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QTL mapping for salt tolerance in barley at seedling growth stage

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Abstract

Barley (*Hordeum vulgare* L.), an important food and fodder crop, is potentially tolerant to salinity. To identify quantitative trait loci (QTLs) controlling salt tolerance, the population of 162 recombinant inbred lines (RILs) derived from F_8 generation of Arigashar (an extremely salt tolerant Iranian six-rowed barley landrace) crossed with Igri (a salt semi-sensitive two-rowed cultivar) were evaluated. The growth of shoots, roots, and coleoptiles, and root numbers are four important growth characteristics severely affected by salt stress at seedling growth stages. A linkage map was constructed using 106 AFLP and SSR markers spanning six barley chromosomes including 2(2H), 3(3H), 4(4H), 7(5H), 6(6H), and 1(7H). Out of totally 26 detected QTLs, 17 QTLs were found effective for salt tolerance at 250 and 350 mM NaCl which localized on chromosomes 2H, 3H, 4H, 6H, 7H, and linkage group L1, whereas considering equivalent overlapped QTLs with a pleiotropic effect led to detection of totally 9 distinctive QTLs (*QClgH2.1b, QSdgH2.1b, QSlgH2.1c, QNrgH2.1b, QTwgH2.2c, QSdg3Hb, QSlg4Hb1, QClg4Hb*, and *QSlg6Hc2*) effective for salinity tolerance. 2(2H), 4(4H), and 6(6H) were major chromosomes harboring QTLs which effectively controlled salt tolerance in the Igri×Arigashar population. An interesting QTL*, QTwg4Hc*, was localized on chromosome 4H in the *XE41-M61* marker distance that controls several traits including shoot and coleoptile lengths and shoot fresh mass under salt stress. A dense marker cluster around a resistance gene could offer a starting point for positional cloning.

Additional key words: AFLP, *Hordeum vulgare*, RILs, SSR.

Introduction

Soil salinity is one of the major environmental constraints limiting crop production in many parts of the world (Munns *et al.* 2006). Salt tolerance is a multigenic trait including salt partitioning within the plant, osmotic adjustment, and morphological changes (Munns 2005). Development and utilization of stress-resistant genotypes is an efficient approach to reduce a yield loss. Current strategies used to create more stress-tolerant crops include genetic engineering, QTLs mapping, and conventional breeding (Zhang *et al.* 1999a,b). Barley is an important crop species and it is an ideal material for genetic studies because of its relatively simple genetic background (Costa *et al.* 2001).

In barley, as in other cereals, the genome consists of a complex mixture of unique and repeated nucleotide sequences (Flavell 1980). Approximately 10 - 20 % of the barley genome is randomly arranged by repeated sequences, whereas 50 - 60 % of repeated sequences is interspersed among one another or among unique nucleotide sequences (Rimpau *et al.* 1980). The total genetic length of the barley maps ranges from 970 to 1873 cM, whereas the length of the most comprehensive

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Abbreviations: AFLP - amplified fragment length polymorphism; LOD - logarithm of the odds value; QTL - quantitative trait locus; PAGE - polyacrylamide gel electrophoresis; RIL – recombinant inbred line; SSR - simple sequence repeat.
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consensus map is 1060 cM. In the consensus map, the lengths of seven linkage groups range from 131 to 195 cM. One cM on the barley maps corresponds to approximately 1 000 - 5 000 bp (Pedersen and Linde-Laursen 1995).

QTLs for salt tolerance at a germination stage have been described for the Harrington×TR306 population on chromosomes 1H and 5H (Mano and Takeda 1997) and for the Steptoe×Morex population on chromosomes 4H, 5H, and 6H (Mano and Takeda 1997). At germination and an early seedling growth stage, three chromosome regions were found to correlate with salinity evaluation scores of the Oregon Wolfe barley (OWB) population including two regions on chromosome 5H and one region on chromosome 7H (Dadshani *et al.* 2004). In Steptoe×Morex barley at a seedling stage and at four salinity levels (0, 5, 10, and 15 dS m^{-1}), Siahsar and Narouei (2010) found overall 29 QTLs for chlorophyll content, chlorophyll fluorescence (F_0, F_v, F_m), proline, water-soluble sugars, and water content. Thirteen, 17, 18, and 22 QTLs were found at 0, 5, 10 and 15 dS m^{-1}, respectively. A phenotypic variance was between 8.63 and 44.69.

RILs population was developed from F_1 hybrids followed by repeated self polination to constitute a permanent mapping population. RILs population is very useful in plant breeding and genetic research (Burr *et al.* 1988) and has been extensively used to construct linkage maps (Simpson 1989), make an inheritance analysis of resistance (Groh *et al.* 1998, Mucella *et al.* 2000), screen QTLs for yield traits (Zhuang *et al.* 2002), and carry out a marker-assisted selection (MAS; Berloo and Stam 1998).

Salt tolerance at a seedling stage is important because an initial plant stage affects final production. In order to acquire some basic information about genes controlling salt tolerance at the seedling stage in barley, we used RILs mapping population of Arigashar×Igri with an amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) genetic markers in conjunction with a salt tolerance test. The objective of this study was to identify QTLs of salt tolerance in barley which may be used in salinity breeding programs.

Materials and methods

A set of 162 F_8 RILs population derived from a cross of *Hordeum vulgare* L. genotypes Igri×Arigashar by a single seed descent (SSD) was prepared in the Plant Improvement Institute, Karaj, Iran. Arigashar, Iranian six-rowed landrace barley, is extremely salinity tolerant, whereas Igri, two-rowed barley, is semi-sensitive to salinity.

The parents along with its RILs population were subjected to salt stresses at 0 (control), 250, and 350 mM NaCl using a randomized complete block design with four replications. Seeds (30) of each line were disinfected in a 1 % (m/v) sodium hypochlorite solution for 10 min followed by three times washing by distilled water. Then the seeds were placed between two layers of filter paper in glass beakers containing 100 cm^3 of water or NaCl solutions placed in an incubator at a temperature of 21 ± 1 °C in the dark. Desired traits were measured after 8 d. Measured traits were shoot (SL), root (RL), and coleoptile (CL) lengths, a root number (RN), a germination percentage (GP), shoot (SFM) and root (RFM) fresh masses, shoot (SDM) and root (RDM) dry masses (after drying at 70 °C until a constant mass), and a total fresh mass (TFM). A salt tolerance index (STI) was calculated for all the traits of each cultivar using an equation STI = Ys/Yc, where Ys is the mean of the cultivar trait under the salt stress and Yc is the mean of cultivar trait under the control conditions (Goudarzi and Pakniyat 2008).

DNA of the RILs population and of the two parents was extracted from leaves of 10 to 14-d-old seedlings grown in a greenhouse. The DNA extraction was conducted using the cetyltrimethylammonium bromide (CTAB) method described in detail by Saghai-Maroof *et al*. (1984). In total, 100 pairs of SSR primers and 25 pairs of AFLP primer combinations were used to determine the parental genotype.

The AFLP analysis of RILs population was performed according to Vos *et al.* (1995) with a minor modification using restriction enzymes *Mse*I and *Eco*RI. The restricted fragments were first amplified with primers each having one selective nucleotide on the 3'-end. Then, the diluted pre-amplified PCR products were used as template for the second amplification using primers containing three selective nucleotides. *Eco*RI selective primers were 5′-end labeled with either a *TAMRA* or *HEX* fluorescent dye. Following the amplification, reaction products were mixed with 2 mm^3 of a formamide dye (98 %, m/m, formamide, 10 mM EDTA and 0.02 %, m/v, bromophenol blue). After denaturation in a thermocycler (*PeqSTAR*, Erlangen, Germany) at 90 °C for 3 min, 2 mm^3 of each sample was loaded on a 5 % (m/v) denaturing polyacrylamide gel in a 38 × 20 cm electrophoresis (PAGE) by using a *Gel-Scan 2000* DNA fragment analyzer (*Corbett Research*, Sydney, Australia). Mapping data were obtained by visual scoring images. AFLP polymorphic bands were scored as present (1) or absent (0), and unreliable ambiguous bands were scored as missing data (−).

Microsatellite primers described previously (Becker *et al*. 1995, Liu and Somerville 1996, Russell *et al.* 1997) were used. The sequences of these primers are available (http://wheat.pw.usda.gov/cgi-bin/graingenes). For determination of annealing temperature, the oligonucleotide properties calculator software (*Oligocalc v 3.26*) was used

(http://www.basic.northwestern.edu/biotools/OligoCalc.html).

The PCR products were separated on 5 % non-denaturing PAGE (18×20 cm) using the *Gel-Scan 2000* DNA fragment analyzer. A *SYBR gold* dye (*Invitrogen*, Burlington, Canada) was used for detection of PCR products on the gel. To map data, the bands were scored as As, Bs, and Hs representing Arigashar, Igri, and heterozygotes, respectively.

Map distances were estimated based on the Kosambi's map function (Kosambi 1944). For mapping QTLs for salt tolerance, the method of composite interval mapping (CIM) on *Winqtl Cart v. 2.5* (Wang *et al.* 2005) was used and *Model 6* was adopted. A control marker number and a window size were 5 and 10 cM, respectively. A walk speed of 2 cM and a forward regression method were selected. LOD score peaks greater than 2.5 indicated the existence of QTL for all traits reported in this study. The phenotypic variation explained by a QTL (R2) conditioned by CIM cofactors included in the model was calculated at the most likely QTL position. The additive effect of an allelic substitution and of a marker with the greatest effect at each QTL was also obtained. In the final step, the chromosomes and groups of linkage were constructed; as well as, confidence intervals of QTL, position and distance between the QTL were determined.

The analysis of variance (*ANOVA*) and means comparison were performed using the *SAS v. 9.1* software. The *SPSS v. 17.0* software was applied for the *t*-test. The linkage map was constructed by the *Join Map v. 3.0* software (Van Ooijen and Voorips 2001). QTL mapping was conducted using *Winqtl Cart v. 2.5* (Wang *et al.* 2005). The linkage groups and related QTLs were drawn by *Map Chart v. 2.1* (Voorrips 2002).

Results

In the current study, Arigashar was superior to Igri for all studied traits at the seedling stage (Table 1). Significant differences and a transgressive segregation were observed in the RILs population for all the traits (data not shown). This finding indicates that the RILs population was suitable for QTL analysis. Germination and an early seedling growth in response to the different salinity differed significantly among the RILs population and related parents. The *ANOVA* analysis was performed at 0, 250, and 350 mM NaCl for the RILs population and related parents (Tables 2 and 3). Almost all lines germinated at the salinity of 250 mM NaCl, whereas the germination rate severely decreased at 350 mM NaCl. STI was different at various salinities and different studied traits. When STI was closer to 1, the respected line was categorized as salt tolerant. Also, there were differences in STIs between parents, and Igri had the lowest STI for all the traits.

Table 1. Salt tolerance indices (STIs) were calculated for parameters measured on Arigashar and Igri as parents of the RILs population (** - significant at the $P < 0.01$, $n = 4$).

Salinity	Parameters	Arigashar	Igri
250 mM	shoot length	0.282	0.156**
	coleoptile length	0.789	0.454**
	root number	0.896	0.752**
	root length	0.605	0.393**
	germination percentage	0.957	0.802**
	root fresh mass	0.353	0.225**
	shoot fresh mass	0.369	0.172**
	total fresh mass	0.466	0.338**
	root dry mass	0.490	0.297**
	shoot dry mass	0.597	0.302**
350 mM	shoot length	0.056	0.011**
	coleoptile length	0.178	0.061**
	root number	0.744	0.381**
	root length	0.234	0.087**
	germination percentage	0.752	0.416**
	root fresh mass	0.115	0.026**
	shoot fresh mass	0.037	0.004**
	total fresh mass	0.227	0.103**
	root dry mass	0.210	0.046**
	shoot dry mass	0.107	0.010**

As concerns AFLP results, totally 990 bands were evaluated ranging from 100 to 500 bp, whereas 242 bands were polymorphic between the two parents. Maximum and minimum numbers of scored bands were observed in E37-M61 and E36-M47 primer combinations, respectively. The highest and lowest numbers of polymorphic bands were detected in E41-M61 and E36-M47 primer combinations, respectively. There was not any direct correlation between total numbers of bands and the amount of polymorphism in the parents and related RILs population.

One of 100 SSR primer pairs was pre-screened in the parents, only 21 SSR primers (21 %) generated polymorphic bands among the parents and related RILs population including *XEbmac0415, XHvhva1, XHvwaxy4, XGms061, XHvm40, XBmac0209, XBmac31, XBmag0135, XEbmac0684, XBmag120, XHvm54, XGbm1323, XGbm1413, XHvcma, XGbm1464, XBmac0156, XHvltppb, XBmag125, XGbm1419, XBmag138*, and *XBmag382*. A marker information on 14 SSR primers was successfully integrated into a constructed linkage map and remaining 7 SSR primers produced an ignorable short linkage group. Fragment sizes of the SSR markers ranged from 100 to 500 bp. Regarding polymorphic bands per gel and primer

Table 2. The analysis of variance for different parameters in parents at 0, 250, and 350 mM NaCl. DF - degree of freedom, SL - shoot length, CL - coleoptile length, RN - root number, RL - root length, GP - germination percentage, RFM - root fresh mass, SFM - shoot fresh mass, TFM - total fresh mass, RDM - root dry mass, SDM - shoot dry mass (* - significant at $P < 0.05$, ** - significant at $P < 0.01$, ^{ns} - non- significant).

Source of variation	DF	SL	CL	RN	RL	GP	RFM	SFM	TFM	RDM	SDM
Block	3	4.04^{ns}	3.44^{*}	2.32^{*}	14.28^{*}	500.58^{**}	1.23^{*}	1.41^{*}	7.80^{*}	0.004^{*}	0.010^{*}
Salinity	2	257.30^{**}	71.54^{**}	34.29^{*}	330.2^{**}	13541.1^{**}	22.05^{**}	27.75^{**}	143.20^{**}	0.030^{**}	0.080^{**}
Parents	1	12.84^{*}	15.24^{**}	1.80^{ns}	62.85^{**}	1996.2^{**}	2.72^{*}	4.13^{**}	22.99^{**}	0.002^{ns}	0.007^{ns}
Parents×salinity	2	2.69^{ns}	3.90^{*}	0.23^{ns}	10.74^{*}	232.9^{ns}	0.61^{ns}	0.98^{ns}	4.35^{ns}	0.0004^{ns}	0.002^{ns}
Error	15	2.33	0.81	0.50	2.84	131.1	0.35	0.37	1.90	0.001	0.002

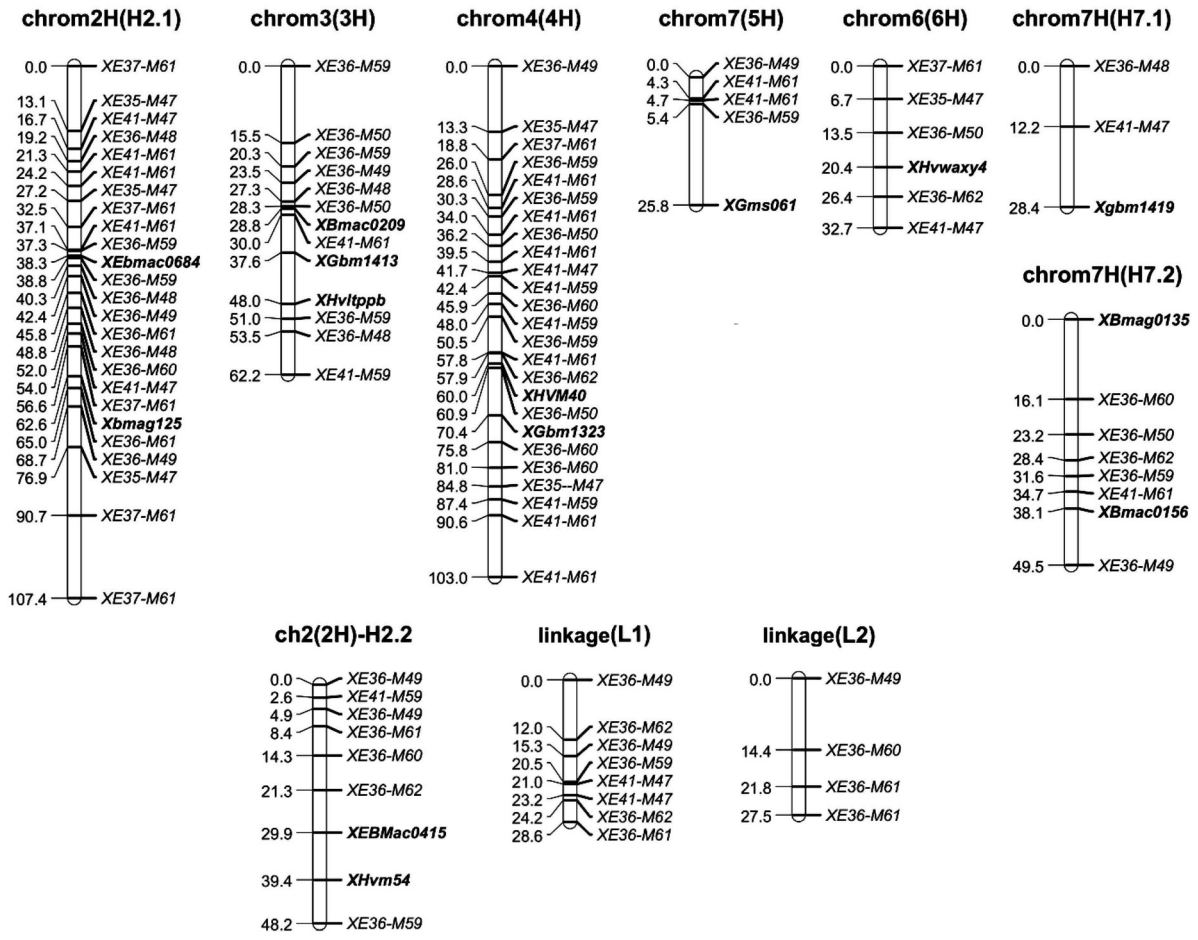

Fig. 1. A linkage map of 6 barley chromosomes including 2(2H) [2H(H2.1), (2H)H2.2], 3(3H), 4(4H), 7(5H), 6(6H), 1(7H) [7H(H7.1), 7H(H7.2)] and two unknown linkage groups (L1 and L2) comprising 14 SSR markers and 92 AFLP loci on an F_8 population developed from an F_1 cross Igri×Arigashar. Scales in cM are shown on the *left* to indicate relative distances among markers. Marker designations are given on the *right side* of each chromosome. SSR markers are shown *in bold*.

combinations, the results show that the SSR primers generated a much lower polymorphism and few numbers of polymorphic bands in comparison to the AFLP technique. However, scoring and analyzing AFLP bands were much more difficult and complicated. Similar finding was seen before by Ridout and Donini (1999).

A genetic linkage map of barley containing 10 separate linkage groups comprising 513.3 cM of the barley genome included 6 chromosomes - 2(2H), 3(3H), 4(4H), 7(5H), 6(6H), 1(7H), and 2 unknown linkage groups (L1 and L2) (Fig. 1). Fourteen SSR and 80 AFLP markers were localized on 6 chromosomes, and 12 AFLP markers were mapped on the 2 unknown linkage groups. The chromosome segments of H2.1 and

H7.1 had gap map with H2.2 and H7.2, respectively, that were separately identified. An average distance between two adjacent markers was 4.84 cM, and the largest distance was 20.4 cM. Dense clusters of markers were observed on chromosomes 2H, 3H, and 4H. A map containing 106 DNA markers was used for QTLs mapping.

The constructed Arigashar×Igri linkage map was used for mapping important quantitative traits related to salinity tolerance. However, QTLs were mapped over all chromosomes, but some interesting clusters of QTLs were co-localized in certain chromosomal regions as well. QTLs affecting several traits are common (Hayes *et al.* 1997) and may be due to either a pleiotropic effect

Table 3. The analysis of variance for different parameters in parents and RILS grown under 250 mM NaCl. CV - coefficient of variation. For other abbreviations see Table 2.

S.O.V.	DF	SL	CL	RN	RL	GP	RFM	SFM	TFM	RDM	SDM
Block	1	$0.0002^{n.s}$	$0.04^{n.s}$	$014^{n.s}$	$0.46^{n.s}$	$0.04^{n.s}$	0.04^{*}	$0.02^{n.s}$	$0.02^{n.s}$	$0.00001^{n.s}$	$0.0007^{n.s}$
Cultivars	163	0.261^{**}	0.34^{**}	1.46^{**}	8.15^{**}	2.79^{**}	0.13^{**}	0.18^{**}	0.23^{**}	0.006^{**}	0.017^{**}
Error	163	0.01	0.01	0.07	0.42	0.15	0.08	0.08	0.07	0.0001	0.001
CV [%]		7.72	6.76	10.06	11.8	4.17	12.11	11.67	14.53	17.23	13.65

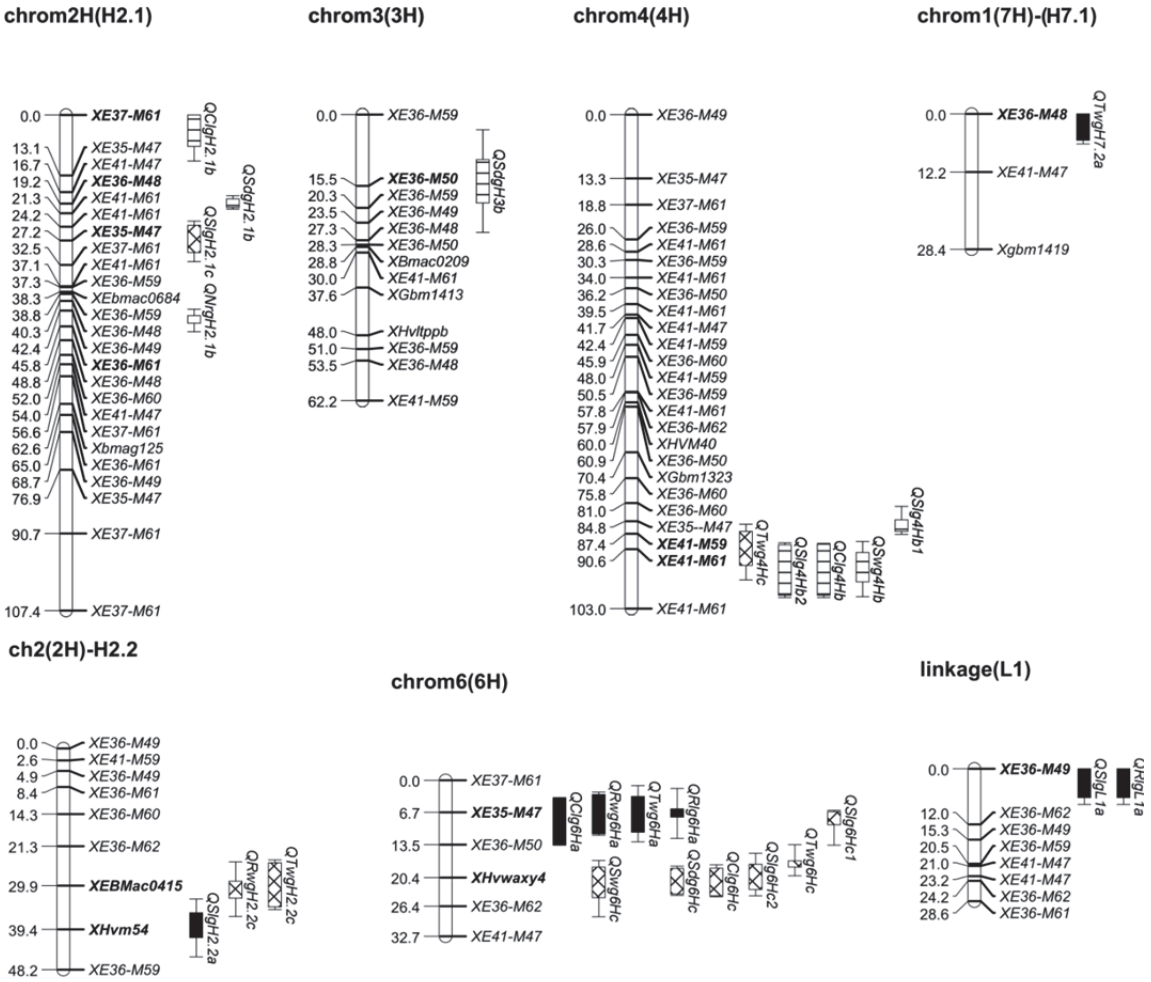

Fig. 2. Positions of different QTLs at 0, 250, and 350 mM NaCl for all studied traits. QTLs locations are indicated with *bars* to the *right* of the significant markers. The markers closest to QTLs are shown *in bold*. QTLs names are based on Q + the trait measured + the level of salinity + the linkage group + the name of university or research center. For example, QRlg6Ha means QTL for root length at 0 mM NaCl on chromosome 6H determined in the research center of Gorgan University of Agricultural Sciences and Natural Resources. ■ - 0 mM NaCl, ▤ - 250 mM NaCl, ▨ - 350 mM NaCl.

and a close linkage. Many of these QTLs overlapped and also controlled different traits. An interesting QTL, *QTwg4Hc*, localized on chromosome 4H in the *XE41-M61* marker distance was observed as a common QTL for several traits including shoot and coleoptile lengths and shoot fresh mass. Clustering QTLs was observed especially on chromosomes 2H (piece H2.2), 4H, and 6H, and on unknown linkage group L1. Clustering QTLs was observed especially on chromosomes 2H (piece H2.2), 4H, and 6H, and on unknown linkage group L1. According to the genetic map of found QTLs, the logarithm of odds (LOD) scores ranged from 2.5 to 4.5 and

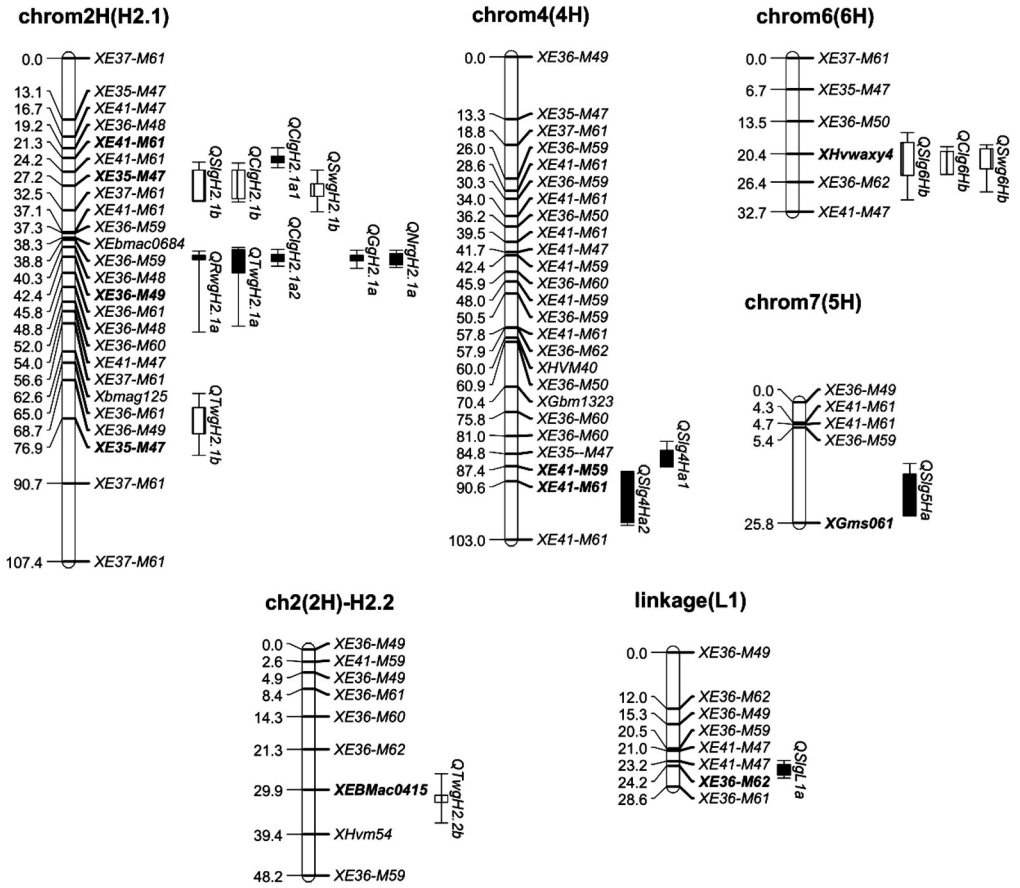

Fig. 3. The salt tolerance QTL mapped in the RILs population of Arigashar×Igri. - Salt tolerance index (STI) at 250 mM NaCl), - salt tolerance index (STI) at 350 mM NaCl.

R^2 from 6 to 17 %. Out of 26 QTLs detected, around 17 QTLs were found only at 250 and 350 mM NaCl on chromosomes 2H, 3H, 4H, 6H, and 7H, and linkage group L1. As overlapped QTLs are equivalent, finally nine QTLs (*QClgH2.1b, QSdgH2.1b, QSlgH2.1c, QNrgH2.1b, QTwgH2.2c, QSdg3Hb, QSlg4Hb1, QClg4Hb*, and *QSlg6Hc2*) were detected for salinity tolerance. The marker distance and position of different QTLs at 0, 250, and 350 mM NaCl for all the traits are shown in Fig. 2. The chromosomal location, QTL name, marker with the greatest effect, peak position, confidence interval, LOD score, additive effect, and variation percentage accounted for the salt tolerance QTLs in the three salinity levels are shown in Table 1 Suppl. For example, *XHVWAXY4F* and *XE35-M47* markers on chromosome 6(6H) had the greatest effect.

STI was calculated and the QTL analysis was performed at 250 and 350 mM NaCl. Eighteen QTLs were mapped on four chromosomes (2H, 4H, 6H, and 5H) and one unknown linkage group (L1) (Fig. 3). Remarkably, there were QTLs affecting several traits, which is consistent with results obtained by Hayes *et al.* (1997). Finally, marking the overlapped QTLs as equivalent, 10 QTLs were considered as specific QTLs (*QClgH2.1a1, QSlgH2.1b, QNrgH2.1a, QTwgH2.1a, QTwgH2.2a, QSlg4Ha1, QSlg4Ha2, QSlg6Hb, QSlg5Ha*, and *QSlgL1a*) that could control salt tolerance. The detailed information on QTLs related to STI at 250 and 350 mM NaCl is shown in Table 2 Suppl. and Fig. 3. QTL mapping based on STI shows that there was a common QTL on chromosome 2H at a marker distance, *XEBMac0415-XHvm54*, for the coleoptile length, root number, germination, RFM, and TFM. This finding shows that it was a major QTL with a pleiotropic effect

that controls the most stable salt tolerance mechanisms. The most effective QTL for the shoot length at 350 mM NaCl (*QSlg6Hc2*) was found on chromosome 6(6H) (LOD = 4.5). The most effective QTL for the shoot length using STI at 350 mM NaCl (*QSlg6Hb*) was also found on chromo-some 6(6H) (LOD = 4.5). The most effective QTL for SFM at 350 mM NaCl (*QSwg6Hc*) was found on chromosome 6(6H) (LOD = 4.1) as well as the most effective QTL for SFM at 350 mM NaCl using STI (*QSwg6Hb*) (LOD = 3.2). In conclusion, these QTLs (*QSlg6Hc2, QSlg6Hb, QSwg6Hc*, and *QSwg6Hb*) were co-localized and could be a single gene with a pleiotropic effect.

Discussion

In the current study, a large number of polymorphic markers have been obtained by the AFLP technique. However, obligating at least one SSR marker into each linkage group is critical for a linkage map construction, but AFLP could be more useful to saturate a linkage map due to a high number and reproducibility of a polymorphic banding pattern. Therefore, the arrangement of applying SSR along with AFLP markers can be one of the best strategies for a rapid and cost effective identification of QTLs responsible for quantitative traits in various mapping populations. Totally, around 513 cM of the barley genome was identified, which covered 55 % of the barley entire linkage map. In this study, two uncharacterized linkage groups were mapped, which probably are unidentified parts of barley chromosomes. Further work is ongoing to identify uncharacterized groups by using more SSR markers. QTLs mapping based on STI shows that 10 QTLs could be considered as specific loci for salt tolerance. One of the major goals of QTLs mapping should be selecting molecular markers that link to genes contributing to a genetic variation in the trait of interest. From this point of view, around eight AFLP and SSR markers including *XE41-M61, XE35-M47, XE36-M49, XEBMAC0415, XE41-M59, XHVWAXY4F, XGMS061*, and *XE36-M62* were co-localized near to salt tolerance QTLs. The result shows that QTLs found on chromosomes 2H, 4H, and 6H described the majority of thephenotypic variation caused by the salinity stress. Therefore, in this study, chromosomes 2(2H), 4(4H), and 6(6H) in the Igri×Arigashar population were major chromosomes harboring QTLs that effectively controlled salt tolerance. This finding is in agreement with an earlier study conducted by Mano and Takeda (1997). However, QTLs for salt tolerance at germination have been described for a Harrington ×TR306 population on chromosomes 1H and 5H and for a Steptoe×Morex population on chromosomes 4H, 5H, and 6H (Mano and Takeda 1997). Flowers *et al.* (2000) reviewed number of reports about QTLs conferring salinity tolerance with various chromosome locations in different mapping populations. The QTLs position on the consensus map of barley suggests that a number and location of identified salt tolerance QTLs will possibly vary depending on different NaCl concentrations and barley genotypic backgrounds (Zaare and Jafary 2013). Associations between QTLs mapping and AFLP polymorphism were applied for localization of QTLs controling a wide range of agronomic traits in barley (Hayes *et al.* 1997).

The result show that the shoot, root, and coleoptile lengths plus the root number were four important growth characteristics that were severely affected by the salt stress at the seedling growth stages of barley and can be selected as reliable characters for QTLs mapping studies. Our data for these traits show that a total of nine QTLs were involved in salt tolerance at 250 and 350 mM NaCl. For example, two QTLs (*QSlg4Hb1* and *QSlg4Hb2*) at 250 mM NaCl were detected for the shoot length and were located on chromosome 4(4H). Three QTLs were reported by Zaare and Jafary (2013) in a L94×Vada population under 200 and 300 mM NaCl at early growth stages for shoot and root lengths; these QTLs are located close to each other on chromosome 4(4H). In this study, consistent with earlier barley studies (Hayes *et al.* 1997, Tinker and Mather 1994), clustering QTLs was detected. Siahsar and Narouei (2010) detected several co-localized QTLs for physiological traits associated with salt tolerance in a Steptoe×Morex mapping population in barley. Also, Peighambari *et al.* (2005) detected several co-localized QTLs for different traits at drought stress conditions in barley**.** The coupled effects detected might be either due to a close linkage or due to pleiotropic effects of a single gene. This finding shows that it is a major QTL with a pleiotropic effect that controls the most stable salt tolerance mechanisms. A future research needs to be navigated to reveal the number and set of genes which play an important role at this locus for salt tolerance. In contrast to Mano and Takeda (1997) who found QTLs on chromosome 5HL, in the current study, QTLs responsible for salt tolerance were found on chromosomes 2H (H2.1 and H2.2), 3H, 4H, 5H, 6H, and7H),and linkage group L1. The objective of this work was to identify and map loci conferring salt tolerance in barley RILs population. The transgressive segregation led to the production of some lines in the RILs population which is superior to Arigashar not only for salt stress tolerance but also for other agronomical traits

References

Becker, J., Vos, P., Kuiper, M., Salamini, F., Heun, M.: Combined mapping of AFLP and RFLP markers in barley. - Mol. gen. Genet. **249**: 65-73, 1995.

Berloo, R.V., Stam, P.: Marker-assisted selection in autogamous RIL populations: a simulation study. - Theor. appl. Genet. **96**: 147-154, 1998.

Burr, B., Burr, F.A., Thompson, K.H., Albertson, M.C., Stuber, C.W.: Gene mapping with recombinant inbred in maize. - Genetics **118**: 519-526, 1988.

Costa, J.M., Corey, A., Hayes, P.M., Jobet, C., Kleinhofs, A., Kopisch-Obusch, A., Kramer, S.F., Kudrna, D., Li, M., Riera-lazarazu, O., Sato, K., Szucs, P., Toojinda, T., Vales, M.I.,Wolfe, R.I.: Molecular mapping of the Oregon Wolfe barleys: a phenotypically polymorphic double-haploid population. - Theor. appl. Genet. **103**: 415-424, 2001.

Dadshani, S.A.W., Weidner, A., Buck-Sorlin, G.H., Börner, A., Asch, F.: QTL Analysis for Salt Tolerance in Barley. - Deutscher Tropenteg, Berlin 2004.

Flavell, R.: The molecular characterization and organization of plant chromosomal DNA sequences. - Annu. Rev. Plant Physiol. **31**: 569 -596, 1980.

Flowers, T.J., Koyama, M,L., Flowers, S.A., Sudhakar, C., Singh, K.P., Yeo, A.R.: QTL: their place in engineering tolerance of rice to salinity. - J. exp. Bot. **51**: 99-106, 2000.

Goudarzi, M., Pakniyat, H.: Evaluation of wheat cultivars under salinity stress based on some agronomic and physiological traits. - J. Agr. Soc. Sci. **4**: 35-38, 2008.

Groh, S., González-de-León, D., Khairallah, M.M., Jiang, C., Bergvinson, D., Bohn, M., Hoisington, D.A., Melchinger, A.E.: QTL mapping in tropical maize: III. Genomic regions for resistance to *diatraea* spp. and associated traits in two RIL populations. - Crop Sci. **38**: 1062-1072, 1998.

Hayes, P.M., Cereno, J., Witsenhoer, H., Kuiper, M., Zabeau, M., Sato, K., Kleinhofs, A., Kudrna, D., Kilian, A., Saghai-Maroof, M.A., Hoffman, D.L.: NABGMP characterizing and exploiting genetic diversity and quantitative traits in barley (*Hordeum vulgare*) using AFLP markers. - J. agr. Genomics **3**: 1-18, 1997.

Kosambi, D.D.: The estimation of map distances from recombination values. - Ann. Eugenics **12**: 172-175, 1944.

Liu, K., Somerville, S.: Cloning and characterization of a highly repeated DNA sequence in *Hordeum vulgare* L. - Genome **39**: 1159-1168, 1996.

Mano, Y., Takeda, K.: Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). - Euphytica **94**: 263-272, 1997.

Mucella, T., Dipak, K.S., Walter, J.K., Fred, J.M.: *Ascochyta* blight resistance inheritance in three chickpea recombinant inbred line populations. - Crop Sci. **40**: 1251-1256, 2000.

Munns, R.: Genes and salt tolerance: bringing them together. - New Phytol. **167**: 645-663, 2005.

Munns, R., James, A.J., Laüchli,A.: Approaches to increasing the salt tolerance of wheat and other cereals. - J. exp. Bot. **57**: 1025-1043, 2006.

Pedersen, C., Linde-Laursen, I.: The relationship between physical and genetic distances at the Hor1 and Hor2 loci of barley estimated by two-colour fluorescent *in situ* hybridization. - Theor. appl. Genet. **91**: 941-946, 1995.

Peighambari, S.A., Yazdi-Samadi, B., Nabipour, A., Charmet, G., Charmet, A.: QTL analysis for agronomic traits in a barley doubled haploids population grown in Iran. - Plant. Sci. **169**: 1008-1013, 2005.

Ridout, C.J., Donini, P.: Use of AFLP in cereal research. - Trends. Plant Sci. **4**: 76-79, 1999.

Rimpau, I., Smith, D.B., Flavell, R.B.: Sequence organization in barley and oats chromosomes revealed by interspecies DNA/DNA hybridization. - Heredity **44**: 131-149, 1980.

Russell, J.R., Fuller, J.D., Macaulay, M., Hatz, B.G., Jahoor, A., Powell, W., Waugh, R.: Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. - Theor. appl. Genet. **95**: 714-722, 1997.

Saghai-Maroof, M.A., Soliman, K.M., Gorgensen, R.A., Allard, R.W.: Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. - Proc. nat. Acad. Sci. USA **81**: 8014-8018, 1984.

Siahsar, B.A., Narouei, M.: Mapping QTLs of physiological traits associated with salt tolerance in Steptoe×Morex doubled haploid lines of barley at seedling stage. - J. Food Agr. Environ. **8**: 751-759, 2010.

Simpson, S.P.: Detection of linkage between quantitative trait loci and restriction fragment length polymorphisms using inbred lines. - Theor. appl. Genet. **77**: 815-819, 1989.

Tinker, N.A., Mather, D.E.: Main effects of quantitative trait loci in Harrington/TR306 two-row barley. - Barley Genet. Newslett. **23**: 72-78, 1994.

VanOoijen, J.W., Voorips, R.E. (ed.): JoinMap 3.0, Software for the Calculation of Genetic Linkage Maps. - Plant Research International, Wangeningen 2001.

Voorrips, R.E.: MapChart: software for the graphical presentation of linkage maps and QTLs. - J. Heredity **93**: 77-78, 2002.

Vos, P., Hgers, R., Bleeker, M., Reijams, M.: AFLP: a new technique for DNA fingerprinting. - Nucl. Acids Res. **23**: 4407-4414, 1995.

Wang, S., Basten, C.J., Zeng, Z.B.: Windows QTL Cartographer 2.5. - Department of Statistics, NCSU, Raleigh 2005.

Zaare, M., Jafary, H.: Quantitative trait loci diversity for salt tolerance at the early growth stage of barley (*Hordeum vulgare L.*). - Crop Breed. J. **3**: 69-77, 2013.

Zhang, J., Nguyen, H.T., Blum, A.: Genetic analysis of osmotic adjustment in crop plants. - J. exp. Bot. **50**: 291-302, 1999a.

Zhang, J., Zheng, H.G., Ali, M.L., Tripathy, J.N., Aarti, A., Pathan, M.S., Sarial, A.K., Robin, S., Nguyen, T.T., Babu, R.C., Nguyen, B.D., Sarkarung, S., Blum, A., Nguyen, H.T.: Progress on the molecular mapping of osmotic adjustment and root traits. - In: O'Toole, J.C., Ito, O., Hardy, B. (ed.): Genetic Improvement of Rice for Water-Limited Environments. International Rice Research Institute, Manila 1999b.

Zhuang, J.Y., Fan, Y.Y., Rao, Z.M., Wu, J.L., Xia, Y.W., Zheng, K.L.: Analysis on additive effects and additive-by-additive epistatic effects of QTL for yield traits in a recombinant inbred line population of rice. - Theor. appl. Genet. **105**: 1137-1145, 2002.