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Research paper

Identification of QTLs controlling some morphological traits in barley under salinity stress by association mapping

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Abstract

The present study used the association mapping method to identify molecular markers associated with morphological traits using 407 SSR and AFLP markers for 148 barley genotypes. This experiment was carried out as an alpha-lattice design with five incomplete blocks in two replications under normal and salinity stress conditions (EC = 12 ds m^{-1}) at the Agriculture and Natural Resources Research Station, Yazd, Iran. The genetic structure of the population was divided into two subpopulations (K = 2) using the Bayesian method and Structure 2.3.4 software. Association mapping was performed based on a mixed linear model using TASSEL4.3.15 software. Association mapping under normal and salinity stress conditions identified 38 and 43 significant marker-trait associations. Also, several common QTLs for the studied traits were identified. Common markers among traits can be due to pleiotropic effects or linkage between genomic regions involved in these traits. Several QTLs were stable for plant height and flag leaf area in different environmental conditions and can be regarded as stable QTLs. Markers HVM40-144, HVM40-147, HVM40-152, and HVM40-162 for plant height and marker Bmag0606-147 for flag leaf area showed a significant association with these traits in both normal and salinity-stress experiments. So, these QTLs can be suggested as stable gene loci. Identifying major gene loci influencing salinity tolerance in barley can assist in the breeding of salinity tolerance in this crop.

Keywords: association mapping; barley; mixed linear model; salinity stress

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Introduction

Salinity in many arid and semi-arid regions is considered a common agricultural problem and a limiting factor in crop yield. Barley (*Hordeum vulgare* L.) is one of the plant species that tolerate salinity (Colmer *et al.* 2005; Munns 2005).

Association mapping is a new and powerful tool to increase the information obtained from the linkage analysis for the genetic study of quantitative traits. Marker information obtained from linkage maps has some constraints such as the unavailability of segregating populations, lack of proper linkage between plant traits and molecular markers, and insufficient time, which reduce the efficiency of these maps in identifying markers related to plant traits (Gupta *et al.* 2005). Association analysis provides appropriate information for researchers by eliminating these

limitations, considering the structure and kinship relationships [mixed linear model (MLM) method], and eliminating false marker-trait associations. Elakhdar *et al.* (2016b), over two years, identified 46 QTLs for 14 traits and one major QTL that controled salinity tolerance on chromosomes 1H, 2H, 4H, and 7H, which are important in improving barley salinity tolerance. A study to determine the QTLs of salinity tolerance in barley was performed with the association mapping method by Sbei *et al.* (2014). In this experiment, a wide range of salinity tolerance was observed among barley genotypes, and seven effective QTLs were located on chromosomes 1H, 2H, 3H, 4H, and 5H. Others such as Eleuch *et al.* (2008), Inostroza *et al.* (2009), EL-Denary *et al.* (2012), Long *et al.* (2013), Elakhdar *et al.* (2016a), and Fan *et al.* (2016) also used association mapping under salinity stress in barley.

Identification of major loci affecting salinity tolerance in barley can increase the efficiency of breeding for this characteristic. Therefore, this study was conducted to determine the markers associated with some morphological traits of barley by association mapping under normal and salinity-stress conditions.

Materials and Methods

Germplasm

In this study 148 modern European two-row spring barley cultivars, representing commercial germplasm from northern and western Europe were investigated (Kraakman *et al.* 2004). The seeds were received from Khorasan Razavi Agricultural and Natural Resources Research and Education Center, Iran.

Phenotyping

The experiment was conducted as an alpha-lattice design with five incomplete blocks in two replications under normal and salinity-stress (EC= 12 ds m^{-1}) environments at the Agriculture and Natural Resources Research Station of Yazd (31° 55' N, 54° 16' E, 1213 m from sea level), Iran. Each block included 30 plots. Salinity treatment was applied with the irrigation water. The field soil in this experiment was naturally saline. Soil salinity was measured regularly during the growth period. The soil salinity was kept constant at the desired level through the amount of water used and the need for soil leaching. The studied traits include biomass, plant height, spike length, flag leaf length, flag leaf width, and flag leaf area. The data normality test was performed based on the Kolmogorov-Smirnov method using SPSS software. Then, the combined analysis of variance was performed with SAS 9.1 software.

Genotyping

In this study, a genetic map of molecular markers, including 407 AFLP and SSR markers, which was prepared by Kraakman *et al.* (2004), Kraakman *et al.* (2006), and Aghnoum *et al.* (Unpublished data) was used. Kraakman *et al.* (2004) used 14 AFLP primers (E33M54, E35M48, E35M54, E35M55, E35M61, E37M33, E38M50, E38M54, E38M55, E39M61, E42M32, E42M48, E45M49, E45M55) for genotyping and identified 286 polymorphic markers. Then, in 2006, 11 SSR primers (Bmac0018, Bmag0009, HVM14, HVM22, HVM65, HVM74, Bmag0223, Bmac0134, HVM54, Bmac0163, Bmac0316) were added to the genotyping map (Kraakman *et al.* 2006). Also,

Aghnoum *et al.* (unpublished data) mapped 21 SSR molecular markers (EBmac0603, GBMS035, HVM36, scssr10559, Bmag0225, Bmag0841, Bmag0606, Bmag0013, HVM40, GBM1482, GBM1015, GBMS062, Bmac0399, EBmac0560, HvHVA1, Bmag0500, GBM1021, Bmag0173, scssr07106, Bmag0357, Bmag0222) in this population. Finally, considering all the different alleles of AFLP and SSR markers, 407 polymorphic markers were used in their population. Aghnoum *et al.* (2010) obtained the sites of mapped QTLs from an integrated barley genetic map consisting of 6990 molecular markers. This integrated genetic map included seven linkage groups and the molecular markers density was 0.125 markers per cM.

Population structure (Q-matrix) and kinship relationships (K-matrix)

Since natural populations are used in the association analysis studies, there should be no structure in the population because the presence of structure may cause unreliable results. Therefore, if in association mapping, the effect of population structure and kinship relationships is not considered, the linkage equilibrium increases. As a result, false-positive results occur, leading to false marker-trait associations (Breseghello and Sorrells 2006; Yu and Buckler 2006; Zhang *et al.* 2012). Therefore, to determine the population structure (Q-matrix), the Bayesian method and Structure 2.3.4 software (Pritchard *et al.* 2000; Falush *et al.* 2003) were used on the genotypic data. The Bayesian method attributes each genotype to hypothetical subpopulations with a probability that in each subpopulation, the linkage disequilibrium is minimum and the gamete equilibrium is maximum. The analysis was performed on 148 barley genotypes in the Admixture model. The length of the Burnin period was 100,000, and the number of Markov Chain Monte Carlo (MCMC) replications was 100,000. K was set from 1 to 10, and 10 iterations was considered. The optimal K was determined based on the delta K method. Finally, the Q-matrix was calculated with the same software by determining the optimal K, related to the highest value of delta K. Also, using genotypic data, the kinship relationships (K-matrix) were determined by TASSEL4.3.15 software.

Linkage disequilibrium and association analysis

To do the associations mapping, the linkage disequilibrium for each pair of markers was estimated by the r^2 statistic for each linkage group with TASSEL 4.3.15 software (Bradbury *et al.* 2007). Marker-trait associations were determined using the MLM with TASSEL 4.3.15 software. In the MLM method, in addition to the genotypic data, the phenotypic data, population structure (Q-matrix), and kinship relationships (K-matrix) were also used as covariates in the model (Yu *et al.* 2006). In the association analysis, only the markers with a frequency of more than 10% were used, and the p-value with 1000 permutations was estimated. Finally, MapChart software was utilized to show the mapped gene loci.

Results

Analysis of variance

The combined analysis of variance in normal and salinity-stress conditions showed significant genetic variability among genotypes in all traits

except biomass, indicating large diversity in the population (Table 1). The genotype × environment interaction was significant for the plant height and spike length. The effect of the environment was significant for biomass, plant height, and spike length.

Population structure

According to Table 2 and Figure 1, the K = 2, which corresponds to the highest value of Delta K, was determined as the optimum K, so it was the most appropriate number to calculate the Q-matrix. Finally, the Q-matrix was obtained by placing K = 2 in the Structure 2.3.4 software.

The bar plot provided by Structure 2.3.4 software for 148 barley genotypes (Figure 2) also confirms the optimum K value. The horizontal axis is related to the genotypes, and the vertical axis shows the share of each genotype in each group. In this bar plot, when the percentage of genotype membership in one cluster was more than or equal to 0.7, the genotype was assigned to that cluster. If the membership percentage was less than this value, it was considered a mixed genotype (Spataro *et al.* 2011). Here, each group was marked with a distinct color and the two separate colors for each genotype indicated that the genotype belongs to one of the two groups or both groups. Then, the number of clusters that better represented the population structure (kinship relationships defined by the K-matrix) was determined by TASSEL4.3.15 software for use in the MLM method.

Table 1. Combined analysis of variance of the studied traits in non-stress and salinity stress conditions

Source of variation	df	Mean squares					
		Bio	PH	SL	FLL	FLW	FLA
Environment (E)	1	60^{**}	10445.8^{**}	497917.5^{**}	$5.24^{n.s}$	$0.06^{n.s}$	$3.75^{n.s}$
Rep / E	2	18.7^{**}	389.7^{**}	$60.4^{n.s}$	9.28^{*}	0.08^{*}	$15.8^{n.s}$
Genotype (G)	147	$2.04^{n.s}$	94.8^{**}	86.1^{**}	6.35^{**}	0.05^{**}	13.14^{**}
G × E	147	$2.15^{n.s}$	41.3^{**}	75.3^{**}	$2.5^{n.s}$	$0.025^{n.s}$	$8.5^{n.s}$
Block	16	$2.44^{n.s}$	83.4^{**}	$76.7^{n.s}$	$3.3^{n.s}$	0.06^{**}	$13^{n.s}$
Error	277	2.04	28.6	51.9	2.44	0.024	8.9
R-square (%)	-	57	81	97.3	67	64.6	58
CV (%)	-	68	11	19.8	25.6	37.2	133.9

n.s, * and **: Not-significant and significant at 5% and 1% probability levels, respectively. Bio: Biomass, PH: Plant height, SL: Spike length, FLL: Flag leaf length, FLW: Flag leaf width, FLA: Flag leaf area, CV: Coefficient of variation.

Table 2. Statistics calculated for optimum K values using Structure software 2.3.4.

K	L(K)	Stdev	L'(K)	L''(K)	|L''(K)|	Delta K
1	-24682.1	1.12	-	-	-	-
2	-23249.7	2.71	1432.41	-611.03	611.03	225.27
3	-22428.3	8.24	821.38	22.84	22.84	2.77
4	-21584.1	218.84	844.22	-289.44	289.44	1.32
5	-21029.4	197.77	554.78	-118.56	118.56	0.599
6	-20593.1	122.63	436.22	-218.4	218.4	1.78
7	-20375.3	50.22	217.82	44.17	44.17	0.88
8	-20113.3	95.84	261.98	-3525.2	3525.2	36.78
9	-23376.6	8275.19	-3263.22	5002.65	5002.65	0.605
10	-21637.1	3430.18	1739.43	-1739.43	1739.43	0.507

L(K): LnP(D) average of all iterations for each K, L'(K): $L(K)_n$- $L(K)_{n-1}$, L''(K): $L'(K)_n$- $L'(K)_{n-1}$, Delta K (ΔK):|L''(K)|/ Stdev.

Linkage disequilibrium and association mapping

The r^2 statistic estimated the linkage disequilibrium associated with each pair of markers for each linkage group (multi-allelic gene locus), and the average of r^2 was 0.02. The results obtained from the MLM identified 38 and 43 significant marker-trait associations ($p< 0.001$) under normal and salinity stress conditions, respectively (Table 3). In the normal conditions, the markers that were associated with different traits were as follows: 10 DNA markers with biomass (on chromosomes 2H, 3H, 4H, 5H, 6H, and 7H), nine markers with the plant height (on chromosomes 4H and 7H), two markers with the flad leaf length (on chromosome 3H), twomarkers with the flag leaf width (on chromosome 3H), and 15 markers with the flag leaf area (3H, 5H, and 7H). Under salinity stress (Table 4), the associated markers were as follows: one DNA marker with biomass, four markers with the plant height (on chromosome 4H), three markers with spike length (on chromosomes 3H and 6H), 10 markers with the flag leaf length (on chromosomes

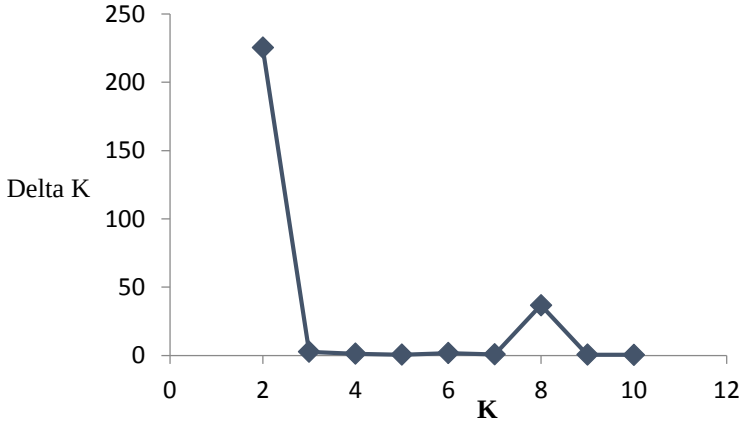

Figure 1. A two-way graph to determine the optimum K value using 2.3.4 Structure software.

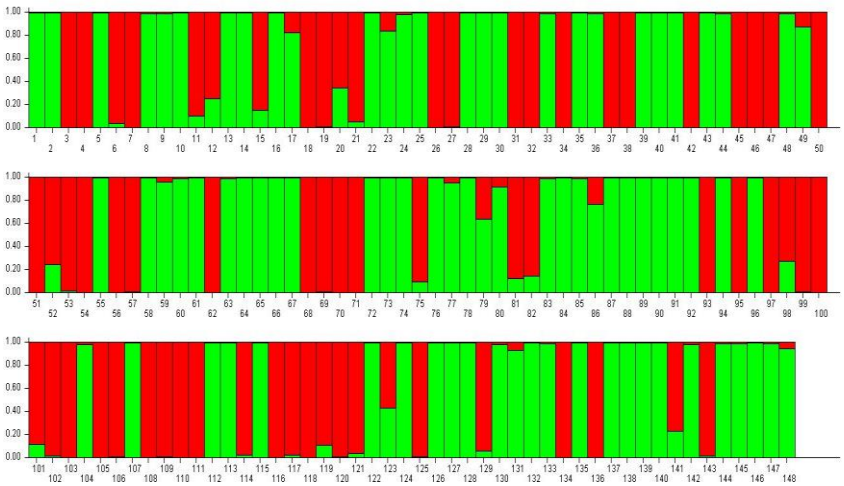

Figure 2. The bar plot was drawn based on 407 AFLP and SSR markers by Structure 2.3.4 software; the horizontal axis is related to the genotypes, and the vertical axis shows the share of each genotype in each group.

Table 3. Markers associated with studied traits in barley genotypes based on the mixed linear model under normal conditions.

Trait	Marker	R^2	P-value	Chromosome	Position (cM)
BY	E33M54-100	0.25	0.000000	77.5	4H
	E37M33-256	0.11	0.00047	-	Unmapped
	E37M33-260	0.12	0.0002	-	Unmapped
	E37M33-583	0.11	0.00034	24	Z
	E42M32-156	0.10	0.0008	-	Unmapped
	E42M32-200	0.13	0.0001	77.8	5H
	E42M32-231	0.13	0.00015	23.5	7H
	E42M32-271	0.15	0.00003	-	Unmapped
	E42M32-338	0.10	0.001	113.5	2H
	Bmag0500-194	0.17	0.00001	29.2	6H
PH	E42M48-087	0.11	0.0005	-	Unmapped
	EBmac0603-183	0.11	0.0007	38.3	7H
	EBmac0603-143	0.10	0.001	38.3	7H
	GBMS035-147	0.11	0.0005	49	7H
	GBMS035-137	0.13	0.00018	49	7H
	HVM40-144	0.13	0.00016	32.3	4H
	HVM40-147	0.14	0.00006	32.3	4H
	HVM40-152	0.13	0.00013	32.3	4H
	HVM40-162	0.14	0.00006	32.3	4H
SL	-	-	-	-	-
FLL	Bmag0606-126	0.11	0.00053	112.5	3H
	Bmag0606-269	0.08	0.00066	112.5	3H
FLW	Bmag0606-126	0.11	0.00055	112.5	3H
	Bmag0606-269	0.08	0.00075	112.5	3H
FLA	EBmac0603-170	0.11	0.00061	38.3	7H
	EBmac0603-183	0.10	0.00082	38.3	7H
	EBmac0603-143	0.10	0.00088	38.3	7H
	EBmac0603-178	0.10	0.00093	38.3	7H
	EBmac0603-153	0.10	0.00094	38.3	7H
	GBMS035-137	0.11	0.00065	49	7H
	Bmag0606-151	0.11	0.00056	112.5	3H
	Bmag0606-138	0.11	0.00055	112.5	3H
	Bmag0606-126	0.13	0.00017	112.5	3H
	Bmag0606-147	0.12	0.00022	112.5	3H
	Bmag0606-118	0.11	0.0005	112.5	3H
	Bmag0606-122	0.11	0.0005	112.5	3H
	Bmag0606-269	0.11	0.00011	112.5	3H
	Bmag0222-153	0.11	0.00063	141.7	5H
	Bmag0222-185	0.11	0.00063	141.7	5H

See Table 1 for the abbreviation of the traits used here. R^2: Coefficient of determination, cM: Centimorgan.

1H, 4H, and 6H), 11 markers with the flag leaf width (on chromosomes 1H, 3H, 4H, and 6H), and 14 markers with the flag leaf area (on chromosomes 1H, 3H, 4H, and 6H). The genetic map of SSR and AFLP markers and the genomic location of markers with significant association with the studied traits are shown in Figure 3.

Discussion

The combined analysis of variance showed large genetic variability among the barley genotypes for the studied traits. The significant genotype × environment interaction for the plant height and spike length indicates different responses of the genotypes to the two environmental conditions for these traits. Zaare and Jafari (2013) and Khalili and Mohammadian (2016) also reported significant genotype × environment interaction for some traits in salinity conditions. G × E interaction usually affects the efficiency of phenotypic selection in

Table 4. Markers associated with studied traits in barley genotypes based on the mixed linear model under salinity stress conditions.

Trait	Marker	R^2	P-value	Chromosome	Position (cM)
BY	E42M32-273	0.10	0.001	-	Unmapped
PH	HVM40-144	0.16	0.00002	32.3	4H
	HVM40-147	0.16	0.00002	32.3	4H
	HVM40-152	0.16	0.00002	32.3	4H
	HVM40-162	0.17	0.00001	32.3	4H
SL	Bmag0606-269	0.08	0.0008	112.5	3H
	HVM65-131	0.1	0.0007	60.7	6H
	HVM65-132	0.1	0.0007	60.7	6H
FLL	HVM40-144	0.11	0.001	32.3	4H
	HVM40-147	0.11	0.0004	32.3	4H
	HVM40-162	0.12	0.0002	32.3	4H
	HvHVA1-140	0.10	0.001	102.49	1H
	Bmag0500-110	0.12	0.0004	29.2	6H
	Bmag0500-146	0.12	0.0002	29.2	6H
	Bmag0500-166	0.12	0.0003	29.2	6H
	Bmag0500-181	0.11	0.0004	29.2	6H
	Bmag0500-192	0.11	0.00037	29.2	6H
	Bmag0500-194	0.11	0.00037	29.2	6H
FLW	Bmag0606-147	0.11	0.0006	112.5	3H
	HVM40-144	0.11	0.00059	32.3	4H
	HVM40-162	0.12	0.00033	32.3	4H
	Bmac0399-152	0.10	0.001	30.7	1H
	Bmag0500-110	0.11	0.0005	29.2	6H
	Bmag0500-146	0.12	0.0003	29.2	6H
	Bmag0500-166	0.12	0.0003	29.2	6H
	Bmag0500-181	0.11	0.0005	29.2	6H
	Bmag0500-192	0.11	0.0005	29.2	6H
	Bmag0500-194	0.11	0.00041	29.2	6H
	Bmag0173-156	0.11	0.00055	57.79	6H
FLA	Bmag0606-147	0.11	0.0007	112.5	3H
	HVM40-144	0.11	0.0005	32.3	4H
	HVM40-147	0.10	0.0007	32.3	4H
	HVM40-162	0.12	0.0002	32.3	4H
	Bmac0399-138	0.10	0.001	30.7	1H
	Bmac0399-143	0.10	0.0007	30.7	1H
	Bmac0399-152	0.11	0.00062	30.7	1H
	Bmag0500-110	0.11	0.0004	29.2	6H
	Bmag0500-146	0.12	0.00022	29.2	6H
	Bmag0500-166	0.12	0.0003	29.2	6H
	Bmag0500-181	0.11	0.0004	29.2	6H
	Bmag0500-192	0.11	0.0004	29.2	6H
	Bmag0500-194	0.11	0.00038	29.2	6H
	Bmag0173-156	0.10	0.0008	57.79	6H

See Table 1 for the abbreviation of the traits used here, R^2: Coefficient of determination, cM: Centimorgan.

breeding programs (Sallam *et al.* 2019).

In genetic studies, population structure describes the relationships of the individuals within and between populations and provides an overview of evolutionary relationships in a population. Ideally, for an association mapping, there should be no structure in the population because the structure can be a barrier to achieving reliable results. Hence, determining the population structure as a prerequisite in association mapping can prevent false-positive associations between markers and traits (Pritchard and Donnelly 2001). This study subdivided barley cultivars into two subpopulations. Some reports suggest that the

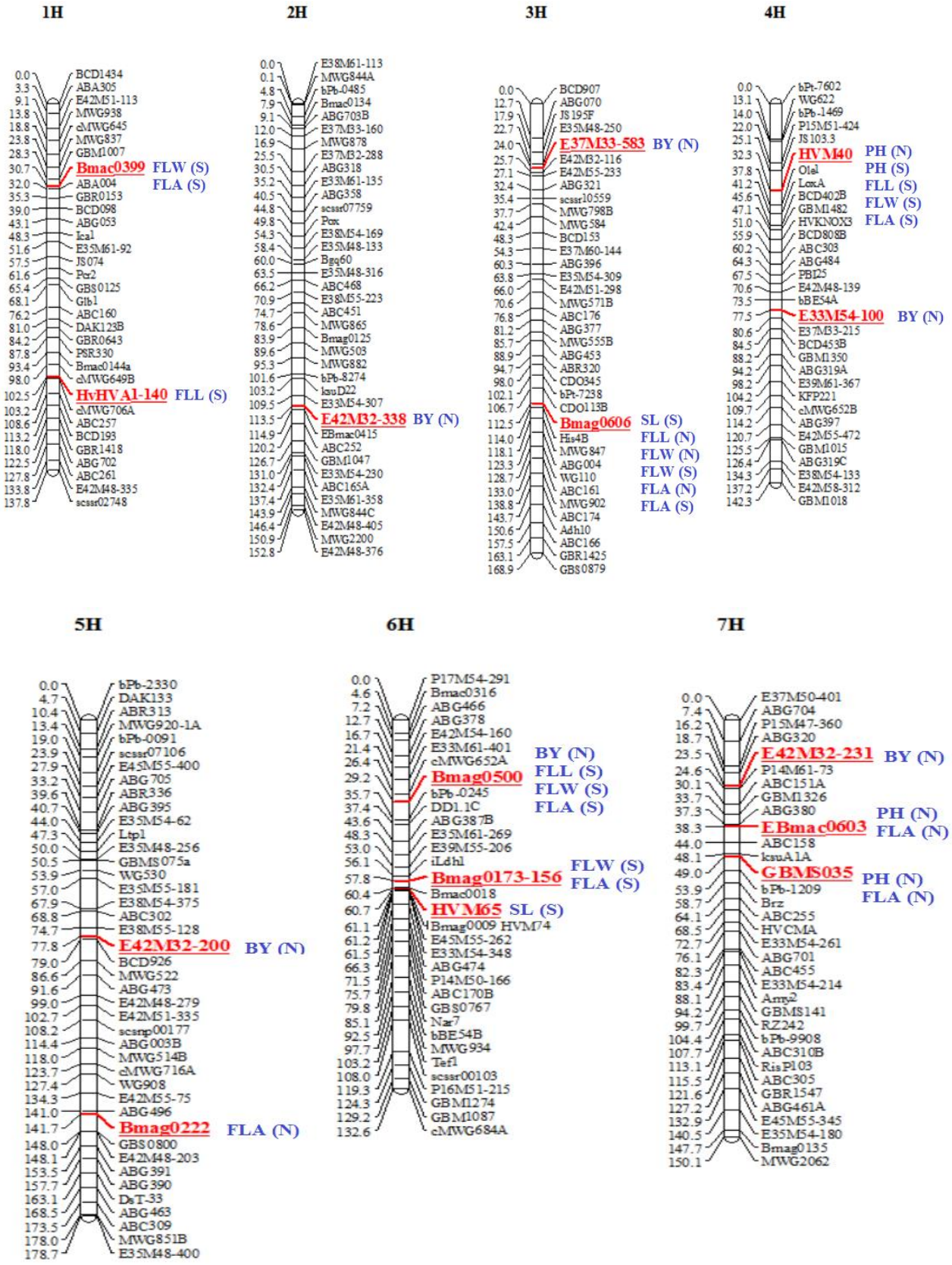

Figure 3. The genetic map of SSR and AFLP markers and genomic location of markers significantly associated with the studied traits in barley (See Table 1 for the abbreviation of the traits used here, S: Salinity stress, N: Normal).

population structure of barley cultivars is related to spike morphology (two-rowed versus six-rowed cultivars) (Pasam *et al.* 2012). In the association mapping method, QTLs are located based on linkage disequilibrium (Gupta *et al.* 2005). In this study, the mean of r^2, an indicator for linkage disequilibrium, was 0.02, which indicates that some loci are in linkage disequilibrium. Several studies have previously reported different rates of linkage disequilibrium in different barley populations (Caldwell *et al.* 2006; Ramsay *et al.* 2011) and among different chromosomes (Rostoks *et al.* 2006). Caldwell *et al.* (2006) reported rapid decay of linkage disequilibrium in barley landraces compared to barley cultivars. Eleuch *et al.* (2008), Inostroza *et al.* (2009), EL-Denary *et al.* (2012), Long *et al.* (2013), Sbei *et al.* (2014), Elakhdar *et al.* (2016a), Elakhdar *et al.* (2016b) and Fan *et al.* (2016) used association mapping under salinity stress in barley.

Eighty-one significant markers were identified for the studied traits in normal and salinity-stress conditions. This study found 10 QTLs for biomass on chromosomes 2H (113.5 cM), 3H (24 cM), 4H (77.5 cM), 5H (77.8 cM), 6H (29.2 cM), 7H (23.5 cM), and four QTLs with unknown gene locations in normal conditions. Gene locations identified were unknown under salinity stress conditions. Elakhdar *et al.* (2016b) identified biomass on chromosomes 4H (58.6 cM), 6H (7.16 cM), 7H (65.9 cM), and 7H (97 cM) under salinity-stress conditions in barley.

This study detected nine and four significant marker-trait associations for plant height under normal and salinity-stress conditions. At salinity-stress conditions, four QTLs on chromosome 4H (32.3 cM) and in normal conditions, five QTLs on chromosome 4H (38.3 cM), two QTLs on 7H (38 cM), and two QTLs on 7H (49 cM) were identified for the plant height. The QTLs identified in two close positions (38.3 and 49 cM) on chromosome 7H, indicated that plant height is probably associated with this position. Xu *et al.* (2012) identified this trait on chromosome 7H under normal conditions in barley, which is consistent with our results. Elakhdar *et al.* (2016b) in a study on barley at normal and salinity stress conditions, showed that this trait had a significant association with marker EBmac0603 on chromosome 7H at 35.39 cM position, which is similar to our results. Sayed *et al.* (2021) identified plant height loci on chromosome 7H, Long *et al.* (2013) on chromosomes 2H (59.2 cM), 6H (60.2 cM), 7H (4.9 cM), and 7H (61.3 cM), Eleuch *et al.* (2008) on 1H (62 cM) and 6H (10 cM), Inostroza *et al.* (2009) on 2H (5, 50, and 44 cM), 4H (78 and 118 cM), 5H (66 and 126 cM), 6H (79), and 7H (80, 85 and 107 cM), EL-Denary *et al.* (2012) on 2H, Xue *et al.* (2009) on 3H under salinity stress conditions in barley.

In this study, two QTLs were identified for the spike length on chromosome 6H at 60.7 cM and one QTL on chromosome 3H at 112 cM in the salinity-stress conditions. Under normal conditions, no significant association was observed with the markers for the spike length. Wang *et al.* (2014) identified loci associated with this trait on chromosomes 2H and 5H in barley. Jabbari *et al.* (2018) identified loci for spike length on chromosome 5H (86.88 and 41.4 cM) under

normal conditions in barley.

Based on the results, two QTLs were identified for the flag leaf length on chromosome 3H (112.5 cM) in normal conditions and six QTLs on chromosome 6H (29.2 cM), three QTLs on chromosome 4H (32.3 cM), and one QTL on chromosome 1H (102.49 cM) in salinity-stress conditions. Jabbari *et al.* (2018), under normal conditions, identified QTLs for this trait on chromosomes 2H, 5H, 5H, and 6H, which were located in the positions of 3.8, 5.55, 157.148, and 121.819 cM, respectively in barley. Koochakpour *et al.* (2021), in a study on barley, identified genomic loci for the flag leaf length on chromosomes 2H, 4H, and 4H, which were located in the positions of 14.77, 120.64, and 125.05 cM, respectively. Also, Gyenis *et al.* (2007) identified three QTLs on 3H, 5H, and 7H, Xue *et al*. (2008) four QTLs on 5H and 7H, Liu *et al.* (2015) seven QTLs on 2H, 3H, and 7H for the flag leaf length.

In this study, two QTLs were identified for the flag leaf width on chromosome 3H (112.5 cM) in normal conditions and one QTL on chromosome 1H (30.7 cM), one QTL on 3H (112.5 cM), six QTLs on 6H (29.2 cM), two QTLs on 4H (32.3 cM), and one QTL on 6H (57.79 cM) in salinity-stress conditions. Jabbari *et al.* (2018), under normal conditions in barley, identified genomic loci for this trait on chromosomes 2H, 3H, 5H, and 7H, which were located at positions 3.8, 10.66, 130.99, and 25.31 cM, respectively. Gyenis *et al.* (2007) found three QTLs on 2H, 4H, and 5H, Liu *et al.* (2015) identified five QTLs on 2H and 4H, and Shahraki and Fakheri (2016) located three QTLs on 2H and 5H which were associated with the flag leaf length.

This study detected 15 and 14 significant marker-trait associations for the flag leaf area in normal and salinity-stress conditions, respectively as follows: seven QTLs on chromosome 3H (112.5 cM), two QTLs on 5H (141.7 cM), five QTLs on 7H (38.3 cM), and one QTL on 7H (49 cM) in normal conditions, and three QTLs on 1H (30.7 cM), one QTL on 3H (112.5 cM), six QTLs on 6H (29.2 cM), three QTLs on 4H (32.3 cM), and one QTL on 6H (57.79 cM) under salinity-stress conditions. ELakhdar *et al.* (2016a) identified genomic loci for this trait on chromosome 1H at 87.83 cM under salinity-stress conditions.

Some of the identified DNA markers were common among several traits in this study. Bmag0606-126 and Bmag0606-269 on chromosome 3H at 112.5 cM were common for the flag leaf length, flag leaf width, and flag leaf area in normal conditions. Also, EBmac0603-183 and EBmac0603-143 on chromosome 7H at 38.3 cM and GBMS035-137 on 7H at 49 cM were common for the plant height and flag leaf area.

Under salinity-stress conditions, HVM40-144 and HVM40-162 on chromosome 4H at 32.3 cM were common for the plant height, flag leaf length, flag leaf width, and flag leaf area. Also, HVM40-147 on chromosome 4H at 32.3 cM was common for the plant height, flag leaf length, and flag leaf area. Bmag0500-110, Bmag0500-146, Bmag0500-166, Bmag0500-181, Bmag0500-194, and Bmag0500-192 on chromosome 6H at 29.2 cM were common for flag leaf length, flag leaf width, and flag leaf area. Bmag0606-147 on chromosome 3H at position 112.5 cM, Bmac0399-152 on chromosome 1H at 30.7 cM, and Bmag0173-156 on chromosome 6H at 57.79 cM were common for

flag leaf width and flag leaf area. Common markers among traits can be due to pleiotropic effects or linkage between genomic regions involved in these traits (Jun *et al*. 2008). Of course, the presence of common markers is valuable when they are associated with large-effect QTLs, and are also stable that can be identified by repeated testing. However, in this experiment, the coefficient of determination was negligible for most traits. However, this phenomenon was not unexpected because the nature of QTLs is such that several positions are involved in one trait, and a high coefficient of determination for a marker is unexpected.

Gene loci that act the same in different environments can be introduced as stable QTLs. The stability of QTLs in different environments is due to the control of traits by a small number of large-effect gene loci. In this case, the marker-assisted selection will be efficient in this population. In our study, a significant association of HVM40-144, HVM40-147, HVM40-152, and HVM40-162 on chromosome 4H at 32.3 cM with plant height and Bmag0606-147 on chromosome 3H at 112.5 cM with flag leaf area was observed in both normal and salinity-stress experiments. So these QTLs can be introduced as stable gene loci.

Conclusion

The present study showed that the MLM method can effectively identify markers associated with morphological traits. In this study, several common QTLs for the measured traits were identified. Common markers among traits can be due to pleiotropic effects or linkage between genomic regions governing these traits. Also, several QTLs were stable for plant height and flag leaf area in different environmental conditions. A significant association of markers HVM40-144, HVM40-147, HVM40-152, and HVM40-162 with plant height and marker Bmag0606-147 with the flag leaf area in both normal and salinity-stress experiments was observed. So these QTLs can be introduced as stable gene loci. Identifying major loci influencing salinity tolerance in barley can help the breeders to efficiently select for salinity tolerance in the breeding programs.

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Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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شناسایی مکان‌های ژنی کنترل کننده برخی صفات مورفولوژیک جو تحت تنش شوری با نقشه‌یابی ارتباطی

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چکیده

پژوهش حاضر در راستای شناسایی نشانگرهای مولکولی مرتبط با صفات مورفولوژیک و با استفاده از ۴۰۷ نشانگر SSR و AFLP روی ۱۴۸ ژنوتیپ جو به روش نقشه یابی ارتباطی انجام شد. این آزمایش در قالب طرح آلفا لاتیس با پنج بلوک ناقص در دو تکرار تحت شرایط بدون تنش و تنش شوری ($EC= 12\ dsm^{-1}$) در مزرعه مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی یزد صورت گرفت. ساختار ژنتیکی جمعیت با روش بیزی و نرم افزار Structure 2.3.4 به دو زیرجمعیت فرعی (K=۲) تقسیم شد. نقشه‌یابی ارتباطی بر اساس مدل خطی مخلوط با استفاده از نرم‌افزار TASSEL4.3.15 انجام شد. به کمک نقشه‌یابی ارتباطی در شرایط نرمال و تنش شوری به ترتیب ۳۸ و ۴۳ ارتباط معنی‌دار نشانگر-صفت مشاهده شد. در این مطالعه چندین QTL مشترک برای صفات مورد مطالعه شناسایی شد. وجود نشانگرهای مشترک در میان صفات می‌تواند ناشی از اثرات پلیوتروپی و یا پیوستگی نواحی ژنومی دخیل در کنترل این صفات باشد. تعدادی از QTLها برای صفات ارتفاع بوته و مساحت برگ پرچم در شرایط محیطی متفاوت پایدار بودند که به عنوان QTLهای پایدار معرفی شدند. نشانگرهای HVM40-144، HVM40-147، HVM40-152 و HVM40-162 با ارتفاع بوته و نشانگر Bmag0606-147 با مساحت برگ پرچم در هر دو آزمایش نرمال و تنش شوری ارتباط معنی‌داری نشان دادند. پس، این QTLها را می‌توان به‌عنوان مکان‌های ژنی پایدار معرفی کرد. شناسایی مکان‌های ژنی اصلی مؤثر در تحمل به شوری در جو می‌تواند به اصلاح تحمل به شوری در این گیاه کمک کند.

واژه‌های کلیدی: تنش شوری؛ جو؛ مدل خطی مخلوط؛ نقشه‌یابی ارتباطی