Identification of QTLs Associated with Agronomic and Physiological Traits under Salinity Stress in Barley

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ABSTRACT

Salinity tolerance is a genetically and physiologically complex trait, controlled by Quantitative Trait Loci (QTLs). In order to map the QTLs associated with agronomic and physiological traits, 149 doubled haploid lines derived from a cross between Clipper (salt susceptible) and Sahara 3771 (salt tolerant) barley genotypes were evaluated under natural saline-stress and non-stress conditions using 14 traits. QTL analysis was performed based on the composite interval mapping method, using the genetic linkage map consisting of 517 molecular markers which spanned a total of 1502.4 cM. A total of 78 QTLs for days to heading, relative water content, chlorophyll content, plant height, spike length, days to maturity, biomass, grain yield, harvest index, grain number per spike, 1,000-kernel weight, Na^+, K^+ concentrations and K^+/Na^+ ratio, were determined, with 40 and 38 QTLs under normal and salinity environments, respectively. Most of the detected QTLs were located on chromosome 2H. The phenotypic variation explained by individual QTLs ranged from 3.3 to 68.6%. A major QTL was identified at both saline-stress and non-stress conditions in the vicinity of *Vrs1* on chromosome 2H, related to biomass, grain number per spike, 1,000 kernel weight, plant height and grain yield. This QTL may be useful in the barley breeding programs for improving salt tolerance by marker-assisted selection. Furthermore, some stable QTLs, were identified for days to heading, biomass, spike length, grain number per spike, 1,000 kernel weight, and K^+ content which can be regarded as promising QTLs for breeding purposes.

Keywords: Doubled haploid, QTL analysis, Salinity tolerance.

INTRODUCTION

Salinity is one of the major abiotic stresses affecting 19.5% of agricultural land (FAO, 2016) and limiting crop production. It affects numerous growth processes at the levels of sub-cell, cell, tissue, whole plant and several physiological process including ionic balance (especially Na^+/K^+ ratio) and distribution (Xue *et al.,* 2009). Barley (*Hordeum vulgare* L.), an important food and fodder crop, is widely cultivated in saline areas as one of the most salt-tolerant crops (Mass and Hoffman, 1997; Munns and Tester, 2008). However, its growth and production is greatly affected by salt stress. Improvement of salinity tolerance is an important aim in barley breeding programs (Colmer *et al.,* 2005). There is a large variation among barley genotypes for salinity tolerance (Haung and Redman, 1995; Mer *et al.,* 2000). Development and utilization of stress-resistant genotypes is an effective approach to prevent yield loss. QTLs can be used in marker

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assisted selection to facilitate selection of salinity tolerant genotypes in conventional breeding programs.

Salinity tolerance is a genetically and physiologically complex trait controlled by Quantitative Trait Loci (QTLs). QTL analysis has been utilized for dissection of different traits in barley (Beecher *et al.,* 2001; Mohammadi *et al.,* 2005; Nguyen *et al.,* 2013). QTLs associated with salinity tolerance have been mapped in barley. Shavrukov *et al.* (2010) identified a single QTL on the short arm of chromosome 7H, *Nax3*, reducing the shoot sodium content of plants grown in 150 mM NaCl about 10-25%. Rivandi *et al.* (2011) mapped a QTL on the long arm of 1H, *HvNax3*, affecting salinity tolerance. Zhou *et al.* (2012) used a combined injury score based on leaf chlorosis and plant survival seven weeks after sowing in a DH population derived from a cross between YYXT genotype (salt tolerant) and Franklin (salt sensitive) and identified five QTLs on chromosomes 1H, 2H, 5H, 6H and 7H for the traits associated with salinity tolerance explaining more than 50% of the phenotypic variation. QTLs for salt tolerance at a late growth stage have been described for the CM72 (salt tolerant)×Gairdner (salt sensitive) population (Xue *et al.,* 2009). In this population, The number of QTLs for tiller number, plant height, spikes per line, spikes per plant, dry weight per plant, grains per plant, grain yield, shoot Na^+ and K^+ concentrations, and Na^+/K^+ ratio were determined as 17 and 13 under, respectively, non-stress and salt stress conditions. They identified the single QTL affecting Na^+ content and Na^+/K^+ ratio.

The objective of this study was to identify QTLs associated with some agronomic and physiological traits under natural salt stress and normal conditions using a barley DH population.

MATERIALS AND MEHTODS

A population of 149 Doubled Haploid lines (DHs) and parental genotypes were evaluated using *α*-lattice design with two replications for two years (2012-2013 and 2013-2014) at two locations: Birjand Agricultural Research Station (As salinity stress condition: Ec_{soil}= 9.8-12 dS m^{-1}, Ec_{water}= 8.8-10 dS m^{-1}) and Research Field of Cereal Department, SPII, Karaj (As normal condition: Soil and water EC: ~2-2.5 dS m^{-1}). The DH lines were derived from a cross between Clipper (an Australian spring, two row and salt sensitive cultivar) and Sahara 3771 (an Algerian winter, six row and salt tolerant landrace) in the University of Adelaide. All DH lines and two parents were planted in early November. Seeds of DH lines and their two parents were sown in two rows of one-meter length and 0.25 meter between rows. Irrigation frequency and amount of fertilizer used were similar in both conditions. The measured traits were Days to Heading (DHA), Relative Water Content (RWC), chlorophyll content (SPAD), Plant Height (PH, cm), Spike Length (SL, cm), Days to Maturity (DMA), Biomass (BY, t ha^{-1}), Grain Yield (GY, t ha^{-1}), Harvest Index (HI), Grain number per Spike (GS), 1,000 Kernel Weight (TKW, g), Na^+ and K^+ contents (mg g^{-1}), and K^+/Na^+ ratio. A SPAD-502 chlorophyll-photometer was used to measure the chlorophyll content, which was measured on three fresh leaves, on the first part, middle part, and last part of the leaf (Munns and James, 2003). Relative Water Content (RWC) was calculated as:

$$RWC = [(f_w - d_w)/(t_w - d_w)] \times 100$$,

Where, f_w, d_w and t_w are leaf fresh, dry, and turgid weight, respectively. Chlorophyll content and relative water content were measured when the plant seeds were at the milky stage. Na^+ and K^+ contents were determined by flame photometry only for one replication every year. Relative efficiency of α-lattice to randomized complete block design was determined for all measured traits. Combined analysis of variance was carried out for the studied traits under saline and normal conditions, separately. Pearson's correlation coefficients were calculated between the traits under both environments, separately.

The genetic linkage map of 1,502.4 cM was constructed using a total of 517 markers (265 SSRs, 217 RFLPs, 18 retrotransposons, 3 morphological markers, 10 ISSRs and 4 IRAPs (average distance of 2.9 cM between two markers) distributed evenly on seven barley chromosomes. A detailed description of the procedure adopted for development of SSR markers and genetic map is given by Ebadi-Segherloo (2013). Briefly, each linkage group corresponded to one of the seven barley chromosomes. The length of each chromosome linkage was 213, 245, 231, 169, 229, 128 and 287 cM, for chromosomes 1H to 7H, respectively. The number of markers was 57, 90, 71, 80, 73, 69 and 77, corresponding to those on chromosomes 1H to 7H, respectively. QTL mapping was performed by composite interval mapping using WinQTL cartographer 2.5 (Wang *et al.,* 2007) with Logarithm of Odds (*LOD*)=> 3, walk speed of 0.5 cM and window size of 10 cM. The percentage of explained phenotypic variance was estimated for each QTL (R^2). Graphical linkage groups were generated using Mapchart 2.2 (Voorrips, 2002).

RESULTS AND DISCUSSION

Phenotypic Variation and Correlations among Traits

Analysis of variance revealed significant differences among DH lines under both salt-stress and normal conditions for the studied traits, except *RWC* and *HI* in both environments and DMA only in the salinity condition. Year×line interaction was significant for all traits, except for SPAD, PH, and GS under normal and *RWC*, SPAD, GY, and GS under salinity condition. Salinity enhanced the *%CV* of the traits, except for *RWC*, PH, BY, and GS (Table 1). Some descriptive statistics of DH and parental lines for the investigated traits are presented in Table 2. Transgressive segregation in both directions was observed for all the traits, except K^+ content under the

stress condition and K^+/Na^+ ratio in both conditions. However, these traits showed transgressive segregation in one direction (Table 2).

Correlations among measured traits are presented in Table 3. Maximum correlation was observed between GY and BY in both conditions (r= 0.7^{**}). Results showed that the sign and magnitude of correlations among measured traits were mostly influenced by the salinity stress. Indeed, some traits showed significant correlation in the normal environment but became non-significant in the saline environment and vice versa. For example, there was a negative and significant correlation between plant height and grain number per spike under normal condition (r= -0.5^{**}) whereas this correlation was positive and significant under salinity condition (r= 0.5^{**}). As the second example, the correlation between days to heading and K^+/Na^+ ratio was negative and significant under normal condition (r= -0.3^{**}) whereas it was non-significant under salinity condition (Table 3).

Salt tolerance is a complex trait and different methods and parameters have been used to assess plant germplasms for salt tolerance. Assessment under saline field condition is one of the methods which are based on natural salinity condition. Various physiological and agronomic traits have been used for salt tolerance evaluation as plant height, chlorophyll content, biomass, Na^+ and K^+ contents (Sbei *et al.,* 2012; Nguyen *et al.,* 2013). Agronomic traits such as leaf area, shoot dry weight, and physiological traits such as Na^+ and Cl^- exclusion, leaf water relation, and chlorophyll content have been studied for salinity tolerance (El-Hendawy *et al*., 2009). We evaluated salinity tolerance of DHs using fourteen agronomical and physiological traits in this study. The results revealed that Na^+ and K^+ levels were increased under salinity condition as compared to the normal environment, whereas the magnitude of other studied traits such as plant height, spike length, and 1,000

Table 1. Combined analysis of variance over years for DH population and their parents under normal and stress conditions.[a]

SOV	df	DHE[a]	RWC[b]	SPAD[c]	PH[d]	SL[e]	DMA[f]	BY[g]	GY[h]	HI[i]	GS[j]	TKW[k]
Normal												
Year	1	2946.0^*	546.0^{ns}	1094.0^*	21207.0^{**}	40.0^{**}	109.0^{ns}	4722.0^{**}	0.4^{ns}	12832.0^*	23.0^{ns}	1677.0^{**}
Block (Year)	2	44.1	2692.0	52.0	52.0	0.2	134.1	29.0	1.7	161.9	58.0	8.6
Line	150	17.0^{**}	52.0^{ns}	34.5^*	117.6^{**}	2.5^{**}	12.5^{**}	17.0^{**}	0.9^{**}	35.0^{ns}	1156.0^{**}	155.1^{**}
YearxLine	150	3.0^*	54.5^{**}	24.0^{ns}	35^{ns}	0.5^{**}	7.7^{**}	7.0^{**}	0.4^*	28.6^{**}	9.3^{ns}	15.6^{**}
Error	300	2.2	39.0	20.0	28.0	0.2	4.6	5.0	0.3	12.8	25.0	7.2
C.V%		0.9	8.0	8.5	7.0	8.0	1.0	16.0	17.6	14.8	14.0	7.2
R^2		89.9	65.0	62.7	84.0	88.5	70.8	84.7	69.0	85.4	96.0	92.6
LSD5%		3.3	14.4	8.7	10.4	1.3	5.4	5.2	1.2	10.4	10.0	7.7
Stress												
Year	1	21912.2^{**}	1854.6^{ns}	1023.8^{**}	4657.8^{**}	68.4^{**}	1551.3^{**}	63.5^*	124.1^{**}	1652.7^{ns}	29.5^*	2476.7^{**}
Block (Year)	2	22.7	444.4	0.1	10.0	0.1	46.3	3.1	3.4	1.4	2.8	4632.3
Line	150	27.0^{**}	36.8^{ns}	61.0^*	101.1^{**}	2.0^{**}	16.7^{ns}	13.6^{**}	0.7^{**}	31.7^{ns}	597.9^{**}	142.1^{**}
YearxLine	150	12.0^{**}	25.4^{ns}	57.2^{ns}	52.8^{**}	0.5^{**}	16.9^*	7.6^{**}	0.4^{ns}	34.6^{**}	2.6^{ns}	17.2^{**}
Error	300	5.6	30.1	46.2	19.1	0.3	6.2	2.5	0.4	9.7	6.1	6.7
C.V%		1.6	7.3	13.3	6.3	11.2	1.3	12.8	26.6	15.6	8.9	7.7
R^2		94.0	57.1	57.5	82.8	80.5	0.2	92.4	71.0	80.0	97.9	94.6
LSD5%		6.7	10.7	13.3	14.2	1.3	8.0	5.4	1.2	11.5	4.8	8.1

[a] Days to Heading; [b] Relative Water Content; [c] Chlorophyll content; [d] Plant Height; [e] Spike Length; [f] Days to Maturity; [g] Biomass; [h] Grain Yield; [i] Harvest Index; [j] Grain number per Spike, [k] 1,000 Kernel Weight; ns, * and **: Indicate non-significant, and significant at 5 and 1% levels of probability, respectively.

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

Trait	Environment	Sahara 3771	Clipper	DH population		
				Mean	Min[n]	Max[o]
DHE[a]	Normal	157.0	154.0	156.3	151.9	162.5
	Stress	146.0	144.0	143.4	137.7	151.2
RWC[b]	Normal	81.9	72.9	77.2	67.9	88.1
	Stress	75.4	72.5	74.7	66.2	83.8
SPAD[c]	Normal	51.7	48.6	52.0	45.4	58.7
	Stress	52.8	51.4	51.0	35.9	61.2
PH (cm)[d]	Normal	76.1	71.3	76.5	63.7	92.6
	Stress	68.3	62.6	69.3	56.1	84.3
SL (cm)[e]	Normal	6.5	5.6	5.8	4.1	7.5
	Stress	5.6	4.5	5.3	3.7	7.0
DMA[f]	Normal	205.0	204.0	206.5	202.7	212.0
	Stress	181.0	182.0	182.4	176.9	187.7
GY (t ha^{-1})[g]	Normal	2.8	4.2	3.2	2.0	4.9
	Stress	2.4	2.2	2.4	1.1	3.7
BY (t ha^{-1})[h]	Normal	10.6	15.1	14.0	8.8	19.7
	Stress	10.0	12.7	12.5	7.4	16.9
HI[i]	Normal	31.5	28.8	24.0	18.3	33.8
	Stress	26.1	18.3	20.0	13.4	32.7
GS[j]	Normal	57.6	23.2	35.0	15.2	65.8
	Stress	45.9	17.4	28.0	11.6	49.1
TKW(g)[k]	Normal	42.7	30.0	36.8	24.6	48.7
	Stress	38.3	26.0	33.6	21.4	45.0
Na$^+$ (mg g^{-1})[l]	Normal	0.6	0.6	3.1	0.2	14.3
	Stress	14.6	9.7	9.8	2.2	26.2
K$^+$ (mg g^{-1})[m]	Normal	16.7	18.4	23.1	9.6	44.2
	Stress	49.1	26.0	25.2	10.1	37.6
K$^+$/Na$^+$	Normal	27.8	30.6	1.5	0.5	2.7
	Stress	3.3	2.68	1.6	0.9	2.7

[a] Days to Heading; [b] Relative Water Content; [c] Chlorophyll content; [d] Plant Height; [e] Spike Length; [f] Days to Maturity; [g] Grain Yield; [h] Biomass; [i] Harvest Index; [j] Grain number per Spike; [k] 1,000 Kernel Weight; [l] Na content; [m] K content; [n] Minimum, [o] Maximum.

kernel weight were reduced (Table 2). Abid *et al.* (2001) reported that Na$^+$ content increased due to salinity stress, but the increased rate was different for different genotypes. Compared with Clipper, Sahara 3771 was more tolerant to salinity based on the reduction percent for GY, BY, *HI*, SL, GS, TKW, and PH in the salinity stress compared to the normal environment.

QTL Analysis

Unraveling the mechanisms underlying salt tolerance in higher plants is a challenging task for plant scientists worldwide. We evaluated the segregating DH population derived from Clipper×Sahara 3771 under natural saline environment to better understand the genetics of the characters under salt tolerance, in particular the concentrations of major ions in shoots, grain yield, relative water content and chlorophyll content. QLT mapping results including type of environment, name of QTL, nearest marker, percentage of explained phenotypic variation, QTL position, LOD and additive effects are summarized in Table 4. A graphical linkage map is shown in Figure 1. A total of 78

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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[a]	1.00	ns	0.2**	0.3**	0.2*	0.3**	0.2**	ns	-0.2*	ns	ns	ns	ns	-0.3**
RWC[b]		1.0	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.2*	ns	-0.2**
SPAD[c]			1.0	0.2*	ns	0.2*	0.2**	0.3**	ns	ns	0.2*	ns	ns	ns
PH[d]				1.0	0.3**	ns	0.4**	ns	-0.2**	-0.5**	0.52**	ns	ns	ns
SL[e]					1.0	ns	0.3**	0.2*	ns	-0.3**	0.4**	ns	ns	ns
DMA[f]						1.0	0.2*	0.2*	ns	-0.3**	ns	ns	ns	ns
BY[g]							1.0	0.7**	-0.2**	-0.3**	0.5**	ns	ns	ns
GY[h]								1.0	0.5**	-0.2*	0.3**	ns	ns	ns
HI[i]									1.0	ns	ns	ns	ns	ns
GS[j]										1.0	-0.9**	0.1 ns	0.2**	ns
TKW[k]											1.0	-0.2*	-0.3**	-0.7**
Na^+[l]												1.0	0.2*	-0.8**
K^+[m]													1.0	0.2**
K^+/Na^+														1.0

[a] Days to Heading; [b] Relative Water Content; [c] Chlorophyll content; [d] Plant Height; [e] Spike Length; [f] Days to Maturity; [g] Biomass; [h] Grain Yield; [i] Harvest Index; [j] Grain number per Spike; [k] 1,000 Kernel Weight; [l] Na content; [m] K content; ns, * and **: Indicate non-significant, and significant at 5 and 1% levels of probability, respectively.

QTLs were mapped for measured traits under two environments, being 40 and 38 QTLs under the normal and salinity stress conditions, respectively (Table 4; Figure 1). Negative and positive additive effects indicated the contribution of desirable alleles at the mapped genomic regions from Sahara 3771 and Clipper, respectively.

The phenotypic variation explained by individual QTLs ranged from 3.3 to 68.6%. Figure 1 shows the positions of QTLs in the linkage map. We mapped 4 and 3 QTLs on chromosomes 2H, 4H, 5H and 7H for days to heading under normal and stress environments, respectively. Peighambari *et al.* (2005) reported a QTL for date of flowering on the same position of qDHE2n. Two QTL, qDHE5n and qDHE5.1s in the vicinity of *wg530* marker, were simultaneously detected under both environments. In this locus, the allele from Sahara 3771 could decrease days to heading 0.93 and 1.06 days under normal and salt stress conditions, respectively.

Two QTLs were detected for relative water content in the normal condition on chromosomes 1H and 5H. In both loci, DH lines having alleles from Sahara 3771, showed increase in RWC as compared with DH lines receiving alleles from Clipper. Two QTLs for RWC on chromosomes 1H (near *abc 257* marker) and 5H (near *5LTR2/Nikita-150* marker) were co-located with the QTLs for harvest index and spike length, respectively. For chlorophyll content, two and four QTLs were mapped under normal and stress environments, respectively, on chromosomes 3H, 4H, 5H and 6H. For plant height, six QTLs on chromosomes 2H, 4H, 5H and 7H in the normal environment and four QTLs on chromosomes 2H, 4H and 7H in the saline environment were identified. These QTLs explained 5.3 to 26.0% of the phenotypic variation for PH under normal condition and 8.0 to 31.3% in the stress condition. The two QTLs, qPH7n and qPH7s, in the vicinity of *5LTR1/Sukkula-150* marker on chromosome 7H, were simultaneously detected under both conditions. Fourteen QTLs were

Table 4. QTLs for the traits identified in the barley DH population derived from a cross between Clipper×Sahara 3771.[a]

	Env[n]	QTL	Chr	Nearest marker	Position	LOD	Additive effect	Var[o] (%)
DHE[a]	N	qDHE2n	2H	mwg892	83.1	3.5	0.5	6.7
		qDHE4n	4H	EBmag0778	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	qDHE4s	4H	cdo669c	116.8	4.3	0.7	8.5
		qDHE5.1s	5H	wg530	189.1	7.4	-1.0	16.0
		qDHE5.2s	5H	Bmag07	196.5	8.01	-1.0	16.6
RWC[b]	N	qRWC1n	1H	abc257	76.7	3.1	-1.0	7.6
		qRWC5n	5H	5LTR2/Nikita-150	174.3	3.08	-1.0	8.7
SPAD[c]	N	qSPAD5n	5H	EBmac0854	150	4.9	-1.0	11.1
		qSPAD6n	6H	Bmac0218(b)	56.9	3.7	1.1	13.9
	S	qSPAD3.1s	3H	HVM27	77.7	4.1	-1.2	9.9
		qSPAD3.2s	3H	EBmac0874(b)	89.3	5.4	-1.4	13.3
		qSPAD4.1s	4H	GMS089	64.3	4.9	1.3	10.8
		qSPAD4.2s	4H	GBM1509	74.3	3.7	1.2	9.3
PH[d]	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	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	qPH2.1s	2H	Ha2	.7	7.8	2.0	16.6
		qPH2.2s	2H	ISSR33-960	95.1	13.5	2.8	31.3
		qPH4s	4H	ISSR8-800	37.5	4.3	1.4	8.0
		qPH7s	7H	5LTR1/Sukkula-150	.9	3.4	1.6	10.2
SL[e]	N	qSL1n	1H	Bmag0345	144	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	qSL1.1s	1H	HVALAAT	142.8	4.8	0.2	7.8
		qSL1.2s	1H	Bmac0063	153.4	3.1	0.1	6.5
		qSL3s	3H	GBM1094	84.3	11.5	0.3	22.6
		qSL4s	4H	GBM1220	127.9	5.7	-0.2	9.7
		qSL7.1s	7H	Bmag0516	105.5	4.7	0.2	8.6
		qSL7.2s	7H	Bmag0110	117.5	4.1	0.1	7.0
DMA[f]	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	qDMA5s	5H	GBM1399	167.0	4.7	-0.7	11.1
		qDMA7s	7H	EBmac0603	72.0	6.5	1.2	19.3
BY[g]	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

[a] Days to Heading; [b] Relative Water Content; [c] Chlorophyll content; [d] Plant Height; [e] Spike Length; [f] Days to Maturity; [g] Biomass; [n] Environment, [o] Explained variance.

Table 4. (continued)

	Env [n]	QTL	Chr	Nearest marker	Position	LOD	Additive effect	Var [o] (%)
BY [g]	S	qBY2.1s	2H	mwg892	85.6	5.3	0.6	11.0
		qBY2.2s	2H	Vrs1	96.6	7.1	0.7	15.5
		qBY5.1s	5H	GBM1399	167.5	3.7	-0.5	7.1
		qBY5.2s	5H	EBmac03	219.8	4.2	-0.6	9.2
GY [h]	N	qGY5n	5H	awwm1-5	221.3	3.5	-0.1	7.8
	S	qGY1.1s	1H	GBM1334	134.0	5.2	0.1	11.1
		qGY1.2s	1H	bcd454	145.6	5.5	0.1	12.7
		qGY2.2s	2H	Vrs1	97.6	3.4	0.1	8.0
		qGY5s	5H	Bmac0306	207.3	4.0	-0.1	8.7
HI [i]	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	qHI1.1s	1H	Bmag0211	139.8	3.2	0.7	7.9
		qHI1.2s	1H	Bmac0063	150.4	3.2	0.8	9.2
GS [j]	N	qGS2n	2H	Vrs1	96.6	53.7	16.2	46.1
	S	qGS2.1s	2H	Vrs1	96.6	52.8	11.0	65.2
		qGS2.2s	2H	GBM1459	121.2	4.4	-3.3	3.3
TKW [k]	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	qTKW2s	2H	Vrs1	96.1	36.9	5.0	68.6
Na^+	N	q Na^+6n	6H	scssr5599	-	3.4	-0.8	9.6
(mgg^{-1}) [l]	S	q Na^+s	4H	GBM1323	32.6	3.2	-1.0	7.6
		q Na^+s	6H	EBmac0708(a)	44.3	3.9	-1.4	14.2
K^+	N	q K^+2n	2H	Bmag0125	88.4	4.03	-1.6	16.7
(mgg^{-1}) [m]	S	q K^+2s	2H	Bmag0125	88.4	4.0	-1.6	9.9
		q K^+6s	6H	Sukkula/Nikita-580	36.6	3.2	-1.5	9.1
K^+/Na^+	S	q K^+/Na^+3s	3H	Bmag0010	143.1	3.8	-0.1	8.7
		qK$^+$/ Na^+6s	6H	GBM19	44.7	5.1	0.1	12.1

[g] Biomass; [h] Grain Yield; [i] Harvest Index; [j] Grain number per Spike; [k] 1,000 Kernel Weight; [l]: Na content; [m] K content; [n] Environment, [o] Explained variance.

detected for spike length under the two environments. There were eight QTLs on chromosomes 1H, 2H, 3H, 4H, 5H and 7H under the normal condition accounting for 4.3 to 24.1% of the total phenotypic variation. Under salt stress, six QTLs were detected on chromosomes 1H, 3H, 4H and 7H accounting for 7 to 22.6% of the total SL variation. The QTLs on chromosome 7H, near *Bmag0110* marker and chromosome 4H, near *GBM1220* marker were mapped in both environments. QTLs for spike length were mapped on chromosomes 1H, 2H, 3H, 4H, 5H and 7H in previous studies (Ren *et*

al., 2013). Four and two QTLs on chromosomes 1H, 5H, 6H and 7H were detected for days to maturity in normal and salinity conditions, respectively. In four out of six identified QTLs, the alleles from Clipper increased days to maturity in DHs. The two QTLs, qDMA5.2n and qDMA5s, on chromosome 5H linked to *GBM1399* marker were simultaneously detected under both environments. Lines receiving allele from Sahara 3771 in this locus were matured 0.53 and 0.72 days earlier under normal and salinity stress, respectively. One QTL for days to heading and one QTL for days to
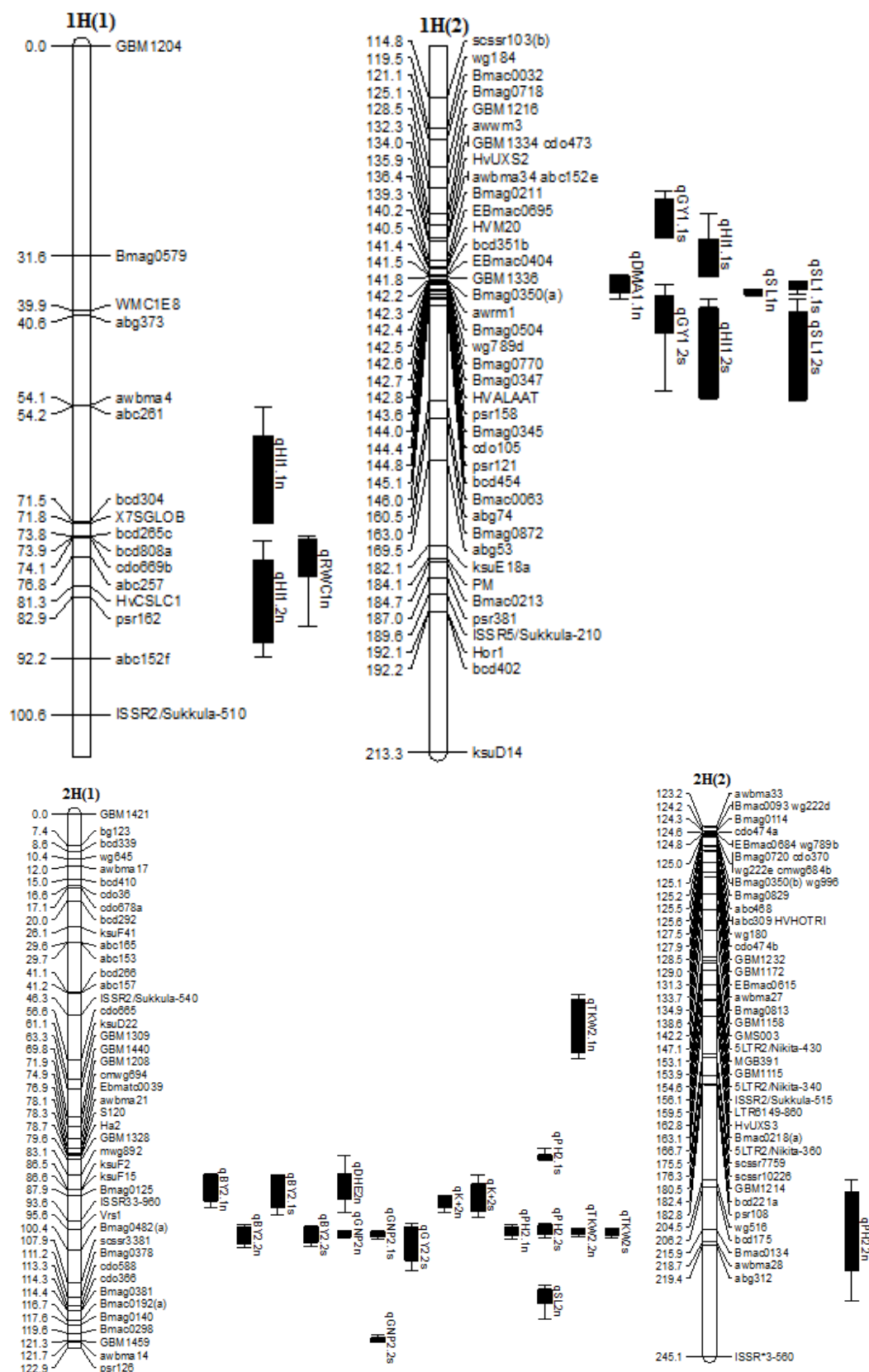
192

Figure 1. Location of QTLs for the traits identified in the barley DH population derived from a cross between Clipper×Sahara 3771. Figure 1. (continued)
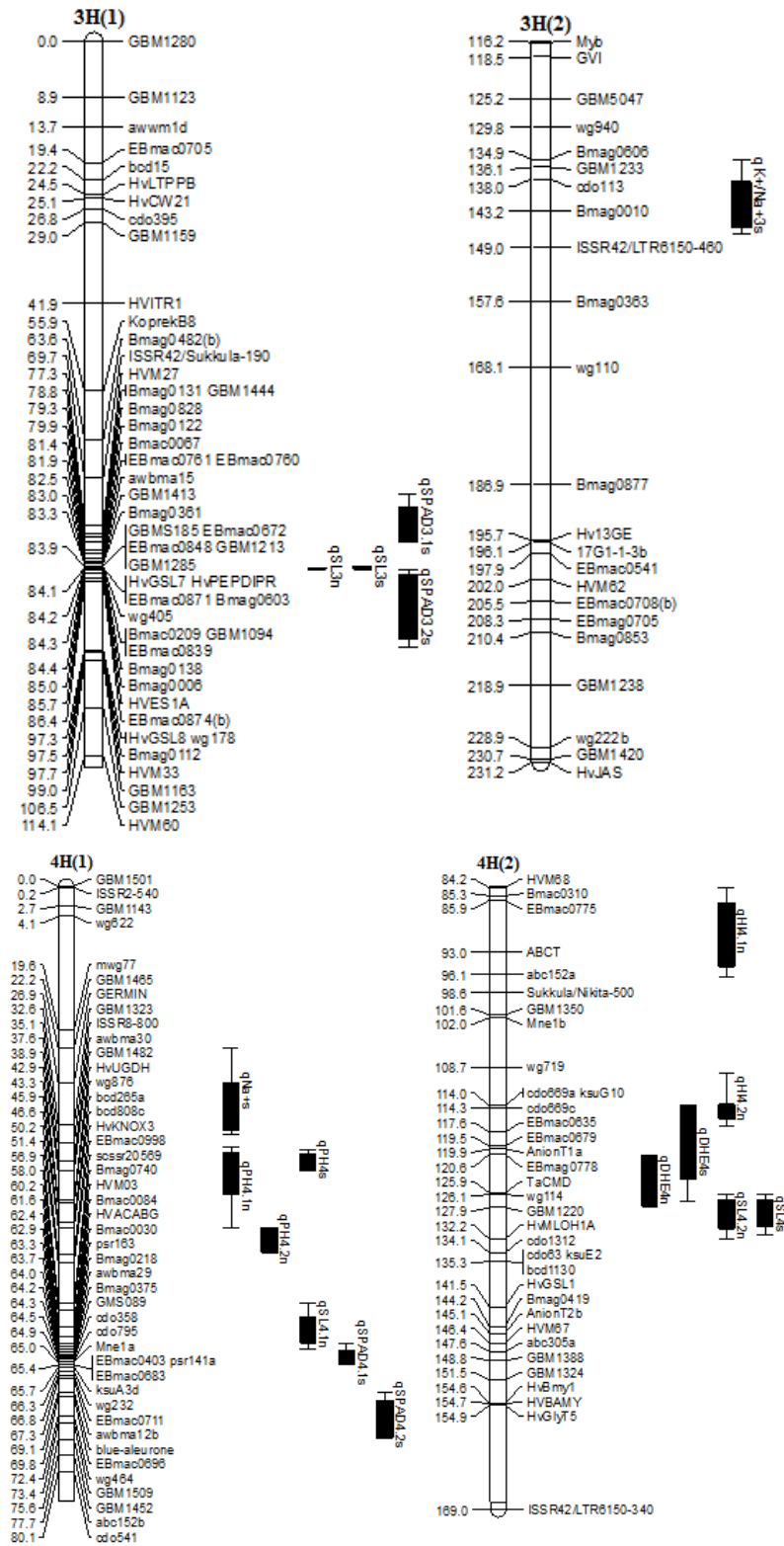
193

continued of Figure 1

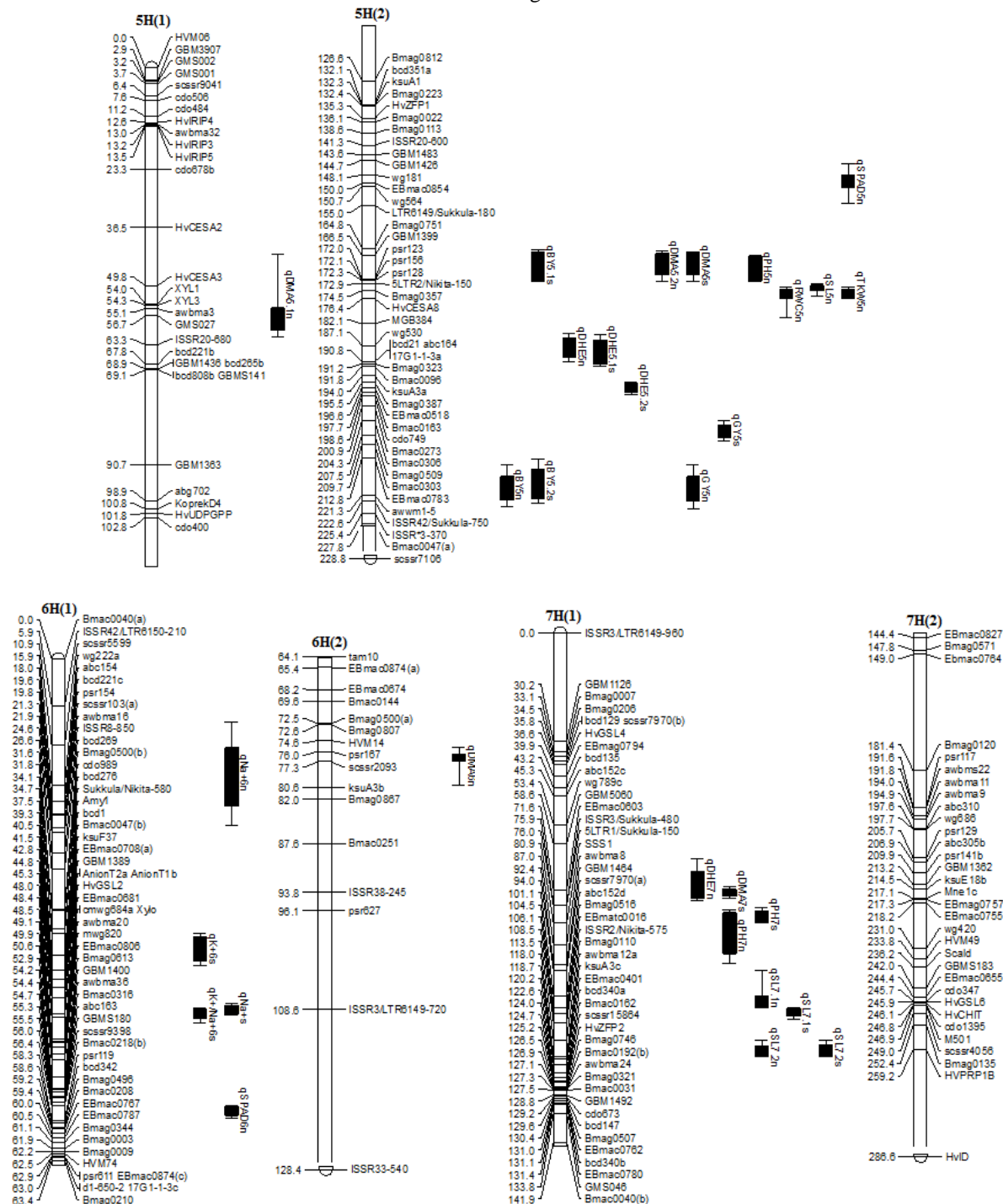**Figure 1. (continued)**

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continued of Figure 1

Figure 1. (continued)

maturity (qDHE7n and qDMA7s, respectively) were simultaneously detected under both conditions on chromosome 7H in the vicinity of *EBmac0603* marker and both had positive additive effect (Table 4). Three and four QTLs were mapped for biomass on chromosomes 2H and 5H under normal and stress environments, respectively. Two QTLs on chromosome 2H (qBY2.1n, qBY2.1s) in the vicinity of the marker *mwg892* and other two (qBY2.2n, qBY2.2s) near the marker *vrs1* were simultaneously detected under both conditions. Only one QTL for grain yield under normal condition was detected on 5H accounting for 7.8% of total phenotypic variation. Whereas, under salinity condition, four QTLs on chromosomes 1H, 2H and 5H were identified. These QTLs explained 40.53% of the total variation for grain yield. Some QTL(s) were common for grain yield and other traits. For example, the QTLs on chromosome 5H, in the vicinity of the marker *awwm1-5*, were observed for grain yield and biomass (qGY5n, qBY5n). Other QTLs on chromosome 2H in the vicinity of the marker *Vrs1* (qGY2.2s), were observed for BY (qBY2.2n, qBY2.2s), GNP (qGNP2n, qGNP2.1s), PH (qPH2.1n), TKW (qTKW2.2n, qTKW2s). Positive and significant correlations of grain yield and SL, BY, *HI* (both environments), SPAD (normal condition), DHE, PH, DMA, GS and TKW (salinity condition) were also observed. This suggested that close linkage or pleiotropy could be the possible causes of the correlations among grain yield and some traits which had similar QTL(s). Based on Paterson *et al.* (1991), pleiotropic effect of major genes and close linkage of many genes are main factors in the appearance of correlation between agronomic traits. Marquez-Cedillo *et al.* (2001) pointed out that the correlation between quantitative traits might be due to the linkage between their QTLs.

For harvest index, six QTLs were detected under the normal and salinity stress conditions, four being located on chromosomes 1H and 4H under normal

condition and two on chromosome 1H in the stress condition. One QTL in the normal and two QTLs in the saline environment on 2H were identified for grain number per spike. Two of the three mapped QTLs were major QTLs accounting for 46.1 and 65.2% of the total GS variation under normal and stress conditions, respectively. These QTLs were located in the same genomic region near *Vrs1* marker. Positive additive effects indicate that in this locus, the alleles from Clipper tended to decrease grain number per plant (16.28 and 11.01) in the normal and salt stress environments, respectively. The QTL on chromosome 2H in the vicinity of *vrs1* marker was common for the two environments. Li *et al.* (2005) mapped QTLs for grain number on chromosome 2H. Three genomic regions on chromosomes 2H and 5H associated with 1000 kernel weight in the normal environment and one on chromosome 2H in the saline condition were detected. Among these QTLs, qTKW2.2n and qTKW2s were detected simultaneously on chromosome 2H in the vicinity of *Vrs1* marker, accounting for 61.8 and 68.6% of the total TKW variation in the normal and stress environments, respectively.

For Na^+, K^+, and K^+/Na^+ ratio, respectively, 3, 3, and 2 QTLs were mapped. Of the three QTLs of Na^+ and three QTLs of K^+, one and two QTLs were identified under normal and salinity environments, respectively. For K^+/Na^+ ratio, no QTL was identified under the normal condition. All identified QTLs for Na^+ and K^+ concentrations had negative additive effects, indicating that their desirable alleles were from Sahara 3771.

In all identified QTLs for Na^+ and K^+, alleles from Sahara 3771 decreased the concentration of the ions in the DH lines as compared with those of Clipper. A common QTL (qK^+2) was mapped for K^+ under both environments on chromosome 2H near *Bmag0125* marker. One QTL for Na^+ content was identified on chromosome 4H and another two on chromosome 6H. Chromosome 4H in barley harbors several loci involved in salt and drought tolerance

(Forster *et al.,* 2000). We mapped a QTL on chromosome 4H, in the vicinity of *GBM1323*. For this QTL, the allele from Sahara 3771 decreased Na^+ and could be utilized to increase salt tolerance in breeding programs. Nguyen *et al.* (2013) used a DH population and assessed salt tolerance according to biomass production and accumulation of K^+ and Na^+ ions as the criterion under saline and non-saline conditions after cultivation in hydroponic system for three weeks. They identified two specific regions on chromosomes 4H and 6H, controlling ion content and salt tolerance, pointing to genes involved in ion homeostasis that contribute to salt tolerance.

A large number of QTLs for salt tolerance have been previously reported in barley germplasms using different mapping populations (Siahsar and Naroui, 2010; Aminfar *et al.,* 2011; Nguyen *et al.,* 2013; Sbei *et al.,* 2014). Most studies for finding QTLs controlling salt tolerance have been carried out under controlled conditions. However, under field condition, few results have been reported. Our study was carried out in the natural salinity condition.

In this study, Sahara 3771 contributed alleles associated with salt tolerance for major traits, such as grain number per spike, relative water content, Na^+ and K^+ contents as well as days to heading and chlorophyll content under stress condition (Table 4). Some QTLs were identified under both environments (stable QTLs) and were not greatly influenced by the environmental condition such as the QTL(s) for days to heading on chromosome 5H near *wg530* marker, days to maturity on chromosome 5H near *GBM1399*, biomass on chromosome 2H near *mwg892* and *Vrs1* markers, spike length on chromosomes 4H and 7H near *GBM1220* and *Bmag0110* markers, respectively, grain number per spike and 1,000 kernel weight on chromosome 2H near *Vrs1* marker, plant height on chromosome 7H near *5LTR1/Sukkula-150* marker and K^+ content on chromosome 2H near *Bmag0125* marker. For grain yield, harvest index, relative water content, chlorophyll and Na^+ contents and K^+/Na^+ ratio, no stable QTL(s) were mapped. This is likely due to the contribution of more

chromosome locations on expression of these traits and, therefore, they are greatly influenced by environmental conditions.

There was a major QTL controlling BY, GS, TKW, PH and GY, and all of these QTLs were mapped on the same region of chromosome 2H. This region which was in the vicinity of *Vrs1* marker, was associated with some QTLs including: qGY2.2s, qBY2.2n, qBY2.2s, qGNP2n, qGNP2.1s, qTKW2.2n, qTKW2s and qPH2.1n (Figure 1). Moreover, these QTLs, except for qGY2.2s and qPH2.1n, were found under both salinity and normal conditions, and all of their positive alleles came from Clipper. Therefore, this region of chromosome 2H can be used as an important target for improving salt tolerance in barley. It may be assumed that there is a QTL cluster for salt tolerance in this region of chromosome 2H (Figure 1). *Vrs1* is a locus on chromosome 2H that controls row type in barley (Komatsuda *et al.,* 2007). This locus affects directly a wide range of morphological traits related to seed yield and grain quality (Turuspekov *et al.,* 2008). Shahinnia *et al.* (2006) identified important QTLs for grain number per plant and 1,000 kernel weight on chromosome 2H between the *Vrs1* and *MWG503* markers. Marquez-Cedillo *et al.* (2001) showed a relationship between *Vrs1* locus and quantitative trait loci. Lin *et al.* (1995) suggested that in the vicinity of *Vrs1* there might be a cluster of linked independent genes, rather than a major gene with pleiotropic effect, that control yield and yield related traits.

The QTLs, qGY5n and qBY5n, were mapped on the same region in the vicinity of *awwm1-5* marker on the chromosome 5H, supporting the significant positive correlation between the two trials under normal and salinity conditions (r= 0.7[**]). Similar results were found in the vicinity of *abc257* marker on chromosome 1H and *5LTR2/Nikita-150 and awwm1-5* markers on chromosome 5H. It can be assumed that the genes in these regions, controlling *HI*, *RWC*, SL, GY, and BY are expressed under normal condition, but their expression is inhibited in the saline condition. The QTL(s) common for some traits were also

detected; for example, a QTL on chromosome 2H near *Vrs1* marker for TKW, BY and GS (both environments), PH (normal condition), GY (stress condition) and a QTL on chromosome 5H near *GBM1399* marker for DMA (normal environment), and BY (stress environment).

Different agronomic and physiological traits in barley are affected by salt stress. Understanding the genetic control of these traits could accelerate the improvement for salt tolerance. On the basis of this study, some markers could be utilized for monitoring these traits in barley breeding programs. Results showed that detected QTLs which can be useful for increasing yield under salinity stress (directly and indirectly) may be transferred to specific lines using the backcross method. Dudly (1993) and Zhou *et al.* (1999) proposed two methods for using identified QTLs in marker-assisted selection: (I) Identification of effective QTLs and transferring them to specific lines using backcrossing, and (II) Designing and pyramiding alleles of desired QTLs in a single genotype. For the validation of our results, the experiment should be repeated in other years, because the power of QTL detection can be affected by the genotype-environment interaction.

ACKNOWLEDGEMENTS

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شناسایی مکان‌های ژنی مرتبط با صفات زراعی و فیزیولوژیکی در جو تحت شرایط تنش شوری

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چکیده

تحمل شوری یک صفت پیچیده ژنتیکی و زراعی می باشد که توسط مکانهای ژنی صفات کمی کنترل می شود. به منظور مکان یابی QTLهای مرتبط با صفات زراعی و فیزیولوژیکی، ۱۴۹ لاین هاپلوئید مضاعف حاصل از تلاقی Clipper (حساس به شوری) و Sahara 3771 (متحمل به شوری) در شرایط شوری طبیعی و نرمال با استفاده از چهارده صفت مورد ارزیابی قرار گرفتند. تجزیه QTL بر اساس روش مکان یابی فاصله ای مرکب و با استفاده از یک نقشه ژنتیکی که مشتمل بر ۵۱۷ نشانگر بود و ۱۵۰۲/۴ سانتی مورگان از ژنوم جو را پوشش داده بود، انجام پذیرفت. در مجموع هفتاد و هشت QTL برای صفات روز تا گلدهی، محتوی آب نسبی، محتوی کلروفیل، ارتفاع بوته، طول سنبله، روز تا رسیدن، عملکرد بیولوژیک، عملکرد دانه، شاخص برداشت، تعداد دانه در سنبله، وزن هزار دانه، محتوی سدیم و پتاسیم و نسبت پتاسیم به سدیم شناسائی شد که چهل QTL مربوط به محیط نرمال و سی و هشت QTL مربوط به محیط شور بود. اکثر QTLهای شناسائی شده بر روی کروموزم 2H قرار داشتند و تغییرات فنوتیپی توجیه شده توسط این QTLها بین ۳/۳ تا ۶۸/۶ درصد بود. بر روی کروموزوم 2H و نزدیک نشانگر *Vrs1* یک QTL بزرگ در هر دو محیط نرمال و شور مشاهده شد که بر روی صفات بیوماس، تعداد دانه در سنبله، وزن هزار دانه، ارتفاع بوته و عملکرد دانه تاثیر داشته و می تواند در برنامه های به نژادی جو برای افزایش تحمل به شوری با استفاده از گزینش به کمک نشانگر مورد استفاده قرار گیرد. تعدادی QTLهای پایدار برای صفات روز تا گلدهی، بیوماس، طول سنبله، تعداد دانه در سنبله، وزن هزار دانه و محتوی پتاسیم نیز QTLهایی شناسائی شدند که می توانند در برنامه های به نژادی مورد استفاده قرار گیرند.