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Determination of QTLs Associated with Agronomic and Physiological Traits under Normal and Salinity Conditions in Barley

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Abstract
Salinity is one of the major abiotic stresses that severely limit barley production worldwide. In the current research, for mapping the QTLs of agronomic and physiological traits, 149 double haploid (DH) lines from a cross between an Australian cultivar, Clipper (salt susceptible), and an Algerian landrace, Sahara3771 (salt tolerant), were evaluated under natural saline (Yazd Station, EC_{soil}=10-12.8 ds/m and EC_{water}= 9-10 ds/m) and normal (Karaj Station, EC_{soil} and EC_{water} ~2-2.5 ds/m) environments. There were remarkable differences between parents and among the lines for studied traits, including days to heading, relative water content, chlorophyll content, plant height, spike length, days to maturity, biomass, grain yield, harvest index, grain number per spike, 1000 grain weight, Na^+ and K^+ contents and K^+/Na^+ ratio. QTL analysis was performed using the genetic linkage map consisted of 517 molecular markers distributed evenly on all seven barley chromosomes spanning 1502 cM of barley genome based on composite interval mapping method. A total of 72 QTLs for the measured traits were determined, from which 40 QTLs were under normal and 32 QTLs were under salinity stress conditions. The phenotypic variation explained by individual QTLs ranged from 2.7 to 61.8%. A major QTL related to biomass, grain number per spike, grain yield, plant height and 1000 grain weight was identified on chromosome 2H in the vicinity of *Vrs1* marker locus. In addition, for plant height, biomass, grain number per spike and 1000 grain weight, some stable QTL(s) under both salinity and normal conditions were identified on that locus which considered as salinity related QTLs. These QTLs can be useful in breeding programs for improving salt tolerance using marker-assisted selection.

Keywords: Agronomic traits; Barley; QTLs; Salinity

Introduction

Salinity is a significant problem that affects agriculture worldwide, resulting in substantial losses in crop yield. More than 800 million hectares of land throughout the world are salt affected (FAO 2008). Despite the improvement in the plant productivity and resistance to a number of pests and diseases, advancement in the salt tolerant crop plants remains elusive, because of the fact that salt tolerance, genetically and physiologically, is a complex inherited trait and it is likely that several QTLs but also several different mechanisms are involved (Eleuch *et al.* 2008).

Many researchers believe that barley is the most salinity tolerant among the cereals (Ceccarelli *et al.* 1987; Munns and Tester, 2008). However, its growth and production is greatly affected by salt stress. Salinity often affects barley during the vegetative growing stage and at flowering stage (Nguyen *et al.* 2013). Identifying the genes or quantitative trait loci whose expression enables plants to tolerate salt stress is essential for breeding

programs, but there is not enough information about the location and inheritance of genes or QTLs that are responsible for salt tolerance (Eleuch *et al.* 2008). Salinity tolerance is regarded as a complex character that is governed by quantitative trait loci (QTLs). QTL analysis is a methodology that combines DNA marker and traits phenotypic data to locate and characterize genes that influence quantitative traits and has been utilized for dissection of different traits in barley (Kleinhof *et al.* 1993; Mohammadi *et al.* 2005; Shahinnia *et al.* 2006; Hearnden *et al.* 2007*;* Nguyen *et al.* 2013). Many QTLs have been reported for salinity tolerance in barley (Jana *et al.* 1980; Shavrukov *et al.* 2010; Rivandi *et al.* 2011; Zhou *et al.* 2012). Mano and Takeda (1997) found QTLs for salt tolerance at germination stage on chromosomes 1H, 4H, 5H and 6H and at seedling stage on chromosomes 1H, 2H, 5H and 6H. Zhou *et al* (2012) used 172 doubled-haploid lines from cross between YYXT (salinity-tolerant genotype) and Franklin (salinity-sensitive genotype) and identified five QTLs for salinity tolerance on chromosomes 1H, 2H, 5H, 6H and 7H. Shahraki *et al.* (2013) evaluated 72 F1-derived doubled haploid lines from the cross between Stetoe×Morex for some phonological traits under normal and salinity stress conditions and identified 53 QTLs controlling different traits. Thomas *et al.* (1998) identified QTLs for physiological traits associated with salinity tolerance on chromosomes 1H, 4H, 5H and 6H. Nguyen *et al.* (2013) used the Steptoe×Morex barley doubled haploid population to screen for genetic variation in response to salinity stress at early development stage, focusing on ion homeostasis. They identified 11 chromosomal regions involved in the control of the variation observed for salt tolerance and various salt-stress response traits, including Na^+, CL^- and K^+ contents in shoots. They found two specific regions on chromosomes 2H and 3H, related to ion content and salt tolerance.

The aim of this program was to identify QTLs in relation to several agronomic and physiological characters and their effects and location under normal and natural salt stress environments in a barley DH population.

Materials and Methods

In this study a barley population consisted of 149 doubled haploid lines together with their parents were evaluated by conducting an α-lattice design with two replications in two growing seasons (2012-2013 and 2013-2014) at two locations, Yazd Agricultural Research Station (salinity stress environment: Ec_{soil}= 10-12.8ds/m, Ec_{water}= 9-10ds/m) and research station of Cereal Department, SPII, Karaj (normal environment: soil and water EC: ~2-2.5 ds/m), Iran. To obtain the doubled haploid lines a cross was made between Sahara3771 (Algerian salt tolerant winter landrace) and Clipper (Australian salt sensitive spring cultivar) in the University of Adelaide, Australia. During the growing season, following characters were measured: grain yield (GY), spike length (SL), plant height (PH), biomass (BY), grain number per spike (GS), 1000 kernel weight (TKW), harvest index (HI), days to heading (DHA), days to maturity (DMA), relative water content (RWC), chlorophyll content (SPAD), Na^+ and K^+ contents and K^+/Na^+ ratio. The following

formula was used to determine the relative water content:

$$RWC = \left(\frac{f_w - d_w}{t_w - d_w}\right) \times 100$$

where, f_w, d_w and t_w are leaf fresh, dry weight and turgid weight, respectively (Fitter and Hay 1987). Chlorophyll content was measured by a SPAD-502 chlorophyll-photometer on three fresh leaves, on three points(first, middle, end) (Munns and James 2003). Due to large number of experimental units, Na^+ and K^+ were only measured in one replication in each year using a flame photometer. Separate combined analyses of variance were used for each environment. Linear correlation coefficients between the characters under study were also calculated separately.

The genetic linkage map of 1502.4cM was constructed by517 markers (265 SSRs, 217 RFLPs, 18 retrotransposons, 10 ISSRs, 4 IRAPs and 3 morphological markers) with the average distance of 2.9 cM between two markers. These markers were distributed evenly on the barley chromosomes. The description about the development of SSR markers and construction of genetic linkage can be seen inEbadi-Segherloo (2013). The numbers of markers on each chromosome were as: 57(1H), 90(2H), 71(3H), 80(4H), 73(5H), 69(6H) and 77(7H). The length of chromosome linkage were 213(1H), 245(2H), 231(3H), 169(4H), 229(5H), 128(6H) and 287(7H) cM. The composite interval mapping was used to map QTLs byWinQTL cartographer 2.5 (Wang *et al*. 2007) with LOD=>3, window size of 10 cM and walk speed of 0.5 cM. Graphical linkage groups were generated through the help of Map chart 2.2 (Voorrips 2002). Coefficient of determination was calculated to measure the percentage of phenotypic variance explained by each QTL.

Results and Discussion

Phenotypic variation and correlation

Investigation of α-lattice relative efficiency to RCBD showed that RCBD design was more efficient for the analysis of experiments. Significant differences were observed among DH lines at both normal and salt-stress environments for the traits under study except HI and RWC in both conditions and DMA in the salinity condition. Year × Line interaction was significant for studied traits except SPAD, PH and GS under normal environment and for DHE, RWC, SPAD, DMA and GY under salinity condition. The coefficient of variation ranged from 0.9% (days to heading) to 17.6% (grain yield) under normal and 3.2%(days to maturity) to 26.8%(grain yield) under saline environments, respectively. Salinity increased the CV% of the traits except spike length (SL) and biomass (Table 1). Means of parents and DH lines with minimum and maximum of DHs lines for investigated traits are presented in Table 2. The differences of parents were remarkable for most of the studied traits. Transgressive segregation in both directions was observed for all the traits except K^+/Na^+ ratio. However, the transgressive segregation for this trait was unidirectional (Table 2).

Table 3 represents linear correlations among traits under study. Highest correlation was obtained between GY and BY at both environments (r=0.69**at normal condition and r=

Table 1. Combined analysis of variance over years for barley DH population and their parents under normal and salt stress conditions

SOV	df	DHE	RWC	SPAD	PH	SL	DMA	BY	GY	HI	GS	TKW
Normal												
Year	1	2946.2*	545.5^{ns}	1094.0*	21207.3**	40.1**	108.5^{ns}	4722.7**	0.4ns	12832.2*	22.9^{ns}	1676.8**
Block (Year)	2	44.1	2691.7	51.8	51.7	0.2	134.1	28.9	1.7	161.9	58.2	8.6
Line	150	17.0**	52.2^{ns}	34.5*	117.6**	2.5**	12.5**	16.9**	0.9**	35^{ns}	1155.9**	155.1**
Year×Line	150	3.0*	54.5**	23.9^{ns}	35.2^{ns}	0.4**	7.7**	7.0**	0.4*	28.6**	9.3^{ns}	15.6**
Error	300	2.2	39.3	19.7	28.2	0.2	4.6	5.0	0.3	12.8	24.9	7.2
C.V%		0.9	8.1	8.5	6.9	7.9	1.0	16.0	17.6	14.8	14.2	7.2
R^2		89.9	65.0	62.7	83.9	88.5	70.8	84.7	69.0	85.4	95.8	92.6
Salt stress												
Year	1	11029.0**	21662.9**	688.5**	29251.2**	126.3**	2239.3**	4496.8**	95.5**	1182.2^{ns}	11768.3^{ns}	678.1**
Block (Year)	2	36.2^{ns}	14.4^{ns}	49.3^{ns}	15.4^{ns}	0.8**	176.5**	26.0**	4.15**	86.3**	2497.7**	6.7^{ns}
Line	150	41.0**	58.3^{ns}	33.9**	90.0**	1.4**	32.8^{ns}	12.6**	0.62**	39.6^{ns}	448.3**	113.1**
Year×Line	150	34.1^{ns}	75.3^{ns}	18.9^{ns}	49.9*	0.4**	26.6^{ns}	8.03**	0.4^{ns}	34.5**	73.0**	11.3**
Error	300	28.3	72.8	20.4	39.0	0.1	27	2.7	0.38	17.4	56.7	6.9
C.V%		4.2	11.4	8.7	10.7	7.9	3.2	14.0	26.8	20.7	24.0	9.2
R^2		72.4	65	58	81	89	58.3	9.0	69	70.5	84	90

^{ns}, * and ** indicate non-significant and significant at 5 and 1% levels of probability, respectively
DHE: days to heading, RWC: relative water content, SPAD: chlorophyll content, PH: plant height, SL: spike length, DMA: days to maturity, BY: biomass, GY: grain yield, HI: harvest index, GS: grain number per spike, TKW: 1000 kernel weight.

0.64**at salinity-stress environment). The sign and magnitude of majority of correlation coefficients were affected by the salinity stress. For example, there was a positive and significant correlation between days to heading and plant height under normal condition (r= 0.33**), whereas this correlation was not significant (0.11^{ns}) under salinity condition (Table 3).

For assessing salt tolerance germplasm, different methods and parameters have been used. One of the methods involves the evaluation of genetic materials under natural saline condition in the field. This method effectively separate tolerant and sensitive varieties/lines at all stages of growth. Na^+ and K^+ contents, chlorophyll content and biomass have been used for the evaluation of salt tolerance (Sbei *et al.* 2012; Nguyen *et al.* 2013). El-Hendawy *et al*. (2009) used agronomic traits such as leaf area, shoot dry weight and physiological traits such as Na^+ and Cl^- exclusion, leaf water relation and chlorophyll content for investigation of salinity tolerance. In our study, Na^+ and K^+ levels were enhanced at the salinity condition as compared with the normal condition. However, other traits were negatively affected by salt (Table 2). Based on Abid *et al.* (2001), Na^+ content increased by the salinity stress, but there was variation in the response of genotypes. Sahara3771

Table 2. Phenotypic values of agronomic and physiological traits in the barley DH population and their parents under salinity stress and normal conditions

Trait	Environment	Sahara3771	Clipper	DH population		
				Mean	Min.	Max.
DHE	Normal	157	154	156.3	151.9	162.5
	Stress	134.2	124.2	125.7	118.9	134.9
RWC	Normal	81.9	72.9	77.2	67.9	88.1
	Stress	73.6	76.1	74.7	61	87.3
SPAD	Normal	51.7	48.6	52.0	45.4	58.7
	Stress	51.0	51.6	51.5	43	58.9
PH (cm)	Normal	76.1	71.3	76.5	63.7	92.6
	Stress	57.7	54.5	58.1	46	72.8
SL(cm)	Normal	6.5	5.6	5.8	4.1	7.5
	Stress	5.1	4.6	5	3.7	7.1
DMA	Normal	205.0	204.0	206.5	202.7	212.0
	Stress	160.0	154.4	157.9	148.7	169.0
GY(t/h)	Normal	2.8	4.2	3.2	2.0	4.9
	Stress	2.141	1.850	2.3	1.4	3.3
BY	Normal	10.6	15.1	14.0	8.8	19.7
(t/h)	Stress	9.466	11.728	11.8	7.2	11.8
HI	Normal	31.5	28.8	24.0	18.3	33.8
	Stress	23.87	15.91	20.1	14.8	35.3
GS	Normal	57.6	23.2	35.0	15.2	65.8
	Stress	43.7	18.1	31.3	14.8	53.3
TKW(g)	Normal	42.7	30.0	36.8	24.6	48.7
	Stress	31.8	24.5	28.7	18.3	40.8
Na^+ (mg g^{-1})	Normal	0.6	0.6	3.1	0.2	14.3
	Stress	16.2	15.9	12.1	1.2	23.2
K^+ (mg g^{-1})	Normal	16.7	18.4	23.1	9.6	44.2
	Stress	33.9	29.2	25.3	9.8	38.6
K^+/ Na^+	Normal	75.4	28.1	1.5	0.5	2.7
	Stress	2	1.8	2.3	0.9	8

DHE: days to heading, RWC: relative water content, SPAD: chlorophyll content, PH: plant height, SL: spike length, DMA: days to maturity, BY: biomass, GN: grain yield, HI: harvest index, GS: grain number per plant, TKW: thousands kernel weight, Na^+: Na content, K^+: K content. Min: minimum, Max: maximum.

Table 3. Linear correlation coefficients among studied traits in the barley DH population under saline and normal conditions averaged over two years

	DHE	RWC	SPAD	PH	SL	DMA	BY	GY	HI	GS	TKW	Na^+	K^+	K^+/Na^+
DHE	1.00	0.17*	0.25**	0.33**	0.18*	0.31**	0.25**	0.06ns	-0.16*	0.01ns	0.15ns	0.15ns	-0.13ns	-0.30**
RWC	0.11ns	1.00	0.02ns	0.02ns	0.08ns	0.11ns	0.04ns	0.03ns	-0.07ns	0.06ns	0.20*	0.01ns	0.02ns	-0.22**
SPAD	0.07ns	0.12ns	1.00	0.12ns	0.07ns	0.18*	0.25**	0.31**	0.09ns	-0.05ns	0.08ns	0.25**	-0.01ns	0.17ns
PH	0.11ns	0.01ns	0.07ns	1.00	0.34**	0.05ns	0.36**	0.09ns	-0.22**	-0.49**	0.05ns	0.52**	-0.13ns	0.11ns
SL	0.12ns	0.04ns	0.01ns	0.34**	1.00	0.09ns	0.33**	0.24**	-0.09ns	-0.28**	0.01ns	0.39**	0.01ns	0.2ns
DMA	0.50**	0.05ns	0.29**	0.21*	0.17*	1.00	0.19*	0.11ns	0.05ns	0.12ns	0.03ns	0.03ns	-0.12ns	-0.11ns
BY	0.14ns	0.06ns	-0.01ns	0.41**	0.36**	0.1ns	1.00	0.69**	-0.25**	-0.34**	0.02ns	0.46**	-0.1ns	-0.13ns
GY	0.10ns	0.05ns	0.01ns	0.31**	0.30**	0.04ns	0.64**	1.00	0.47**	-0.18*	0.01ns	0.29**	0.01ns	-0.07ns
HI	0.04ns	0.04ns	-0.04ns	-0.09ns	0.01ns	0.04ns	-0.24**	0.52**	1.00	0.12ns	0.03ns	-0.09ns	0.11ns	0.08ns
GS	-0.04ns	0.07ns	0.22**	-0.34**	0.27**	0.07ns	-0.37**	-0.32**	-0.09ns	1.00	-0.04ns	-0.83**	0.13ns	0.15ns
TKW	0.10ns	0.12ns	-0.17*	0.49**	0.37**	0.09ns	0.49**	0.40**	0.07ns	-0.80**	1.00	-0.03ns	0.13ns	-0.09ns
Na^+	0.12ns	0.03ns	0.05ns	0.30**	-0.17*	0.02ns	-0.26*	-0.23**	0.12ns	0.13ns	-0.1ns	1.00	0.18*	-0.70**
K^+	0.01ns	0.06ns	0.02ns	-0.25**	0.02ns	0.06ns	-0.17*	-0.11	0.13ns	-0.01ns	-0.01ns	0.38**	1.00	0.12ns
K^+/Na^+	-0.16*	0.02ns	-0.11ns	0.08ns	0.12ns	-0.02ns	0.16*	0.18*	-0.08ns	-0.10ns	0.08ns	-0.70**	0.10ns	1.00

ns, * and ** indicate non-significant and significant at 5 and 1% levels of probability, respectively

Values above the diagonal correspond to the normal environment; Values below the diagonal correspond to the salt stress environment

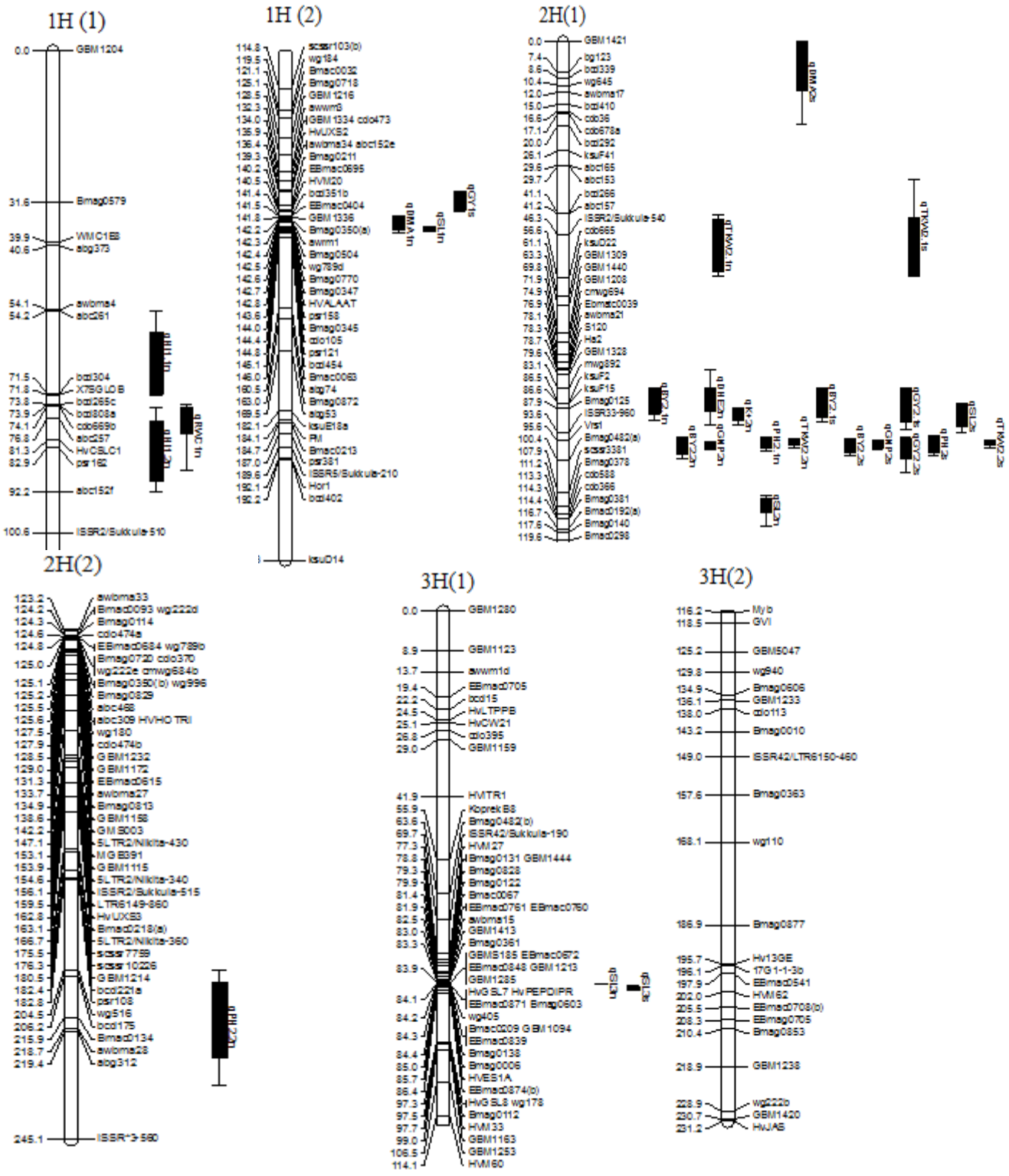
DHE: days to heading, RWC: relative water content, SPAD: chlorophyll content, PH: plant height, SL: spike length, DMA: days to maturity, BY: biomass, GY: grain yield, HI: harvest index, GS: grain number per plant, TKW: 1000 kernel weight, Na^+: Na content, K^+: K content.

was more tolerant to salinity based on the percent reduction in DHE, DMA, GY, BY and HI.

QTL Analysis

The results of QLT mapping are presented in Table 4. Results showed that a total of 72QTLs on seven linkage groups representing seven barley chromosomes were mapped for measured traits under two environments, being 40 and 32 QTLs under the normal and salinity stress conditions, respectively. The percentage of total phenotypic variation explained by individual QTLs ranged from 2.7-61.8%. Of the 72 QTLs, 29 QTLs explained more than 10% of the phenotypic variation (Table 4). Figure 1 represents the position of QTLs in the linkage map. We mapped four and one QTLs on chromosomes 2H, 4H, 5H and 7H for days to heading under normal and salinity environments, respectively. Each QTL accounted for 6.6-19.8% of the total DHE phenotypic variation. Three QTLs received alleles from Sahara3771 and two QTLs from Clipper (Table 4). Peighambari *et al*. (2005) reported a QTL on chromosome 2H at the position of 80 cM, which was close to qDHE2n that identified in this study at the position 83.1 cM on chromosome 2H. For relative water content, two QTLs in the normal condition on chromosomes 1H and 5H, and only one QTL on chromosome 5H in the salinity condition were detected. For all loci, DH lines having alleles from Sahara3771showed decreased RWC compared with DH lines receiving alleles from Clipper (Table 4). For chlorophyll content, two QTLs were mapped under both condition on chromosomes 5H, 6H and 7H, accounting for 11.1, 13.9, 10.7 and 11.2% of the phenotypic variation, respectively (Table 4). One QTL for RWC (qRWC5n) in the vicinity of *5LTR2/Nikita-150* marker, was co-located with the QTL for spike length (Figure 1). For plant height, six QTLs on chromosomes 2H, 4H, 5H and 7H in the normal condition and three QTLs on chromosomes 2H, 4H and 5H in the saline environment were identified. These QTLs explained 5.3-26.0% of PH phenotypic variation under the normal condition and 7.1-18.3% in the stress condition (Table 4). Two QTLs on chromosome 4H (qPH4.1n, qPH4s) in the vicinity of*awbma30* marker, and another QTL (qPH2.1n, qPH2s) near *Vrs1* marker were simultaneously detected under both conditions and are considered as stable (QTLs) which are not influenced by environment and are useful in marker-assisted selection (MAS). Thirteen QTLs were detected for spike length under two environments. There were eight QTLs on chromosomes 1H, 2H, 3H, 4H, 5H and 7H in the normal condition accounting for 4.3 to 24.1% of the total SL phenotypic variation. Under salt stress condition, five QTLs were detected on chromosomes 2H, 3H, 6H and 7H accounting for 5.7-21.9% of the total SL variation. Two QTLs on chromosome 7H (qSL7.2n, qSL7.2s) in the vicinity of *Bmag0110* marker were located in the similar region under salinity and normal conditions (Table 4). Ren *at al.* (2013) reported QTLs for spike length on chromosomes 1H, 2H, 3H, 4H, 5H, 6H and 7H. Four and three QTLs on chromosomes 1H, 2H, 5H, 6H and 7H were detected for days to maturity in the normal and salinity conditions, respectively. QTLs under the normal condition, accounted for 6.4 to 8.8% of the total DMA phenotypic variation, but accounted for 7.3 to 13.5% of the total variation in the salinity condition. In six out of seven identified QTLs, the alleles from Clipper increased days to maturity (Table 4). No similar QTLs were detected for DMA and DHE. Three and four QTLs were

mapped for biomass on chromosomes 2H and 5H under the normal and stress environments, respectively (Table 4).The qBY2.1n and qBY2.1s were simultaneously detected on chromosome 2H in the vicinity of *mwg892* marker. Alleles of these QTLs from Clipper, could increase the spike length



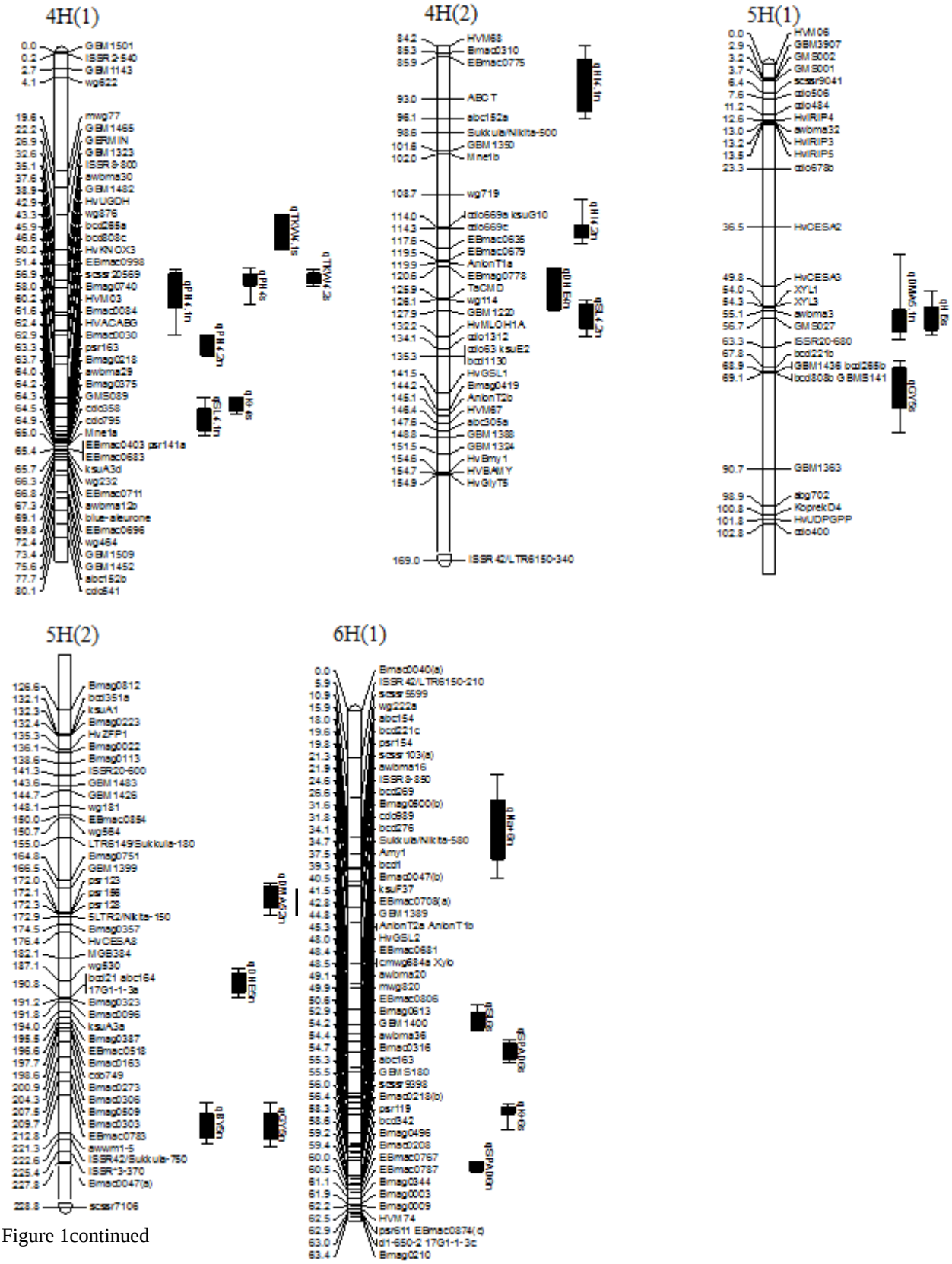


Figure 1continued

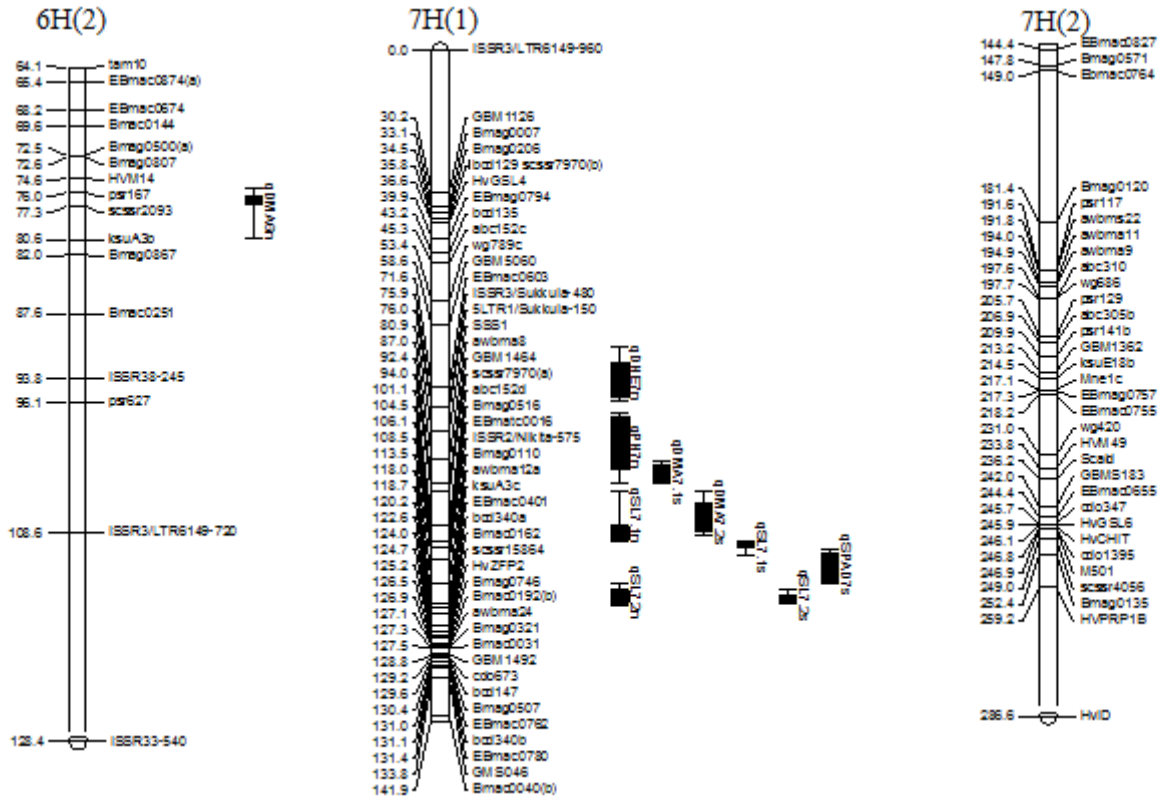


Figure 1. Chromosomal location of the QTLs identified for studied traits in barley

Table 4. QTLs for the traits identified in the barley DH population derived from a cross between Clipper × Sahara3771.

Trait	Environment	QTL	Chr	Nearest marker	Position	LOD	Additive effect	Var (%)
DHE	N	qDHE2n	2H	mwg892	83.1	3.5	0.5	6.7
		qDHE4n	4H	EBmag07	123.1	3.4	-0.5	6.6
		qDHE5n	5H	wg530	187.1	10.4	-0.9	19.8
		qDHE7n	7H	EBmac0603	72.5	6.8	0.7	14.6
	S	qDHE5s	5H	Bmac0306	204.3	4.5	-1.0	9.9
RWC	N	qRWC1n	1H	abc257	76.7	3.1	-1.0	7.6
		qRWC5n	5H	5LTR2/Nikita-150	174.3	3.0	-1.0	8.7
	S	qRWC5s	5H	Bmac0303	209.7	3.9	-1.22	9.6
SPAD	N	qSPAD5n	5H	EBmac0854	150.0	4.9	-1.0	11.1
		qSPAD6n	6H	Bmac0218(b)	56.9	3.7	1.1	13.9
	S	qSPAD6s	6H	EBmac0708(a)	42.8	4.3	-0.9	10.7
		qSPAD7s	7H	ISSR2/Nikita-575	110.5	4.0	1	11.2
PH	N	qPH2.1n	2H	Vrs1	95.6	12.4	2.8	26.0
		qPH2.2n	2H	Bmac0134	215.9	3.1	-1.2	5.3
		qPH4.1n	4H	awbma30	37.6	4.1	1.5	7.3
		qPH4.2n	4H	bcd808c	50.0	3	1.3	5.5
		qPH5n	5H	GBM1399	169.5	3.4	-1.4	6.4
		qPH7n	7H	5LTR1/Sukkula-150	79.4	4.2	1.7	10.3
	S	qPH2s	2H	Vrs1	96.1	7.4	2.0	18.3
		qPH4s	4H	awbma30	37.6	3.0	1.29	7.1
		qPH5s	5H	Bmag0751	166.3	3.1	-1.32	7.4
SL	N	qSL1n	1H	Bmag0345	144.0	8.7	0.2	13.1
		qSL2n	2H	Bmag03	111.1	4.0	0.1	5.5
		qSL3n	3H	Bmac0209	84.2	14.7	0.4	24.1
		qSL4.1n	4H	HVM03	60.7	3.1	0.1	4.3
		qSL4.2n	4H	GBM1220	127.9	5.4	-0.2	7.7
		qSL5n	5H	5LTR2/Nikita-150	173.8	4.0	-0.2	7.5
		qSL7.1n	7H	abc152d	103.1	5.2	0.2	8.3
		qSL7.2n	7H	Bmag0110	117.5	4.3	0.2	6.5
	S	qSL2s	2H	Bmag0125	88.4	6.5	0.14	5.7
		qSL3s	3H	Bmag0138	85.0	8.2	0.29	21.9
		qSL6s	6H	Amy1	39.0	3.4	-0.16	7.2
		qSL7.1s	7H	Bmag0110	117.5	5.2	0.19	9.9
		qSL7.2s	7H	Bmag0516	105.5	4.5	0.22	13.9
DMA	N	qDMA1.1n	1H	Bmag0350(a)	142.2	3.1	0.45	6.4
		qDMA5.1n	5H	GMS027	57.2	3.1	0.47	7.2
		qDMA5.2n	5H	GBM1399	166.5	4.2	-0.5	8.7
		qDMA6n	6H	psr167	76.4	3.5	0.54	8.8
	S	qDMA2s	2H	bcd339	8.6	3.2	0.805	7.3
		qDMA7.1s	7H	awbma8	88.5	3.9	1.08	10.5
		qDMA7.2s	7H	abc152d	101.1	5.6	1.13	13.5

Table 4 continued

Trait	Environment	QTL	Chr	Nearest marker	Position	LOD	Additive effect	Var (%)
BY	N	qBY2.1n	2H	mwg892	84.6	5.5	0.7	12.6
		qBY2.2n	2H	Vrs1	96.6	5.3	0.7	13.0
		qBY5n	5H	awwm1-5	22.1	3.0	-0.5	6.5
	S	qBY2.1s	2H	mwg892	86.1	5.8	0.71	15.5
		qBY2.2s	2H	Vrs1	96.6	6.9	0.8	21.2
		qBY5.1s	5H	EBmac0518	196.6	13.2	-0.5	6.8
		qBY5.2s	5H	Bmac0306	207.3	14.1	-0.57	8
GY	N	qGY5n	5H	awwm1-5	221.3	3.5	-0.1	7.8
	S	qGY1.1s	1H	abc152e	138.4	3.2	0.11	7.7
		qGY2.1s	2H	ksuF2	86.5	3.9	0.12	9.1
		qGY2.2s	2H	Vrs1	97.6	6.5	0.16	16.7
		qGY5s	5H	bcd808b	69.1	3.5	-0.15	7.7
HI	N	qHI1.1n	1H	abc261	67.2	5.6	1.1	15.6
		qHI1.2n	1H	abc257	79.7	7.6	1.2	18.8
		qHI4.1n	4H	ABCT	93.0	4.1	-1.2	9.1
		qHI4.2n	4H	cdo669c	114.3	5.8	1.4	13.1
	S	qHI5s	5H	GMS027	56.7	3.9	-0.93	8.5
GS	N	qGS2n	2H	Vrs1	96.6	53.7	-16.2	46.1
	S	qGS2s	2H	Vrs1	96.1	6.9	-8.55	60.2
TKW	N	qTKW2.1n	2H	ISSR2/Sukkula-540	49.7	5.2	1.4	4.7
		qTKW2.2n	2H	Vrs1	96.1	43.5	5.0	61.8
		qTKW5n	5H	Bmag0357	174.5	5.2	-1.4	4.1
	S	qTKW2.1s	2H	ISSR2/Sukkula-540	48.3	3.9	1.08	3.6
		qTKW2.2s	2H	Vrs1	96.1	41.3	4.34	60.1
		qTKW4.1s	4H	GERMIN	26.9	4.1	1.03	3.6
		qTKW4.2s	4H	ISSR8-800	37.6	6.2	1.29	5.5
		qTKW5s	5H	Bmag0357	174.5	3.3	-0.89	2.7
Na^+ (mgg^{-1})	N	q Na^+6n	6H	scssr5599	15.9	3.4	-0.8	9.6
K^+ (mgg^{-1})	N	q K^+2n	2H	Bmag0125	88.4	4.0	-1.6	16.7
	S	q K^+4s	4H	Bmag0740	58	4.8	2.26	12.1
		q K^+6s	6H	mwg820	49.9	3.6	-1.65	9.1

Var: Variance, DHE: days to heading, RWC: relative water content, SPAD: chlorophyll content, PH: plant height, SL: spike length, DMA: days to maturity, BY: biomass, GY: grain yield, HI: harvest index, GS: grain number per plant, TKW: 1000 kernel weight, Na^+: Na content, K^+: K content.

about 0.74 and 0.71, under the normal and salinity conditions, respectively. Another QTL simultaneously detected under both normal (qBY2.2n) and stress (qBY2.2s) conditions, was located on chromosome 2H in the vicinity of *Vrs1* marker. Only one QTL for grain yield under normal condition was detected on chromosome 5H accounting for 7.8% of total GY phenotypic variation. Under salinity condition four QTLs on chromosomes 1H, 2H and 5Hwere identified. Under salinity condition, the identified QTLs explained 49.4% of the total GY phenotypic variation. Some QTL(s) were common for grain yield and other traits. For example, the QTL on chromosome 5H in the vicinity of *awwm1-5* marker was common for biomass and grain yield (qBY5n, qGY5n). Another example was a QTL on chromosome 2H in the vicinity of *vrs1* marker (qGY2.2s) that was co-located with QTLs of BY (qBY2.2n, qBY2.2s), GS (qGS2n, qGS2s), PH (qPH2.1n, qPH2s) and TKW (qTKW2.2n, qTKW2s). Positive and significant correlations of grain yield and SL, BY, HI and TKW (both environments), SPAD (normal condition) PH and K^+/Na^+ ratio (salinity condition) were also observed. Pleiotropic effect of major genes and

close linkage of genes are main factors in the appearance of correlation between agronomic traits (Paterson *et al.* 1991). According to Marquez-Cedillo *et al.* (2001) the correlation between quantitative traits might be due to the linkage between their QTLs. For harvest index, five QTLs were detected under the normal and salinity stress conditions, four being located on chromosomes 1H and 4H under normal condition and on 5H chromosome in the salinity condition (Table 4). One QTL in the normal and one QTL in the saline environment on 2H were identified for grain number per spike. Both of them were major QTLs accounting for 46.1 and 60.2% of total GS variation under the normal and stress conditions, respectively. These QTLs located in the same region near *Vrs1* marker. Negative additive effect indicates that in this locus, allele from Sahara3771 tended to decrease grain number per plant by 16.28 and 8.55 in the normal and salt stress conditions, respectively. Li *et al.* (2005) reported QTLs for grain number in a spike on chromosome 2H.Three genomic regions in chromosome 2H and 5H associated with 1000 kernel weight in the normal condition and five on chromosomes 2H, 4H and 5H in the saline environment were detected. These QTLs accounted 4.1 to 61.8% and 2.7 to 60.1% of total TKW phenotypic variation in the normal and salinity environment, respectively (Table 4). Two QTLs (qTKW2.2n, qTKW2.2s) were simultaneously detected on chromosome 2H in the vicinity of*Vrs1* marker, accounting for 61.8 and 60.1% of the total TKW phenotypic variation in the normal and stress environments, respectively. For Na^+, only one QTL was detected under the normal environment, located on chromosome 6H and accounted for 9.6% of the total Na^+ phenotypic variation. The allele of this QTL came from sahara3771 and decreased the Na^+ content. For Na^+, no QTL was identified under salinity condition (Table 4). Three genomic regions related to K^+ content, were detected. Of them, one QTL on chromosome 2H was detected under the control condition, whereas two other QTLs were found under salinity stress environment on chromosomes 4H and 6H (Table 4). Each QTL accounted for 9.1 to 16.7% of the total K^+ phenotypic variation. No QTL was detected for K^+/Na^+ ratio in this study. Based on Forster *et al.* (2000), chromosome 4H in barley harbours several loci governing salt and drought tolerance. We mapped a QTL on chromosome 4H, in the vicinity of *Bmag0740* marker for K^+ content. This QTL received its allele from Clipper parent which increases K^+ content and may be utilized to increase salt tolerance in breeding programs. Nguyen *et al.* (2013) assessed salt tolerance of a DH population based on K^+ and Na^+ ions accumulation and biomass in a hydroponic system for three weeks. They reported two regions on chromosomes 2H and 3H that controlled ion content and salt tolerance, explaining 12% and 14.7% of variation for shoot Na+/K+ ratio, respectively.

Many QTLs for salt tolerance in barley have been reported in the literature (Taghipour and Salehi 2008; Xue *et al.* 2009; Shavrukov *et al.* 2010; Aminfar *et al.* 2011; Zhou *et al.*2012). Most studies for detecting QTLs involved in salt tolerance were carried out in controlled environments. However, under field condition the research results are scarce. Our experiment was conducted under natural salinity condition.

Out of 72 QTLs 6, 20, 2, 11, 18, 6 and 9 were located on chromosomes 1 to 7, respectively. However, some QTLs were similar under both environments (stable QTLs). Stable QTL(s) were not greatly influenced by the environmental conditions, including the QTL(s) for plant height on chromosome 4H in the vicinity of*awbma30* and *Vrs1* markers, biomass on chromosome 2H in the vicinity of *mwg892* and *Vrs1* markers, grain number per spike on chromosome 2H in the vicinity of Vrs1 marker and 1000 kernel weight on chromosome 2H in the vicinity of *Vrs1* marker.

On the chromosome 2H, in the vicinity of *Vrs1* marker a major QTL was identified, controlling BY, GY, PH, GS and TKW. This region contained qBY2.2n, qBY2.2s, qPH2s, qPH2.1n, qGS2n, qGS2s, qTKW2.2n, qTKW2.2s andqGY2.2s (Figure 1). Moreover, these QTLs, except qGY2.2s, were found under both the salinity and normal conditions, and alleles came from both parents (Table 4). Thus, this region of chromosome 2H in barley can be regarded as a useful target in the improvement of salt tolerance. It is possible for the existence of a QTL cluster for salt tolerance in this chromosomal region (Figure 1). Kicherer *et al.* (2000) reported that exclusive gathering of QTLs in a region of chromosome 2H, controlling heading date, plant height and grain weight is due to multi-locus gene clusters in the barley genome. *Vrs1*affects many characters related to grain yield and quality (Turuspekov *et al.* 2008). This locus which is located on chromosome 2H, governs row type in barley (Robertson *et al.* 1965). Marquez-Cedillo *et al.* (2001) reported the relationship of *Vrs1* locus with some QTLs. Some important QTLs related to 1000 kernel weight and number of grains per plant, have been reported on chromosome 2H between the *Vrs1* and *MWG503* markers (Shahinnia *et al.* 2014). Based on Lin *et al.* (1995), the existence of linked genes on chromosome 2H close to *Vrs1* marker is more possible than the pleiotropic effect of a major gene.

The QTL(s) common for some traits were also detected; for example a QTL on chromosome 2H in the vicinity of *5LTR2/Nikita-150* marker for SL and RWC, or a QTL on the chromosome 2H in the vicinity of *Vrs1* marker for biomass, plant height, grain number per spike, 1000 kernel weight (both environments) and grain yield (salinity environment).

Our results showed the usefulness of several markers for monitoring the agronomic characters, especially grain yield, in barley breeding programs conducted under salinity stress. Two methods have been proposed for utilizing QTLs in marker-assisted selection (Dudly 1993; Zhou *et al.* 1999): 1) Pyramiding alleles of useful QTLs in a single line and 2) Transferring these QTLs to specific genotypes by the backcross method. However, for validation of the results obtained in this program, the DH lines should be evaluated in several years.

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References

Abid M,Qayyum AA, Dasti A and Abdilwajid R, 2001. Effect of salinity and SAR of irrigation water on yield, physiological growth parameters of maize and properties of the soil. Journal of Research12: 26-33.

Aminfar Z, Dadmehr M, Korouzhdehi B, Siahsar B and Heidari M, 2011. Determination of chromosomes that control physiological traits associated with salt tolerance in barley at the seedling stage. African Journal of Biotechnology 10: 8794-8799.

Ceccarelli S, Grando S and van Leur JAG, 1987.Genetic diversity in barley landraces from Syria and Jordan. Euphytica 36: 389-405.

Dudly JW, 1993.Molecular markers in plant improvement.Manipulation of genes affecting quantitative traits. Crop Science 33: 660-668.

EbadiSegherloo A, 2013. Construction of barley doubled haploid population microsatellite linkage map and identification of genetic regions associated with agronomic traits and some micronutrients accumulation. Unpublished Ph.D. thesis, Faculty of Agriculture, University of Tabriz,Tabriz, Iran (In persian with English abstract).

Eleuch L, Jilal A, Grando S, Ceccarelli S, Schmisisng MvK, Tsujimoto H, Hajer A, Daaloul A and Baum M, 2008. Genetic Diversity and Association Analysis for Salanity Tolerance, heading Date and Plant Height of Barley. Journal of Integrative Plant Biology 50: 1004-1014.

EL-Hendawy SE, Ruan Y, Hu Y and Schmidhalter U, 2009. A comparison of screening criteria for salt tolerance in wheat under field and controlled environmental conditions. Journal of Agronomy and Crop Science 195:356-367.

FAO, 2008. FAO land and plant nutrition management service. http://www.faoorg/ag/agl/agll/spush.

Fitter AH and Hay RKM, 1987. Environmental physiology of plants. Academic press 227-228.

Forster BP, Ellis RP, Thomas WTB, Newton AC, Tuberosa R, This D, El-Enein RA, Bahri MH and Ben Salem M, 2000.The development and application of molecular markers for abiotic stress tolerance in barley. Journal of Experimental Botany 51: 19-27.

Hearnden PR, Eckermann PJ, McMichael GL, Hayden MJ, Eglinton JK and Chalmers KJ, 2007. A genetic map of 1000 SSR and DArT markers in a wide barley cross. Theoretical and Applied Genetics 115: 383–391.

Jana MK, Jana S and Ascharya SN, 1980. Salt stress tolerance in heterogeneous populations of barley. Euphytica 29: 409-417.

Kicherer S, Backes G, Walther U and Jahoor A, 2000. Localizing QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics 100: 881-888.

Kleinhofs A, Kilian A, Maroof MAS, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick AT, Blake K, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R and Steffenson BJ, 1993. A molecular, isozyme and morphological map of the barley (*Hordeum vulgare* L.) genome. Theoretical and Applied Genetics 86: 705-712.

Li JZ, Haung XQ, Heinrichs F and Ganal MW, 2005. Analysis of QTLs for yield, yield components and malting quality in a BC3-DH population of spring barley.Theoretical and Applied Genetics 110: 356-363.

Lin YR, Schertz F and Paterson A, 1995. Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141: 391-411.

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

Marquez-Cedillo LA, Hayes PM, Kleinhof A, Legge WG, Rossangel BG, Sato K, Ullrich SE and Wesenberg DM, 2001. QTL analysis of agronomic traits in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. Theoretical and Applied Genetics 103: 625-637.

Mohammadi M, Taleei A, Zeinali H, Naghavi MR, Ceccarelli S, Grando S and Baum M, 2005. QTL analysis for phonologic traits in doubled haploid population of barley. International Journal of Agriculture and Biology 5: 820-823.

Munns R and James RA, 2003. Screening methods for salinity tolerance, a case study with tetraploid wheat. Plant and Soil 253: 201-218.

Munns R and Tester M, 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology l59: 651-681.

Nguyen VL, Ribot SA, Dolstra O, Niks RE, Visser RGF and van der Lindeh CG, 2013. Identification of quantitative trait loci for ion homeostasis and salt tolerance in barley (*Hordeum vulgare* L.). Molecular Breeding 31: 137-152.

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HO, Linclon SE, Lander ES and Tanksley SD, 1991. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations and environments. Genetics 127: 181-197.

Peighambari SA, YazdiSamadi B, Nabipour AR, Charmet G and Sarrafi A, 2005. QTL analysis for agronomic traits in a barley doubled haploids population growth in Iran. Plant Science169: 1008-1013.

Ren X, Sun D, Sun G, Li C and Dong W, 2013. Molecular detection of QTL for agronomic and quality traits in a doubled haploid barley population. Australian Journal of Crop Science 6: 878-886.

Rivandi J, Miyazaki J, Hrmova M, Pallotta M, Tester M and Collins NC, 2011. A SOS3 homologue maps to HvNax4, a barley locus controlling an environmentally sensitive Na^+ exclusion trait. Journal of Experimental Botany 62: 1201–1216.

Robertson DW, Wiebe GA, Shands RG and Hagberg A, 1965. A summary of linkage studies in cultivated barley, *Hordeum* species: Supplement III, 1954– 1963. Crop Science 5: 33–43.

Sbei H, Hammami Z, Trifa Y, Hamza S and Harrabi M, 2012. Phenotypic diversity analysis for salinity tolerance of Tunisian barley populations (*Hordeum vulgare* L.). Journal of Arid Land Studies 22: 57–60.

Shahinnia F, Rezai A, Sayed-Tabatabaei BE, Omatsuda TK and Mohammadi SA, 2006. QTL mapping of heading date and plant height in Barley cross "Azmamugi×KantoNakate Gold. Iranian Journal of Biotechnology 4: 88-94.

Shahraki H, Fakher BA and Allahdou M, 2013. Genomic regions mapping for some phonological traits associated with salt tolerance in a doubled haploid lines of barley. International Journal of Agriculture and Crop Science 7: 403-409.

Shavrukov Y, Gupta NK, Miyazaki J, Baho MN, Chalmers KJ, Tester M, Langridge P and Collins NC, 2010. HvNax3a locus controlling shoot sodium exclusion derived from wild barley (*Hordeum vulgare* ssp. spontaneum). Functional and Integrative Genomics 10: 277–291.

Taghipour F and Salehi M, 2008. The study of salt tolerance of Iranian barley (*Hordeum vulgare* L.) genotypes in seedling growth stages. American-Eurasian Journal of Agriculture and Environment Science 4: 525-529.

Thomas WTB, Baird E, Fuller JD, Lawrence P, Young GR, Russell J, Ramsay L, Waugh R and Powell W, 1998. Identification of QTL decreasing yield barley linked to Mlo powdery mildew resistance. Molecular Breeding 4: 381-393.

Turuspekov Y, Martin JM, Bowman JGP, Beecher BS and Giroux MJ, 2008.Association between Vrs1 alleles and grain quality traits in spring barley (*Hordeumvulgare* L.). Cereal Chemistry 6: 817-823.

Voorrips RE, 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. Journal of Heredity 93: 77-78.

Wang S, Basten CJ and Zeng ZB, 2007: Windows QTL Cartographer V2.5. Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/wQTLcart.htm.

Xue D, Haung Y, Zhang X, Wei K, Westcott S, Li C, Chen M, Zhang G and Lance R, 2009. Identification of QTL sassociated with salinity tolerance at late growth stage in barley. Euphytica 169: 187-196.

Zhou H, Briecero G, Dovel R, Hayes PM, Liu BH, and Ulrich SE, 1999. Molecular breeding for grain yield in barley. An evaluation of QTL effects in a spring barley. Theoretical and Applied Genetics 98: 772-779.

Zhou G, Johnson P, Ryan PR, Delhaize E andZhou M, 2012.Quantitative trait loci for salinity tolerance in barley (*Hordeum vulgare* L.). Molecular Breeding 29: 427-436.