	0.447 ^{ns}	32.666 ^{ns}	67.920 ^{ns}	-0.005 ^{ns}	0.670 ^{ns}	0.090 ^{ns}	5.083 ^{ns}
^{†††} h ²	47.00	61.90	22.20	37.30	38.00	42.80	51.60

* and **, significant at 0.05 and 0.01 probability level, respectively; ns, non-significant.

Ch: Chlorophyll content; Fv, Fo and Fm/Fv: Chlorophyll fluorescence parameters; Pr: Proline; WSC: Water-soluble carbohydrate; RWC: Relative water content.

[†]Negative Genetic Gain = GG_N = Worst DHs - Worst Parent; ^{††}Positive Genetic Gain = GG_P = Best DH - Best Parent; ^{†††}Heritability.

Table 3. Simple correlation of 7 physiological traits in a population of 72 Steptoe/Morex doubled haploid lines of barley and their two parents.

	Ch	Fo	Fv	Fm/Fv	Pr	WSC
Fo	0.41**					
Fv	0.42**	0.55**				
Fm/Fv	0.41**	0.92**	-0.34**			
Pr	0.39**	0.33*	-0.47**	0.45**		
WSC	0.32*	0.34**	-0.33*	0.30*	0.53**	
RWC	-0.34**	-0.36**	-0.40**	0.39**	-0.33*	0.46**

* and **, significant at 0.05 and 0.01 probability level, respectively.

From 2 to 6 QTLs were detected for each of the 7 physiological traits studied using the winQTL cartographer 2.5 program (Table 4). In general we found 29 QTLs for all studied traits that 23 of them were arisen in the mean of four salinity conditions, 13 of them appeared in free salinity condition and 17, 18 and 22 of them in first, second and third salinity levels, respectively. Phenotypic variations that were explained by these QTLs changed from 8.63 to 44.69%. The highest and the lowest phenotypic variations were related to Fv and chlorophyll content QTLs (*QFv2H* and *QCh7Ha*) in mean of four conditions and third salinity level, respectively. LOD score ranged from 3.01 to 15.97. The lowest and the highest LOD scores were attained for the QTLs of chlorophyll content in mean of four conditions (*Qch2Ha*) and WSC in second salinity level (*QWSC2H*).

QTL models explained about 43.57, 72.45, 82.42, 90.89 and 80.00% of the total variation of chlorophyll content in four salinity levels and mean of them, respectively. Eight QTLs were identified for chlorophyll content in four salinity levels and means of them were detected on chromosomes 1H, 2H, 5H and 7H, respectively. These QTLs (*Qch1Ha*, *Qch1Hb*, *Qch2Ha*, *Qch2Hb*, *Qch5Ha*, *Qch5Hb*, *Qch7Ha* and *Qch7Hb*) were located near BCD351C, G1b1, Adh8, ABC165, WG908, ABG496, iPgd1A and ABC310b markers,

respectively. *Qch1Hb*, *Qch2Hb* and *Qch7Hb* QTLs are coincident in four salinity level and means of them.

There were five QTL identified on chromosomes 2H and 5H for Fo controlling approximately 30.78, 13.16, 31.41, 36.08 and 54.22 of the total phenotypic variation for the trait, in four salinity levels and means of them, respectively. These five QTL, *QFo2Ha*, *QFo2Hb*, *QFo5Ha*, *QFo5Hb* and *QFo5Hc* were located on chromosomes 2H and 5H near ABG072, ABG316E, ABC706, WG644 and ABC482 markers, respectively. *QFo5Ha* was stable in four salinity levels and means of them.

Fv-QTLs, *QFv2H*, *QFv3H* and *QFv5H* were located on chromosomes 2H, 3H and 5H near ABC165, MWG571B and ABC482 markers and controlled about 34.61, 62.76, 70.23, 74.75 and 92.72% of the total variation in four salinity levels and means of them, respectively. Major Fv-QTL, *QFv2H* that explained about 34.61, 34.37, 35.02, 34.99 and 44.69% of the total variation in four salinity levels and mean of them, was stable in four salinity levels and their mean.

QTLs for Fm/Fv accounted about 28.64, 41.33, 46.55, 52.68 and 45.45% of the total variation for this trait in four salinity levels and mean of them, respectively. *QFmv1H*, *QFmv2H* and *QFmv5H* were located on chromosomes 1H, 2H and 5H near G1b1, ABC165 and ABC482 markers, respectively. Major Fm/Fv-QTL, *QFmv5H* was stable in four salinity levels and their mean.

Four QTLs affecting proline content were found on chromosomes 1H, 2H and 5H controlling approximately 43.89, 46.28, 27.20, 31.61 and 45.87% of the total variation in four salinity levels and their mean, respectively. These QTL (*QPr1H*, *QPr2H*, *QPr5Ha* and *QPr5Hb*) were near ABR335 ABG703b, Adh6 and ABC482 markers, respectively. *QPr2H* was constant in four salinity levels and mean of them.

Table 4. Quantitative trait loci (QTL) for 7 physiological traits identified by composite interval mapping for 72 Steptoe/Morex doubled haploid (DH) barley lines population.

Trait	QTL name	Chr. name	Nearest marker	[‡] QTL pos. ^a	QTL interval	[†] LOD score ^b	Allelic effect	[†] R ^{2c}	Total R ^{2d}
Ch	QCh1Hb	1H	G1b1	72.4	67.0-86.5	4.57	0.36	18.67	
	QCh2Ha	2H	Adh8	56.0	53.9-63.3	3.01	-0.12	11.67	
	QCh2Hb	2H	ABC165	167.1	165.6-170.9	3.49	-0.25	12.09	
	QCh5Ha	5H	WG908	153.5	149.7-157.8	3.77	0.22	10.64	
	QCh5Hb	5H	ABG496	166.6	158.2-169.4	3.54	-0.39	12.54	
	QCh7Hb	7H	ABC310b	120.8	126.2-130.8	3.95	0.26	14.39	
	Fo	QFo2Ha	2H	ABG072	123.0	113.3-133.1	3.74	4.46	12.10
QFo2Hb		2H	ABG316E	171.1	166.5-174.1	4.22	-5.23	14.27	
QFo5Ha		5H	ABC706	62.3	54.9-75.4	3.73	-1.25	15.51	
QFo5Hb		5H	WG644	146.8	140.7-148.8	3.70	-4.22	12.34	
Fv		QFv2H	2H	ABC165	167.0	161.1-177.1	12.88	-28.09	44.69
	QFv3H	3H	MWG571B	87.9	80.1-98.0	6.52	-17.34	28.88	
	QFv5H	5H	ABC482	174.5	171.7-178.8	4.75	-0.21	19.15	
Fm/Fv	QFmv2H	2H	ABC165	165.2	162.8-175.7	13.91	-0.79	24.81	
	QFmv5H	5H	ABC482	173.5	169.7-177.8	4.77	1.22	20.64	
Pr	QPr2H	2H	ABG703b	6.7	0.0-7.7	3.92	1.80	13.73	
	QPr5Ha	5H	Adh6	43.5	32.4-44.2	3.27	0.69	13.86	
	QPr5Hb	5H	ABC482	173.0	169.3-187.2	4.00	0.23	18.28	
WSC	QWSC2H	2H	ABC165	167.1	154.9-175.4	3.73	-0.45	20.51	
	QWSC5Hb	5H	MEG813a	194.5	191.7-198.8	4.75	-0.21	19.15	
RWC	QRWC2H	2H	ABG703b	7.3	5.5-13.8	3.60	-1.38	14.92	
	QRWC5Ha	5H	ABC706	62.3	60.8-66.5	3.21	1.95	13.99	
	QRWC5Hb	5H	ABC302	78.2	73.8-86.2	3.91	-1.27	12.83	

^aQTL position expressed in cM, from origin of the linkage group (end of short arm). ^bPeak value of the LOD. ^cProportion of phenotypic variance explained by the QTL. ^dTotal phenotypic variance explained by the model. Ch: Chlorophyll content; Fv, Fo and Fm/Fv: Chlorophyll fluorescence parameters; Pr: Proline; WSC: Water-soluble carbohydrate; RWC: Relative water content.

Table 5. Quantitative trait loci (QTL) for 7 physiological traits identified by composite interval mapping for 72 Steptoe/Morex doubled haploid (DH) barley lines population in salt stress and non-stress conditions.

Trait	QTL name	Chr. name	Nearest marker	QTL pos.	*LOD score					Allelic effect					†R ²				
					0	5	10	15	0	5	10	15	0	5	10	15			
Ch	QCh1Ha	1H	BCD351C	62.3	-	-	-	4.27	-	-	-	-	0.30	-	-	-	-	-	15.28
	QCh1Hb	1H	Glb1	72.4	5.27	4.36	4.99	5.02	0.36	0.28	0.31	0.32	0.31	19.08	18.02	21.77	20.15	20.15	
	QCh2Ha	2H	Adh8	56.0	-	3.16	3.24	4.01	-	-0.19	-0.21	-0.27	-	-	12.89	13.09	14.15	14.15	
	QCh2Hb	2H	ABC165	167.1	3.26	3.56	3.36	4.09	-0.24	-0.18	-0.24	-0.25	-	11.99	15.63	14.23	12.17	12.17	
	QCh5Hb	5H	ABG496	166.6	-	3.71	3.28	3.31	-	-0.16	-0.32	-0.29	-	-	12.32	13.99	11.22	11.22	
	QCh7Ha	7H	iPgd1A	6.0	-	-	-	3.85	-	-	-	0.23	-	-	-	-	-	8.63	8.63
	QCh7Hb	7H	ABC310b	120.8	3.71	3.05	4.47	3.85	0.23	0.20	0.28	0.13	0.28	12.50	13.59	19.34	9.29	9.29	
Fo	QFo5Ha	5H	ABC706	62.3	3.26	3.47	3.71	3.91	-0.94	-1.3	-2.86	-2.1	13.58	13.16	12.89	16.51	16.51		
	QFo5Hb	5H	WG644	146.8	-	-	5.08	4.68	-	-	-4.60	-6.81	-	-	18.52	19.57	19.57		
Fv	QFo5Hc	5H	ABC482	173.0	3.08	-	-	-	-12.91	-	-	-	-	17.20	-	-	-	-	
	QFv2H	2H	ABC165	167.0	8.09	9.0	8.42	10.09	-23.49	-18.45	-20.70	-22.02	34.61	34.37	35.02	34.99	34.99		
	QFv3H	3H	MWG571B	87.9	-	5.73	7.22	6.88	-	-18.95	-14.9	-18.45	-	28.39	22.41	24.37	24.37		
Fm/Fv	QFv5H	5H	ABC482	173.0	-	-	3.58	4.87	-	-	-14.83	-18.95	-	-	12.80	15.39	15.39		
	QFmv1H	1H	Glb1	72.7	-	-	-	3.82	-	-	-	0.30	-	-	-	8.97	8.97		
	QFmv2H	2H	ABC165	167.1	-	3.23	5.67	3.63	-	-0.31	-0.56	-0.30	-	15.61	24.40	17.99	17.99		
Pr	QFmv5H	5H	ABC482	173.5	5.97	4.81	5.02	4.81	1.70	1.40	1.32	1.40	28.64	25.72	22.15	25.72	25.72		
	QPr1H	1H	ABR335	65.1	3.23	-	-	-	0.31	-	-	-	15.61	-	-	-	-		
	QPr2H	2H	ABG703b	6.7	3.87	4.45	3.89	3.6	1.08	2.20	1.60	1.38	12.67	19.53	13.13	14.92	14.92		
	QPr5Ha	5H	Adh6	43.5	-	3.60	3.42	3.54	-	0.37	0.45	0.47	-	9.91	14.07	16.69	16.69		
	QPr5Hb	5H	ABC482	173.0	4.49	3.39	-	-	0.31	0.41	-	-	15.61	16.84	-	-	-		
WSC	QWSC2H	2H	ABC165	167.1	13.81	15.97	14.41	13.56	1.16	1.47	1.37	1.38	20.97	28.64	18.79	29.63	29.63		
	QWSC5Ha	5H	Ubi2	50.2	-	-	-	3.56	-	-	-	-0.34	-	-	-	13.68	13.68		
	QWSC5Hb	5H	MWG813a	194.5	-	3.71	3.78	4.49	-	-0.25	-0.24	-0.24	-	16.83	17.51	19.02	19.02		
RWC	QRWC2H	2H	ABG703b	7.3	3.61	3.89	3.85	4.69	0.17	0.16	0.23	0.20	19.28	13.13	14.63	20.67	20.67		
	QRWC5Ha	5H	ABC706	62.3	4.27	3.65	5.67	3.91	-1.20	-1.02	-1.56	-1.02	12.60	15.34	24.40	19.51	19.51		

*QTL position expressed in cM; from origin of the linkage group (end of short arm). *Peak value of the LOD. †Proportion of phenotypic variance explained by the QTL. ‡Total phenotypic variance explained by the model.
Ch: Chlorophyll content; Fv, Fo and Fm/Fv: Chlorophyll fluorescence parameters; Pr: Proline; WSC: Water-Soluble carbohydrate; RWC: Relative water content.

Three QTLs affecting WSC were found on chromosomes 2H and 5H controlling approximately 20.97, 45.47, 36.30, 62.33 and 39.66% of the total variation in four salinity levels and their mean. *QWSC2H*, *QWSC5Ha* and *QWSC5Hb* QTLs were located near ABC165, Ubi2 and MWG813a markers, respectively. The major QTL, *QWSC2H* was coincident in four salinity levels and mean of them.

RWC mapped to a region of chromosome 2H and two regions of chromosome 5H near ABG703b, ABC706 and ABC302 markers, respectively. These QTLs (*QRWC2H*, *QRWC5Ha* and *QRWC5Hb*) accounted about 31.88, 28.47, 39.03, 40.18 and 41.74% of the total variation in four salinity levels and their mean. *QRWC2H* and *QRWC5Ha* were stable in four salinity levels and their mean.

Several QTL co-locations confirm the observed correlations among the traits. For example, in mean of four environments, *QCh2Hb*, *QFv2H*, *QFmv2H* and *QWSC2H* QTLs concurred in their genomic locations with each other. The positive correlation between chlorophyll content, Fv, Fm/Fv and WSC would come from these common QTLs with congruent effects. QTLs associated with proline and RWC (*QPr2H* and *QRWC2H*, respectively) were also co-localized. Other examples of co-locations were the QTL near the marker of ABC706 on chromosome 5H which affected Fo (*QFo5Ha*) and RWC (*QRWC5Ha*) or the QTL near the marker of ABC482 on chromosome 5H which affected Fv (*QFv5H*), Fm/Fv (*QFmv5H*) and proline (*QPr5Hb*). The negative correlation between these traits could come from those common QTLs with opposing effects. Thus, we may reach the breeding goal of decreasing one trait and increasing other trait simultaneously through selection for either of the traits. Han and Ullrich¹⁵ also reported several co-locations for different traits, for example, QTLs for kernel weight and grain protein were located in the same region on 2H. These co-locations could be either because of linkage between two genes or the pleiotropy effect of one gene. In the later case, the correlation between traits will never be broken. Pleiotropy controls the common sub-fraction of the traits, and therefore, would result in the concurrent increase or decrease of the correlated traits when we select for only one trait. The large effect of QTL near the ABC165 marker on chromosome 2H on chlorophyll content, Fv, Fm/Fv and WSC traits or the large effect of QTL near ABC482 marker on chromosome 5H on Fo, Fv, Fm/Fv and proline, raises an intriguing question about whether a single gene or a cluster of genes for salinity tolerance traits is located on these chromosomes. The answer to this question requires a detailed study using a more densely saturated map. Because, there is one difficulty that must be faced in attempting to discern the nature of QTL governing of physiological traits. The best confidence interval with which mapping can localize QTL, regardless of the size of the mapping population, is perhaps 10 cM²². With a mapping population constrained by the difficulties of phenotyping it may be 20-30 cM. Under the most hopeful conditions the best map location of a QTL is probably only to the nearest one hundred genes. This is an impossible large window in which to look for a gene without some clear idea of what it might be. All in all, this region of chromosome 2H and 5H is important for salt tolerance in barley, and thus the region may be used as an important target for improving salt tolerance of barley.

One of the major goals of QTL mapping is to select markers that linked to genes contributing to variation in the trait of interest. QTL consistency across different environments and background

is important component for MAS. Coincident QTLs provide relative stability of genetic control which would overcome the problem associated with the interaction of QTL by environment. In this study 29 QTLs were detected for physiological traits associated with salt tolerance and some of these QTLs appear to be quite stable between four salinity levels, therefore gain through marker-assisted selection (MAS) in this population would be unlimited. In previous studies the utility of marker-assisted selection for traits such as yield and quality in barley has been demonstrated^{1,48}.

We detected a number of variable QTLs for the same trait in different environment. Moreover, fewer QTLs were detected in lower salinity levels when compared with higher levels, suggesting the presence of QTL×environment interaction variation. Mano and Takeda³⁵ in mapping QTLs for salt tolerance stated that in the genetic background of Steptoe and Morex, salt tolerance at the seedling stage was controlled by a number of minor genes and the expression of these genes seems to be affected by the environment. In the case of salt tolerance the minimum physiological requirements are a balance between growth rate and net ion transport combined with sufficient intracellular compartmentation to permit the leaf cytoplasm to function effectively at the ionic concentration needed in the vacuole to provide balanced water relation. This must be achieved at an acceptable cost in osmolyte/osmoprotectant synthesis. If any one of these components fails, the whole system fails, certainly in an agricultural context. A comparable (and never simple) list of minimum requirements could be drawn up for saline and other environmental stresses. The number of structural genes that must contribute to a tolerant phenotype are evidently legion. This brings us back to ask if it is not surprising that such small number of QTL is often found in one condition other than another condition. The interaction of QTLs and environment were observed in many studies^{6, 29, 44}. Salinity affects the physiological trait values measured. Therefore, the magnitude of variations might be different in different salinity conditions, which causes the inconsistency of the QTL detection. Since environment plays a tremendous role in the phenotypic expression of traits associated with salinity tolerance, thus QTL analysis must be repeated in different environments, because a QTL may be expressed in one environment but not in another, or expressed strongly in one environment but weakly in another, and or expressed very differently and with opposite effects in different environments.

This study is one of few reports of QTL analysis in barley evaluated in Iran. We had focused on chlorophyll content, chlorophyll fluorescence (Fo, Fv, Fm/Fv), proline, WSC and RWC traits, which were supposed to be associated with salinity tolerance. The means of all DH lines were close to the mid-parental values for all traits. Although phenotypic distribution of DH population was normal, transgressive segregation was also observed in both directions for all traits. Significant variation and normal distribution of all traits measured in this study suggest the suitability of the DH population for QTL analysis. Despite, the small sample size used for technical reasons, a total of 29 significant QTLs were found for the 7 physiological traits. Some of detected QTLs appear to be quite stable between four salinity levels. Several QTL co-locations confirm the observed correlations among the traits. Although the detected regions need to be more precisely mapped, the information obtained should help in marker-assisted selection.

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