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Genetic structure of germination parameters in Iranian wheat RILs under salinity stress

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ABSTRACT

In order to increase global wheat production, it is necessary to examine different ways of increasing yield. One of the solutions is to identify the genes that control different stress tolerance indices. This research aims to identify the genes controlling quantitative traits under normal conditions and salt stress in the germination stage. The experiments were carried out in a factorial form using a completely randomized design with 3 replications, on 107 lines resulted from the crossing of Gonbad and Zagros cultivars at Gonbad Kavous University, 2021. A linkage map was obtained using 519 SSR, 8 CAAT, 33 IJS, 47 iPBS, 3 IRAP, 17 RAPD, 8 SCoT and 12 ISSR markers on 21 wheat chromosomes. The length of the linkage map was 4918.94 cM and the distance between two adjacent markers about 5.55 cM. A total of 84 QTLs were detected in normal and salinity stress conditions (control, 6 dS/m, 12 dS/m), of which seven QTLs were related to control condition, 42 QTLs were related to 6 dS/m salinity stress and 35 QTLs were related to 12 dS/m stress condition. qLR-B3, qMGT-A5, qR/SDW-B2, qR/SDW-A3, qR/SDW-B7, qLS-A5 and qILVS-A5 were detected under control condition. qSLI-D6 was identified as a major QTL for SLI under 6 dS/m salinity stress by explaining more than 44% of the phenotypic variation of the trait. In the 12 dS/m salinity stress, several gene loci of large effect QTLs were detected, among which qIWVS-B3 explained more than 55% of the phenotypic diversity of the trait. After validation, the results of this research can introduce suitable candidates for marker-assisted selection programs in the population of Iranian wheat RILs.

Key words: Abiotic stress, Linkage map, SSR, QTL.

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INTRODUCTION

In recent years, with the increase in the world population, the demand for food has increased. However, climate change, pests and environmental pollution cause many challenges to respond to this increase. These factors can affect agricultural production and seed quality. Wheat (*Triticum aestivum* L.) is one of the most important food products in the world (Asseng *et al*., 2020), and is the source of energy for more than half of the world's population (Lian *et al*., 2020). Quality seeds are an important tool in the germination and rapid growth of the wheat (Meng *et al*., 2017). Seed germination in wheat is the most important period of its life due to its effect on the quality and quantity of the grain yield. Undoubtedly, one of the most sensitive stages of plant growth to salinity stress is the germination stage (Kader *et al*., 2004). Because this stage is the basis of the initial establishment of the plant and has a great impact on the final yield and the occurrence of stress at this stage can have irreparable consequences for the plant (Rauf *et al*., 2007).

Abiotic stresses such as salinity, UV, radiation, high and low temperatures, drought and heavy metals can affect plants at different stages of their life cycle. These stresses have a great impact on plant morphology, growth and production (Quraan *et al*., 2019). Salinity along with drought are two the reasons for the limitations in the world food security. The reason for this can be attributed to factors such as stunted growth. (Ziemann *et al*., 2013; Long *et al*., 2013; Shrivastava *et al*., 2015). The results show that salinity affects 20% of cultivated lands and 50% of irrigated lands and reduces the crop below their genetic potential (Flowers, 2004; Jones, 2007; Munns *et al*. 2006). Salinity stress impairs plant growth and development by reducing water potential (osmotic stress), accumulation of sodium and chlorine ions (ion toxicity), damage to reactive oxygen groups, and disturbing the balance of nutrient ions in the root environment (Arzani, 2008; Arzani and Ashraf, 2016).

Saline soils contain large amounts of soluble salts. The reason for the accumulation of these salts is poor management in soils of agricultural areas, which causes salinity stress (Liu *et al*., 2022). Salinity stress significantly reduces plant yield and thus reduces grain quality and crop yield (Pitman and Läuchli, 2002). It has been identified in many studies that tolerance to salinity is a multi-gene and complex trait that includes various biochemical and physiological mechanisms (Flowers and Colmer, 2008). Modification for tolerance to salinity stress should focus on trait-based selection. The use of quantitative trait mapping (QTL) to withstand salinity stress and marker-assisted selection (MAS) helps to increase the duration for the breeding method (Masoudi *et al*., 2015). In the study of Li *et al*. (2019) using 660 K array, 18 gene locations for the studied traits were identified in 150 double haploid (DH) lines obtained from the cross between Hanxuan 10 and Lumai 14 and QESNP-DS-R2 on chromosome 5D, which could justify 29.0% of the phenotypic variance.

Quantitative trait loci (QTL) analysis is an effective strategy for dissecting QTL and has been successfully applied for gene mining in crops (Liu *et al*., 2019). Previous studies have reported drought and salinity tolerance at the wheat germination stage using QTL mapping (Yuan *et al*., 2011; Czyczyło-Mysza *et al*., 2014; Nagel *et al*., 2014; Ashraf *et al*., 2015; Liu *et al*., 2017; Azadi *et al*.,2015; Ghaedrahmati *et al*., 2014; Rehman *et al*., 2020; Ren *et al*., 2018). Gregorio *et al*. (1997) believe that susceptible and tolerant cultivars can be distinguished by observing the external symptoms caused by salt stress. But according to Fernandez *et al*. (1992), in addition to phenotypic observations, cultivars can be divided into four groups by using various stress tolerance or sensitivity indices. In the study of Batool *et al*. (2018) on the population resulted from the intersection of Pasban (salinity resistant) and Frontana (salinity sensitive) using the CIM method in the stage of rejuvenation in wheat, 44 genetic locations were identified. Among these, 26 QTLs at 150 mM salinity and 18 QTLs under control conditions were detected. Eleven major QTLs were located on chromosomes 1B, 2B, 3B, 5B, 6B and 7B under stress. Also, gene loci detected on chromosomes 6A, 3B, 6B and 6D for root and stem length, fresh and dry weight were identified as the main QTLs under control conditions. It is important to identify salt tolerance at the germination stage. Markers closely related to some of the identified major QTLs can be used in salinity breeding programs and pave the way for map-based cloning in wheat. In addition, the use of stress tolerance indices is also a useful solution in separating and selecting tolerant and sensitive lines. The purpose of this research is to identify gene locations that control traits related to salinity stress and to identify a suitable index for evaluating salinity tolerance at the germination stage in wheat.

MATERIALS AND METHODS

Phenotypic evaluations

The plant material used in the present study was 107

lines from the F_8 population of recombinant inbred lines of Gonbad (salt tolerance)×Zagros (salt sensitive) crosses. Evaluation of genetic diversity and crossing programs and genetic population development were carried out at Gonbad Kavous University (Amraei, 2014; Enchebroun, 2016; Sabouri *et al*., 2019; Sabouri *et al*., 2022). Factorial experiment was performed in a completely randomized design with 9 cm diameter petri dishes with 3 replications.

The first factor was 107 lines of F_8 RIL population and the second factor was salinity stress level at three levels of control, 6 and 12 dS/m. One hundred seeds were selected from each line. The seeds were sterilized with 2% sodium hypochlorite solution for 10 min and then washed three times with sterile distilled water.

The seeds were placed in sterilized petri dishes (temperature 121 °C, 1.5 atmospheres pressure for 20 min) on sterile filter papers and salinity treatments were applied. Petri dishes were placed in a germinator at 25 ° C, 70% humidity and dark for one week. The number of germinated seeds for each line was counted 24, 48, 72, 96, 120, 144, 168 h after placement in the petri dishes. Root length, stem length and the weight of roots and shoots were measured. At the end of germination, percentage (GP) and rate (GR), germination percentage per day (GPD), germination index (GI), seed vigor index (SVI), mean germination time (MGT), coefficient of germination rate (CGR), root length index (RLI), root dry weight index (RDWI), shoot length index (SLI), shoot dry weight index (SDWI), root to shoot ratio by length (R/SL), root to shoot ratio by dry weight (R/SDW), root/shoot ratio by length index (R/SLI) and root/shoot ratio by dry weight index (R/SDWI) were calculated according to the relationships presented in Table 1.

Genotypic evaluation

Genomic DNA was extracted from 107 lines of the RILs population and their parents by a modified CTAB method (Saghi *et al*., 1994). The polymerase chain reaction for 519 SSR primers was performed using a BioRad thermocycler. Each PCR reaction contained, PCR buffer 1X, 0.25 µl $MgCl_2$ 1.5 mM, 1 µl dNTPs, 0.5 µl of each primer (5 mM concentration), Taq polymerase and 50 ng of template DNA. The PCR condition was set as 5 min for the initial denaturation at 94 °C, then 35 cycles were performed including: 1 min at 94 °C, 45 sec at 55 °C for annealing, 1 min at 72 °C and final expansion at 72 °C for 7 min. PCR products were separated by electrophoresis on a 6% polyacrylamide gel and visualized by a simplified silver staining method (Xu *et al*., 2002). Touchdown

Table 1. Formulas used to calculate germination indices.

Traits	Calculations	References
Germination Percentage (GP)	(Number of germinated seeds / Total number of seeds incubated)*100	Mwando *et al.,* 2021
Germination Percentage in Day (GPD)	GP/D	Mwando *et al.,* 2021
Germination Index (GI)	n/D	Mwando *et al.,* 2021
Germination Rate Index (GRI)	(G1-N1)+(G2-N2)+…+(Gn-Nn)	Mwando *et al.,* 2021
Seed of Vigor Index (SVI)	SDW×GP	Elias and Copleland, 2001
Mean Germination Time (MGT)	∑ ((n1*d1)/n)	De and Kar, 1994
Germination Rate (GR)	1/MGT	Mwando *et al.,* 2021
Seedling weight index (IWVS)	Seedling dry weight×germination ability	Abdul Baki and Anderson, 1973
Coefficient of Germination rate (CGR)	(100/n)*∑ (n1*d1)	
Longitudinal index of seedling stem (ILVS)	Seedling length × germination ability	Abdul Baki and Anderson, 1973
Root length index (RLI)	(salt treated root L/control root L)*100	Mwando *et al.,* 2021
Root dry weight index (RDWI)	(salt treated root DW/ control root DW)*100	Mwando *et al.,* 2021
Shoot length index (SLI)	(salt treated shoot L/control shoot L)*100	Mwando *et al.,* 2021
Shoot dry weight index (SDWI)	(salt treated shoot DW/ control shoot DW)*100	Mwando *et al.,* 2021
Root to shoot ratio by Length (R/SL)	root length/ shoot length	Mwando *et al.,* 2021
Root to shoot ratio by DW (R/SDW)	root DW/ shoot DW	Mwando *et al.,* 2021
Root/shoot ratio by length Index (R/SLI)	((R/SL Treated)/(R/ SL Control))	Mwando *et al.,* 2021
Root/shoot ratio by DW Index (R/SDWI)	((R/SDW Treated)/(R/ SDW Control))	Mwando *et al.,* 2021

Table 2. Mathematical formulas of tolerance and susceptibility indices calculated by iPASTIC software (Pour-Aboughadareh *et al.,* 2019).

Index	Formula	Pattern of selection	Reference
Tolerance	$TOL=Y_p-Y_s$	Minimum value	Rosielle and Hamblin (1981)
Mean productivity	$MP=\frac{Yp+Ys}{2}$	Maximum value	Rosielle and Hamblin (1981)
Geometric mean productivity	$GMP=\sqrt{Ys \times Yp}$	Maximum value	Fernandez (1992)
Harmonic mean	$HM=\frac{2(Ys \times Yp)}{(Ys+Yp)}$	Maximum value	Bidinger *et al.* (1987)
Stress susceptibility index	$SSI=\frac{1-(Ys/Yp)}{1-(\bar{Y}s/\bar{Y}p)}$	Minimum value	Fischer and Maurer (1978)
Stress tolerance index	$STI=\frac{Ys \times Yp}{(Yp)\hat{}2}$	Maximum value	Fernandez (1992)
Yield index	$YI=\frac{Ys}{\bar{Y}s}$	Maximum value	Gavuzzi *et al.* (1997)
Yield stability index	$YSI=\frac{Ys}{Yp}$	Maximum value	Bouslama and Schapaugh (1984)
Relative stress index	$RSI=\frac{(Ys/Yp)}{(\bar{Y}s/\bar{Y}p)}$	Maximum value	Fischer and Wood (1979)

program was used for other primers. In this way, the primer annealing temperature was considered 10 °C higher than the actual annealing temperature, and 1 °C was reduced from the annealing temperature in each cycle until the primer anneal temperature was obtained. Also, polymerase chain reaction for 8 CAAT, 33 IJS, 47 iPBS, 3 IRAP, 17 RAPD, 8 SCoT and 12 ISSR primers was performed. PCR products were separated by electrophoresis on a 0.8% agarose gel. Linkage analysis was conducted with Map Manager QTX17 for the segregating polymorphic markers.

Data analysis

Analysis of variance was performed by SAS 9.4. Linkage map was provided using QTLmapmanager. A $\chi 2$ test ($P<0.005$) was performed on each marker to verify the expected 1:1 segregation ratio. A logarithmic odds (LOD) score of 2.5 was used to determine both the linkage groups and the order of markers QTX17X (Manly and Olson, 1999). Finally, data analysis was performed using the QTL.gCIMapping.GUI v2.0 software (Zhang *et al*., 2020) in R software with the map length based on the Kosambi (1994) function equal to 4918.94 cM and the distance between two adjacent markers equal to 5.55 cM. Plant abiotic stress indices were calculated using iPASTIC (Plant Abiotic Stress Index Calculator) software. Table 2 shows the mathematical formulas and selection pattern for each index. iPASTIC is a suitable software for screening stress sensitive and tolerant genotypes and is available as a web application (https://mohse nyous efian.com/ipast ic/). The main function of iPASTIC is to calculate several indices and percentages of relative changes due to stress compared to non-stress environment for a set of genotypes. This software has the ability to calculate the ranking patterns of genotypes based on each index. As a result, users can place any genotype in groups A, B, C and D using the grouping carried out by Fernandez (1992) (Pour-Aboughadareh *et al*., 2019).

RESULT

Analysis of variance

Analysis of variance for investigated traits under normal condition and salt stress showed that the difference between lines was significant for all traits (Table 3). This result indicated the presence of phenotypic diversity for the evaluated traits at the germination stage under salt and normal stress conditions in the studied lines.

The different reactions of the studied lines to the stress made the interaction effect of the line×cultivation conditions significant at the probability level of 1% for most of the traits except MGT and GR. The significance of the interaction effect showed the different behavior of the lines in normal and salt stress conditions in terms of the examined traits and probably showed different mechanisms between them in response to different conditions. This can be used for the selection of suitable cultivars for each cultivation condition, separately (stressed and normal). Analysis of variance on tolerance and sensitive indices in 6 and 12 dS/m

Table 3. Variance analysis of investigated traits under salinity stress conditions in 107 lines resulting from Gonbad×Zagros cross.

Sources of variation	df	Mean of square										
		GP	GPD	GI	GRI	MGT	GR	LS	LR	WDS	WDR	WDT
R	2	12.078^{ns}	0.248^{ns}	0.0155^{ns}	72.188^{ns}	0.255*	0.00000166*	1.921^{ns}	3.214^{ns}	0.00000540^{ns}	0.000155^{ns}	0.000113^{ns}
line	106	156.561**	3.192**	0.20001**	488.821**	0.128**	0.00000082**	9.202**	13.934**	0.00249**	0.00173**	0.00630**
salinity	2	126812.827**	2589.948**	161.578**	420584.761**	6.377**	0.00000042^{ns}	1499.652**	2821.176**	0.498**	0.347**	1.698**
line×salinity	212	65.958**	1.345**	0.0843**	202.405**	0.053^{ns}	0.000000021^{ns}	2.342**	5.777**	0.000565**	0.000679**	0.00176**
Error	640	14.845	1.303	0.0188	46.434	0.0604	0.00000041	0.673	1.625	0.000238	0.000195	0.000520
Coefficient of variation (%)		4.865	4.869	4.857	6.237	0.884	1.605	8.605	13.612	13.079	13.103	10.177

GP: Germination percentage, GPD: Germination percentage per day, GI: Germination index, GRI: Germination Rate Index, MGT: Mean germination time, GR: Germination rate, LS: Shoot length, LR: Root length, WDS: Shoot dry weight, WDR: Root dry weight, WDT: Total dry weight.
*,**: Probability levels at 0.05 and 0.01, respectively, ^{ns}: no significant difference.

Table 3 (Continued). Variance analysis of investigated traits under salinity stress conditions in 107 lines resulting from Gonbad×Zagros cross.

Sources of variation	df	Mean of square										
		ILVS	IWVS	SVI	R/SL	R/SDW	RLI	RDWI	SLI	SDWI	R/SLI	R/SDWI
R	2	573.878^{ns}	0.0185^{ns}	0.0641^{ns}	0.00698^{ns}	0.1858^{ns}	67.473^{ns}	370.7648*	8.151^{ns}	70.3079^{ns}	0.0468^{ns}	0.1917^{ns}
line	106	4186.638**	1.115**	44.283**	0.1754**	0.2924**	174.336**	461.773**	360.108**	358.299**	0.11114**	0.2100**
salinity	2	2533016.429**	611.354**	3373.17**	2.4166**	1.4019**	678810.110**	486921.476**	628283.296**	439798.561**	88.8715**	133.2110**
line×salinity	212	1351.984**	0.315**	16.915**	0.0655**	0.14609**	171.351**	253.4174**	151.385**	184.4708**	0.0534**	0.1285**
Error	640	362.027	0.106	4.107	0.02201	0.1047	78.478	89.966	47.989	79.206	0.0245	0.06889
Coefficient of variation (%)		9.7099	13.266	10.858	15.0802	34.0724	33.035	21.884	13.998	21.7577	25.851	35.3681

ILVS: Longitudinal index of seedling stem, IWVS: Seedling weight index, SVI: Seed vigor index, R/SL: Root to shoot ratio by length , R/SDW: Root to shoot ratio by dry weight, RLI: Root length index, RDWI: Root dry weight index, SLI: Shoot length index, SDWI: Shoot dry weight index, R/SLI: Root/shoot ratio by length index, R/SDWI: Root/shoot ratio by DW Index.
*,**: Probability levels at 0.05 and 0.01, respectively, ^{ns}: no significant difference.

Table 4. Variance analysis of tolerance and sensitive indices in 6 dS/m salinity stress in 107 lines obtained from Gonbad×Zagros cross.

Sources of variation	df	Mean of square								
		TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
line	106	3.275*	3.053*	3.089*	3.127*	0.000419*	0.000095*	0.000077*	0.000087^{ns}	0.000086^{ns}
Error	214	0.000124	2.269	2.311	2.352	0.000123	0.000071	0.000058	0.000068	0.000067
Coefficient of variation (%)		0.637	0.419	0.423	0.427	0.747	0.836	0.765	0.822	0.822

TOL: Tolerance, MP: Mean productivity, GMP: Geometric mean productivity, HM: Harmonic mean, SSI : Stress susceptibility index, STI: Stress tolerance index, YI: Yield index, YSI: Yield stability index, RSI : Relative stress index.
*,**: Probability levels at 0.05 and 0.01, respectively, ^{ns}: no significant difference.

Table 5. Variance analysis of tolerance and sensitive indices in 12 dS/m salinity stress in 107 lines obtained from Gonbad×Zagros cross.

Sources of variation	df	Mean of square								
		TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
line	106	4.369*	6.609*	6.721*	6.837*	0.000589*	0.00021*	0.00017*	0.00018^{ns}	0.00018^{ns}
Error	214	0.000259	0.00000005	0.00000005	0.00000005	0.00011	0.00015	0.00013	0.00014	0.00014
Coefficient of variation (%)		0.798	0.609	0.619	0.628	0.957	1.215	1.166	1.205	1.205

TOL: Tolerance, MP: Mean productivity, GMP: Geometric mean productivity, HM: Harmonic mean, SSI : Stress susceptibility index, STI: Stress tolerance index, YI: Yield index, YSI: Yield stability index, RSI : Relative stress index.
*,**: Probability levels at 0.05 and 0.01, respectively, ^{ns}: no significant difference.

salinity stress conditions showed that the difference between the studied lines was significant for Ys, MP, GMP, HM, STL, YI and YSI traits (Tables 4 and 5).

The values of the indices for resistant and sensitive lines are presented in Tables 6 and 7. According to Fernandez (1992), an index will increase performance in both stress and non-stress conditions if it has a significant and high correlation with grain yield in both conditions. In this research, the correlation of stress resistance indices with germination traits including GR was investigated in two conditions of salinity stress of 6 and 12 dS/m. Based on the results shown in Table 6 and the estimation of the sensitivity of wheat lines based on GR, lines 6, 96 and 82 showed the highest resistance to the salinity stress 6 dS/m. However, based on the investigated index, in 12 dS/m salinity stress, lines 96, 2 and 98 had the highest resistance to stress (Table 7). However, lines 40, 80 and 81 were recognized as sensitive to salinity stress in both salinity conditions of 6 and 12 dS/m.

The most appropriate index for selecting stress-tolerant cultivars is an index with a relatively high correlation to grain yield in both non-stressed and stressed conditions. Therefore, it is possible to identify the most suitable index by evaluating the correlation between stress tolerance indices and grain yield in two environments, normal and salt stress. In this research, the results of the correlation between the mentioned indices and the GR under normal condition, stress condition of 6 and 12 dS/m are given in Figures 1 and 2. In both conditions (6 and 12 dS/m), the GR under stress conditions (Ys) had a positive and significant correlation with TOL (0.982, 0.959 for 6 and 12 dS/m, respectively), MP (0.982, 0.960), GMP (0.985, 0.966), HM (0.987, 0.971), STI (0.983, 0.961), YI (1, 1), YSI (-0.990, -0.978) and RSI (-0.990, -0.978) indices (Figures 1 and 2).

Linkage map construction

Out of the 671 SSR marker pairs tested, 519 produced polymorphic bands between the genomic DNAs of parents. Also, 21 CAAT, 41 IJS, 62 iPBS, 12 IRAP, 23 RAPD, 11 SCoT and 15 ISSR markers were used for the parental survey. Eight CAAT (21 polymorphic band), 33 IJS (75 polymorphic band), 47 iPBS (138 polymorphic band), 3 IRAP (5 polymorphic band), 17 RAPD (54 polymorphic band), 8 SCoT (20 polymorphic band) and 12 ISSR (53 polymorphic

Table 6. The value of different tolerance and sensitive indices based on GR in 6 dS/m for six tolerant and sensitive lines.

Genotype code	Yp	Genotype code	Ys	Genotype code	TOL	Genotype code	MP	Genotype code	GMP	Genotype code	HM	Genotype code	SSI	Genotype code	STI	Genotype code	YI	Genotype code	YSI	Genotype code	RSI
G40	0.0258	G81	0.0350	G40	-0.0101	G40	0.0309	G40	0.0304	G40	0.0300	G81	-2.5034	G40	0.7273	G81	0.9728	G81	0.9792	G81	0.9711
G64	0.0357	G80	0.0351	G6	-0.0007	G81	0.0354	G81	0.0354	G81	0.0354	G80	-2.0754	G81	0.9824	G80	0.9763	G80	0.9828	G80	0.9747
G89	0.0357	G92	0.0358	G53	-0.0006	G80	0.0354	G80	0.0354	G80	0.0354	G96	-0.8095	G80	0.9860	G92	0.9935	G96	0.9933	G96	0.9851
G98	0.0357	G25	0.0358	G78	-0.0005	G25	0.0357	G25	0.0357	G25	0.0357	G122	-0.3406	G25	1.0033	G25	0.9939	G122	0.9972	G122	0.9889
G108	0.0357	G17	0.0358	G3	-0.0005	G92	0.0358	G92	0.0358	G92	0.0358	G72	-0.1822	G92	1.0044	G17	0.9948	G72	0.9985	G72	0.9902
G109	0.0357	G23	0.0358	G82	-0.0005	G16	0.0358	G16	0.0358	G16	0.0358	G23	-0.1762	G16	1.0051	G23	0.9951	G23	0.9985	G23	0.9903
G58	0.0359	G3	0.0362	G23	0.0001	G79	0.0360	G79	0.0360	G79	0.0360	G82	1.5747	G79	1.0175	G3	1.0064	G82	1.0131	G82	1.0047
G84	0.0359	G50	0.0362	G72	0.0001	G51	0.0360	G51	0.0360	G51	0.0360	G3	1.5819	G51	1.0177	G50	1.0066	G3	1.0131	G3	1.0047
G72	0.0360	G78	0.0363	G122	0.0001	G96	0.0360	G96	0.0360	G96	0.0360	G78	1.7968	G96	1.0193	G78	1.0077	G78	1.0149	G78	1.0065
G122	0.0360	G82	0.0363	G96	0.0002	G53	0.0361	G53	0.0361	G53	0.0361	G53	2.1403	G53	1.0211	G82	1.0090	G53	1.0178	G53	1.0093
G6	0.0360	G53	0.0364	G80	0.0006	G82	0.0361	G82	0.0361	G82	0.0361	G6	2.2675	G82	1.0218	G53	1.0110	G6	1.0188	G6	1.0104
G96	0.0362	G6	0.0367	G81	0.0007	G6	0.0364	G6	0.0364	G6	0.0364	G40	47.0414	G6	1.0377	G6	1.0197	G40	1.3901	G40	1.3786

TOL: Tolerance, MP: Mean productivity, GMP: Geometric mean productivity, HM: Harmonic mean, SSI : Stress susceptibility index, STI: Stress tolerance index, YI: Yield index, YSI: Yield stability index, RSI : Relative stress index.

Table 7. The value of different tolerance and sensitive indices based on GR in 12 dS/m for six tolerant and sensitive lines.

Genotype code	Yp	Genotype code	Ys	Genotype code	TOL	Genotype code	MP	Genotype code	GMP	Genotype code	HM	Genotype code	SSI	Genotype code	STI	Genotype code	YI	Genotype code	YSI	Genotype code	RSI
G40	0.0258	G80	0.0348	G40	-0.0103	G40	0.0310	G40	0.0305	G40	0.0301	G80	-2.1161	G40	0.7317	G80	0.9620	G80	0.9728	G80	0.9604
G64	0.0357	G81	0.0348	G98	-0.0012	G80	0.0353	G80	0.0353	G80	0.0353	G81	-2.0926	G80	0.9760	G81	0.9623	G81	0.9731	G81	0.9607
G89	0.0357	G35	0.0357	G2	-0.0011	G81	0.0353	G81	0.0353	G81	0.0353	G122	-0.4412	G81	0.9763	G35	0.9884	G122	0.9943	G122	0.9817
G98	0.0357	G89	0.0357	G117	-0.0008	G89	0.0357	G89	0.0357	G89	0.0357	G35	-0.2249	G89	1.0018	G89	0.9884	G35	0.9971	G35	0.9845
G108	0.0357	G122	0.0358	G55	-0.0008	G32	0.0358	G32	0.0358	G32	0.0358	G89	0.0371	G32	1.0046	G122	0.9899	G89	1.0005	G89	0.9878
G109	0.0357	G44	0.0358	G68	-0.0008	G91	0.0358	G91	0.0358	G91	0.0358	G44	0.0603	G91	1.0047	G44	0.9907	G44	1.0008	G44	0.9881
G58	0.0359	G117	0.0366	G44	0.0000	G82	0.0362	G82	0.0362	G82	0.0362	G68	1.6509	G82	1.0290	G117	1.0116	G68	1.0212	G68	1.0083
G84	0.0359	G82	0.0366	G89	0.0000	G55	0.0362	G55	0.0362	G55	0.0362	G55	1.8072	G55	1.0297	G82	1.0116	G55	1.0232	G55	1.0102
G72	0.0360	G55	0.0366	G35	0.0001	G96	0.0362	G96	0.0362	G96	0.0362	G117	1.8205	G96	1.0312	G55	1.0134	G117	1.0234	G117	1.0104
G122	0.0360	G6	0.0367	G122	0.0002	G98	0.0363	G98	0.0363	G98	0.0363	G2	2.2987	G98	1.0338	G6	1.0139	G2	1.0296	G2	1.0165
G6	0.0360	G98	0.0369	G81	0.0010	G6	0.0363	G6	0.0363	G6	0.0363	G98	2.5193	G6	1.0364	G98	1.0200	G98	1.0324	G98	1.0193
G96	0.0362	G2	0.0369	G80	0.0010	G2	0.0364	G2	0.0364	G2	0.0364	G40	30.9947	G2	1.0391	G2	1.0212	G40	1.3985	G40	1.3808

TOL: Tolerance, MP: Mean productivity, GMP: Geometric mean productivity, HM: Harmonic mean, SSI : Stress susceptibility index, STI: Stress tolerance index, YI: Yield index, YSI: Yield stability index, RSI : Relative stress index.

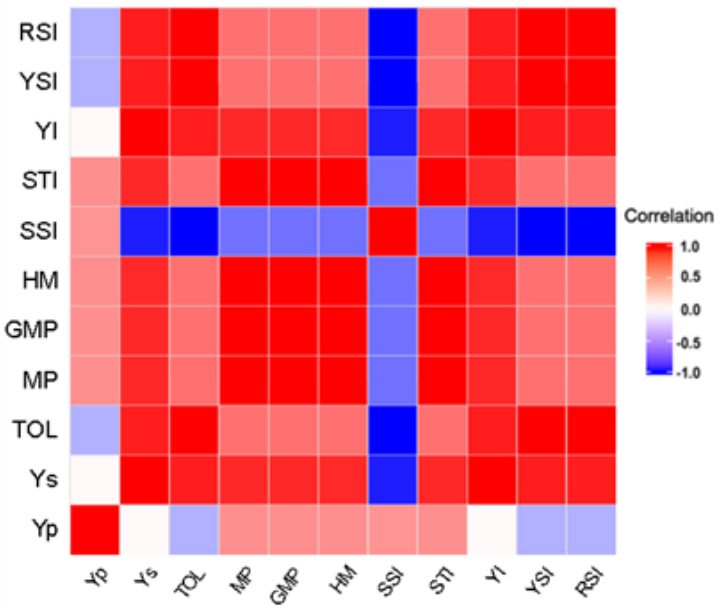

Figure 1. Correlation of tolerance and sensitive indices in 6 dS/m salinity based on GR.

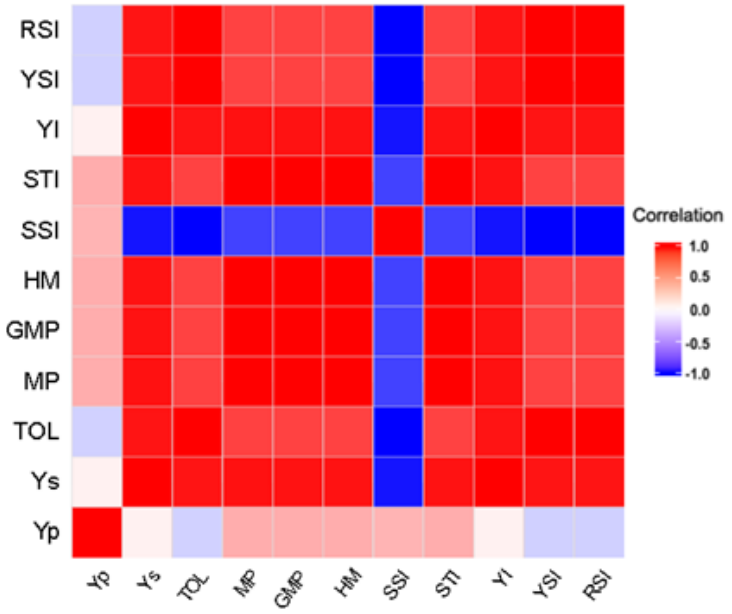

Figure 2. Correlation of tolerance and sensitive indices in 12 dS/m salinity based on GR.

band) were polymorphic and used for providing the linkage map. This map covered 4918.94 cM of the wheat genome. The marker distances for genomes A, B, and D were found to be 5.68, 5.71, and 5.27 cM, respectively. The length of genome A was 1732.53 cM, and genomes B and D were 1668.28 and 1518.13 cM in the total map length, respectively. A total of 305, 292, and 288 markers were distributed on genomes A, B, and D, respectively. In the prepared map, the average distance between the flanked markers for the whole genome was 5.55. Chromosome 3D had the maximum map length (293.28 cM) and chromosome 7D had the minimum map length (154.32 cM). The genomic and chromosomal regions of the located gene locations in the evaluated conditions are observed in Figure 3.

Mapping of QTLs

In total, 84 gene loci were identified for the three conditions, which separately included seven QTL for five traits in normal condition, 42 QTL for 17 traits in

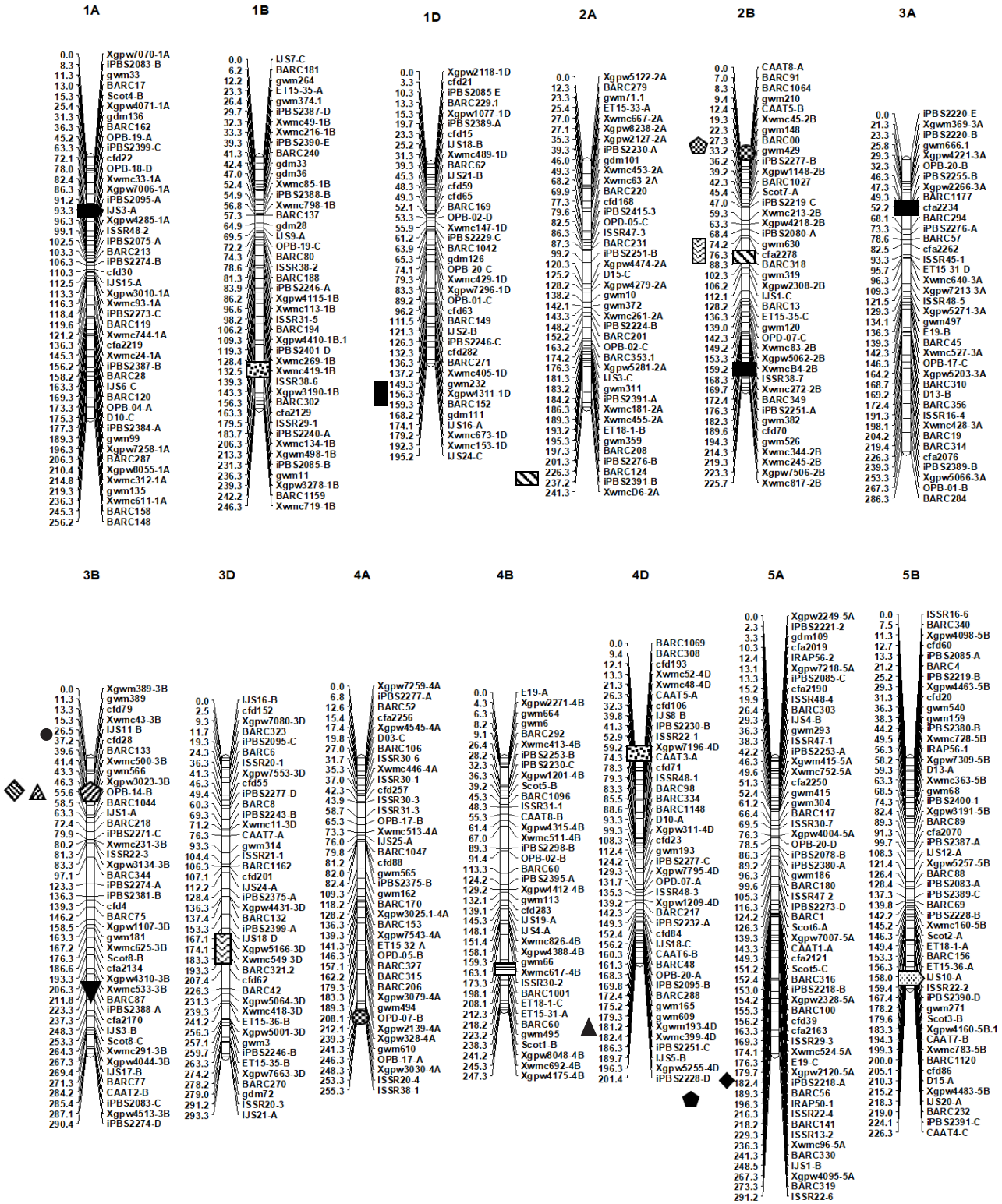

Figure 3. Genetic linkage maps of QTLs identified under normal stress and salt stress in the germination stage of the F_8 population derived from Dome in Zagros (**A:** normal, **B:** salt stress 6 dS/m, **C:** salt stress 12 dS/m).

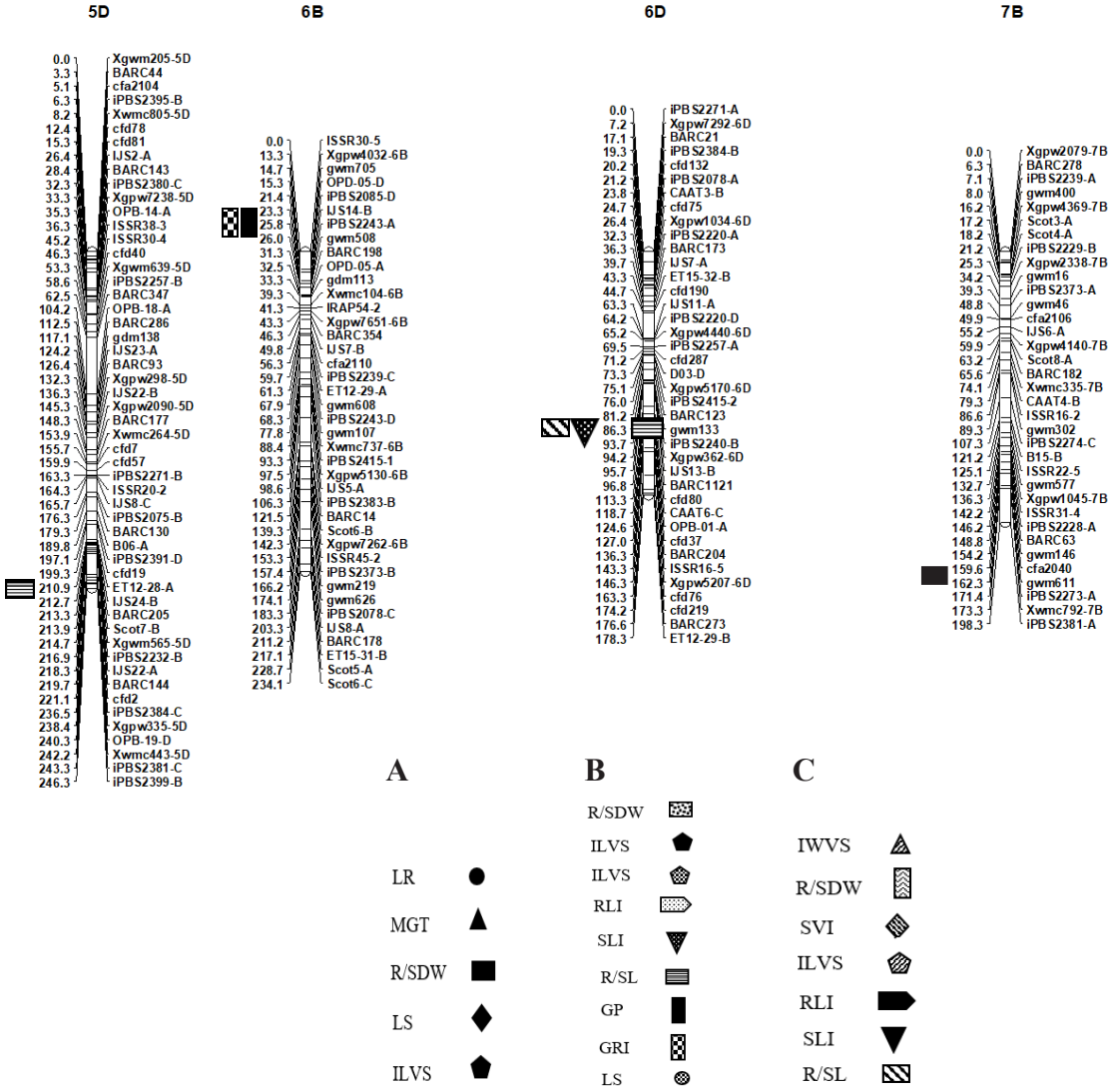

Figure 3 (Continued). Genetic linkage maps of QTLs identified under normal stress and salt stress in the germination stage of the F_8 population derived from Dome in Zagros (**A:** normal, **B:** salt stress 6 dS/m, **C:** salt stress 12 dS/m).

6 dS/m salinity condition, and 35 QTL for 17 traits in 12 dS/m salt stress condition.

For root length under normal condition, one gene locus was traced, being located on chromosome B3 at 21.36 cM. No QTL was detected for this trait under salt stress conditions. qMGT-A5 was detected for the average germination time on chromosome A5 under normal condition (Figure 4) and with LOD equal to 3.34 and between two markers Xg+pw2120-5A and iPBS2218-A. For R/SDW, three, two and two QTLs were detected for normal, 6 and 12 dS/m salinity stress conditions, respectively.

qR/SDW-B2 in normal (Table 8) condition and qR/SDW-B2 in 12 dS/m salt stress were traced on the same chromosome but in different positions, which explained more than 19 and 31% of the phenotypic variation of the trait, respectively. For LS, a total of three gene locations were traced on chromosome A5 for normal condition and chromosomes B2 and A4 under stress conditions of 6 dS/m. The LOD was 3.52, 3.06 and 3.16, respectively (Tables 8 and 9). For ILVS, a number of gene loci were observed in all three conditions. qILVS-A5 was detected under normal condition, qILVS-B2 under 6- dS/m salt stress (Figure 5) and ILVS-B3 under 12 dS/m salt stress, which were 41.66, 42.51 and 30.54%, respectively (Table 10). For RLI, in each one of 6 and 12 dS/m salinity stress conditions, one QTL was detected, which were located on chromosomes B5 and A1, respectively, with the LOD scores of 3.01 and 3.06, respectively. qSLI-D6 and qSLI-B3 were detected on D6 and B3 chromosomes under salinity stress conditions of 6 and 12 dS/m, respectively. These gene locations had

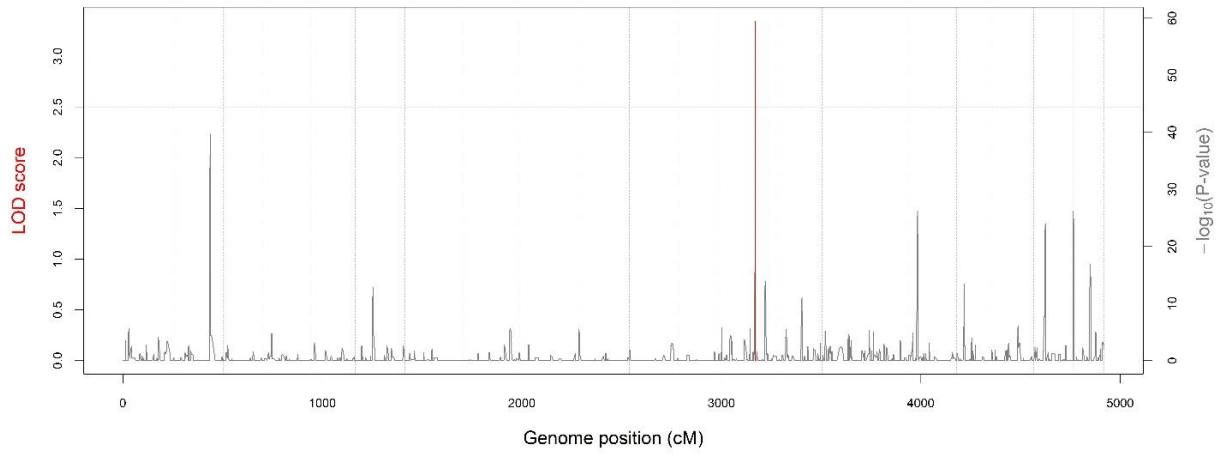

Figure 4. LOD plot of MGT on chromosome A5 under normal condition.

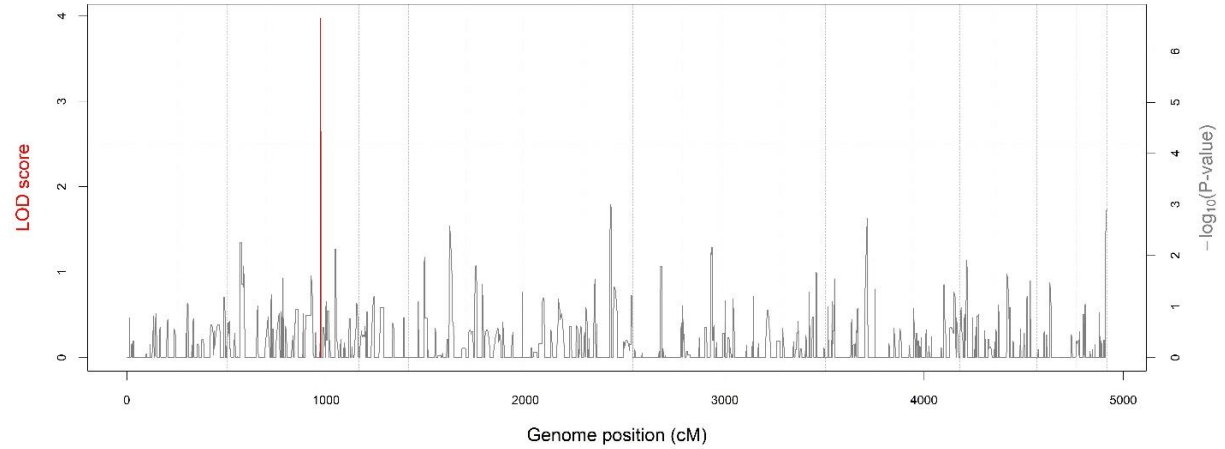

Figure 5. LOD plot of ILVS on chromosome B2 under 6 dS/m stress condition.

Table 8. QTLs identified for the examined traits under normal condition.

Trait	QTL	Chr	Position (cM)	Additive effect	LOD	Left_marker	Right_marker	r^2 (%)
LR	qLR-B3	B3	36.21	-2.19	3.24	IJS11-B	cfd28	44.69
MGT	qMGT-A5	A5	181.46	0.07	3.34	Xg+pw2120-5A	iPBS2218-A	49.84
	qR/SDW-B2	B2	159.22	0.16	3.46	XwmcB4-2B	XwmcB4-2B	19.19
R/SDW	qR/SDW-A3	A3	60.16	-0.21	3.62	cfa2234	BARC294	33.13
	qR/SDW-B7	B7	162.25	-0.18	3.76	gwm611	gwm611	25.20
LS	qLS-A5	A5	181.46	1.09	3.52	Xgpw2120-5A	iPBS2218-A	44.71
ILVS	qILVS-A5	A5	181.46	29.80	3.79	Xgpw2120-5A	iPBS2218-A	41.66

an additive effect of -6.72 and 10.32, respectively, explaining 44 and 50% of the trait phenotypic variation. For R/SL in both stress conditions, three gene loci were identified, among them, qR/SL-D6 in both conditions was located on chromosome D6 positions (in 6 dS/m at 86.25 and 12 dS/m at 85.41 cM). For GP, two QTLs were detected on D1 and B6 chromosomes in salinity stress of 6 dS/m, with the LOD values of 3.04 and 3.56, respectively (Table 9). In the same situation, for GRI, a gene locus was detected with justification of more than 24% of the phenotypic variation of the trait on chromosome B6 and at the position of 23.29 cM. In the salinity stress of 12 dS/m, one QTL was detected for each one of the IWVS and SVI traits, both of which

Table 9. QTLs identified for the examined traits and tolerance and sensitive indices under 6 dS/m stress.

Trait	QTL	Chr	Position (cM)	Additive effect	LOD	Left_marker	Right_marker	r^2 (%)
ILVS	qILVS-B2	B2	33.21	-22.51	3.97	gwm429	gwm429	42.51
RLI	qRLI-B5	B5	162.0433	-10.12	3.01	ISSR22-2	iPBS2390-D	40.91
SLI	qSLI-D6	D6	89.995	-6.72	3.24	gwm133	iPBS2240-B	44.71
R/SL	qR/SL-D5	D5	211.795	-0.11	3.40	ET12-28-A	IJS24-B	19.17
	qR/SL-D6	D6	86.25	0.14	6.51	gwm133	gwm133	34.42
	qR/SL-4	B4	162.17	0.08	3.08	gwm66	Xwmc617-4B	13.30
R/SDW	qR/SDW-B1	B1	131.698	0.10	3.44	Xwmc269-1B	Xwmc419-1B	27.71
	qR/SDW-D4	D4	74.26	0.11	3.06	CAAT3-A	CAAT3-A	32.11
GP	qGP-D1	D1	154.25	6.90	3.04	gwm232	Xgpw4311-1D	40.41
	qGP-B6	B6	25.75	-2.90	3.56	iPBS2243-A	iPBS2243-A	7.15
GRI	qGRI-B6	B6	23.29	10.13	3.03	IJS14-B	IJS14-B	24.99
LS	qLS-B2	B2	33.21	-0.87	3.06	gwm429	gwm429	17.75
	qLS-A4	A4	208.14	-1.09	3.16	OPD-07-B	OPD-07-B	27.71
MP	qMP-A7a	A7	12.33	1.2	3.10	BARC275	gwm233	19.89
	qMP-A7b	A7	176.25	-1.40	4.68	ET12-28-B	ET12-28-B	27.24
	qMP-A6	A6	94.042	1.29	5.52	CAAT4-A	CAAT3-C	23.22
GMP	qGMP-A7	A7	176.25	-53.84	4.58	ET12-28-B	ET12-28-B	27.08
	qGMP-A6	A6	94.042	50.46	5.55	CAAT4-A	CAAT3-C	23.78
HM	qHM-B5	B5	23.23	10.21	4.43	BARC4	iPBS2219-B	13.13
	qHM-A7a	A7	12.33	12.76	4.04	BARC275	gwm233	20.50
	qHM-A7b	A7	176.25	-13.03	6.75	ET12-28-B	ET12-28-B	21.38
	qHM-A6	A6	93.24	10.13	3.85	CAAT4-A	CAAT4-A	12.94
STI	qSTI-A7a	A7	12.33	-0.33	3.32	BARC275	gwm233	20.26
	qSTI-A7b	A7	176.25	0.38	5.02	ET12-28-B	ET12-28-B	27.43
	qSTI-A6	A6	94.84	-0.33	5.54	CAAT4-A	CAAT3-C	21.39
YI	qYI-D5	D5	52.41	-4.07	4.89	cfd40	Xgwm639-5D	20.79
	qYI-A7a	A7	12.33	3.33	3.05	BARC275	gwm233	13.95
	qYI-A7b	A7	176.25	-4.08	5.07	ET12-28-B	ET12-28-B	20.91
	qYI-A6	A6	96.44	2.77	3.23	CAAT4-A	CAAT3-C	9.65
YSI	qYSI-B5	B5	22.23	2.79	3.89	BARC4	iPBS2219-B	15.53
	qYSI-A7a	A7	12.33	3.89	4.04	BARC275	gwm233	30.29
	qYSI-A7b	A7	176.25	-4.24	7.56	ET12-28-B	ET12-28-B	35.95
RSI	qRSI-B5	B5	225.54	-0.32	4.34	iPBS2391-C	CAAT4-C	22.02
	qRSI-A7a	A7	12.33	-0.38	3.68	BARC275	gwm233	32.29
	qRSI-A7b	A7	176.25	0.33	4.18	ET12-28-B	ET12-28-B	24.10

were located on the B3 chromosome at 53.69 and 54.62 cM and were able to increase 55.8, respectively (Figures 6 and 7). Also, these QTLs explained 54.41% of the trait phenotypic variation (Table 10). qRDWI-D3 was detected on chromosome D3 and at 29.221 cM and could explain more than 55% of the phenotypic variance of the trait.

Mapping of tolerance and sensitive indices was also done, and for each index, several QTLs were identified on different chromosomes. For MP under 6 dS/m salinity stress, three QTLs were detected on the A7 (two QTLs) and A6 chromosomes, with the LOD values of 3.10, 4.68, 5.52, respectively. Also, for this trait, under salinity stress of 12 dS/m, four QTLs were detected on chromosomes D5, A7, B1 and A6, among which qMP-76 had the highest LOD (4.66). qHM-A7b was also detected with LOD=6.75 which explained 21% of the trait phenotypic variation, on A7 chromosome for HM under 6 and 12 dS/m stress. Chromosome A7 seems to be very important for stress tolerance indices. Because for most indices, in 6 and 12 dS/m salinity

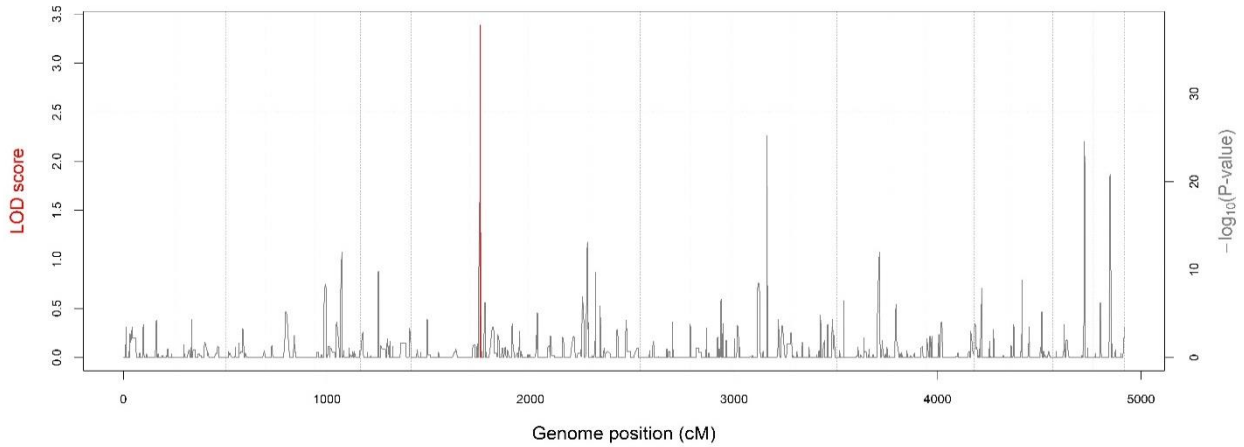

Figure 6. LOD plot of IWVS on chromosome B3 under 12 dS/m stress condition.

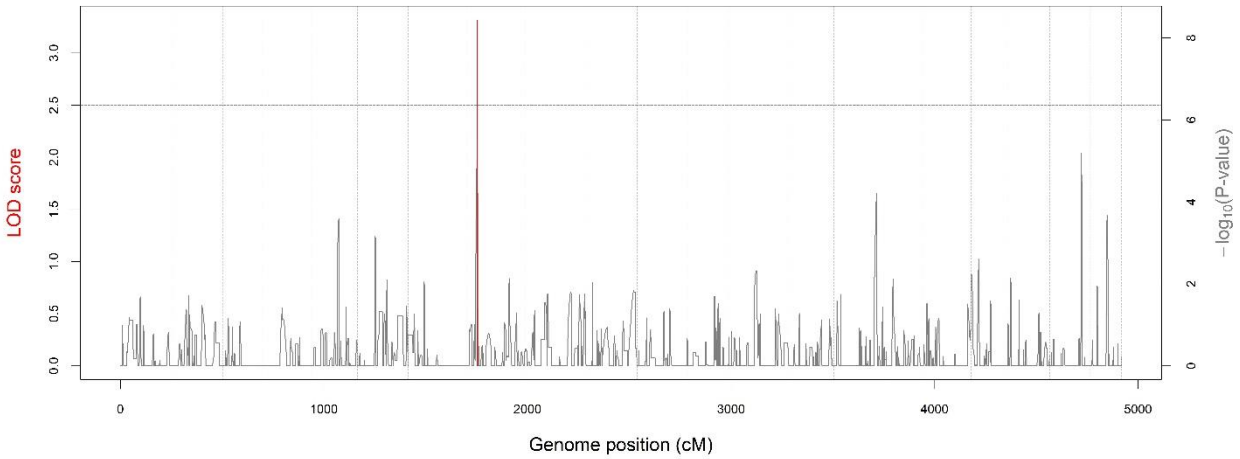

Figure 7. LOD plot of SVI on chromosome B3 under 12 dS/m stress condition.

stress, the QTLs were detected on this chromosome. Under salinity stress of 6 dS/m, qYI-A7b with LOD=5.07, qYSI-A7b with LOD=7.56 and qRSI-a7b with LOD=4.18 were detected at 176.25 cM from the top of chromosome A7. The same cases were observed in 12 dS/m stress. In these conditions, qMP-A7 with LOD=4.25, qGMP-A7 with LOD=7.14, qSSI-A7b with LOD=4.58 and qYI-A7b with LOD=4.70 were identified on A7 chromosome.

DISCUSSION

One of the stages sensitive to salinity stress is the germination stage and it is very important in the growth stages of the plant (Feizi and Aghakhani, 2007; Moursi, 2014; Wu *et al*., 2019). For QTL mapping, it is necessary to measure the appropriate phenotypic parameters. Several attempts have been made to map the genes involved in resistance to salt stress in wheat, using different indices of salt tolerance and different populations (Wyn Jones *et al*., 1984; Shan *et al*., 1987; Dvorak *et al*., 1994; Munns *et al*., 2002), which causes differences in the results obtained in different wheat cultivars. Therefore, it is very important to evaluate genotypes in studies related to salt stress in order to develop resistant cultivars (Sallam *et al*., 2019). Several gene loci for germination percentage were traced on chromosomes 2A and 4B of recombinant inbred lines (Marouf and Mohammadi, 2015) and QTLs were identified for germination percentage in non-stressed and stressed environments (Wang *et al*., 2011). Czyczyło Mysza *et al*. (2014) recorded germination percentage related QTLs under non-stressed and stressed conditions on chromosome 5A, 1B, 3B, 4B and 6B of the mapping population (doubled haploid lines).

In this study, qGP-B6 was detected at 6 dS/m salt stress for germination percentage. Also, gene

Table 10. The identified QTLs for the examined traits and tolerance and sensitive indices under 12 dS/m stress.

Trait	QTL	Chr.	Position (cM)	Additive effect	LOD	Left_marker	Right_marker	r^2 (%)
RDWI	qRDWI-D3	D3	221.29	13.99	3.07	cfd62	BARC42	55.49
SLI	qSLI-B3	B3	209.91	10.32	3.45	Xwmc533-3B	BARC87	50.32
RLI	qRLI-A1	A1	94.25	12.94	3.06	IJS3-A	Xgpw4285-1A	40.17
ILVS	ILVS-B3	B3	54.62	-29.67	3.32	Xgpw3023-3B	OPB-14-B	30.54
R/SL	qR/SL-B2	B2	81.32	0.24	3.73	cfa2278	BARC318	18.56
	qR/SL-D6	D6	85.41	0.22	3.36	BARC123	gwm133	14.88
	qR/SL-A2	A2	231.24	0.22	3.41	BARC124	iPBS2391-B	15.08
R/SDW	qR/SDW-B2	B2	76.32	0.28	3.00	cfa2278	cfa2278	31.35
	qR/SDW-D3	D3	170.12	-0.28	3.71	IJS18-D	Xgpw5166-3D	32.96
IWVS	qIWVS-B3	B3	53.69	-0.43	3.38	Xgpw3023-3B	OPB-14-B	55.8
SVI	qSVI-B3	B3	54.62	-2.81	3.31	Xgpw3023-3B	OPB-14-B	54.41
TOL	qTOL-A1	A1	94.25	-66.60	3.18	IJS3-A	Xgpw4285-1A	9.01
	qTOL-A7	A7	176.25	-109.15	5.41	ET12-28-B	ET12-28-B	24.22
MP	qMP-D5	D5	51.53	-1.05	4.62	cfd40	Xgwm639-5D	19.99
	qMP-A7	A7	177.63	-0.94	4.25	ET12-28-B	gwm332	15.94
	qMP-B1	B1	83.06	0.59	3.58	BARC188	iPBS2246-A	6.45
	qMP-A6	A6	96.44	0.82	4.66	CAAT4-A	CAAT3-C	12.34
GMP	qGMP-B5	B5	22.23	5.63	3.75	BARC4	iPBS2219-B	15.68
	qGMP-A7	A7	177.63	-7.79	7.14	ET12-28-B	gwm332	30.01
HM	qHM-A7a	A7	12.33	35.29	3.23	BARC275	gwm233	19.69
	qHM-A7b	A7	176.25	-40.61	4.76	ET12-28-B	ET12-28-B	26.08
	qHM-A6	A6	94.042	43.11	6.83	CAAT4-A	CAAT3-C	29.38
SSI	qSSI-B5	B5	224.83	27.32	5.16	iPBS2391-C	CAAT4-C	22.43
	qSSI-A7a	A7	12.33	29.58	3.34	BARC275	gwm233	26.30
	qSSI-A7b	A7	177.63	-26.62	4.58	ET12-28-B	gwm332	21.30
STI	qSTI-D1	D1	55.89	35.10	3.94	Xwmc147-1D	Xwmc147-1D	11.39
	qSTI-A7a	A7	12.33	49.13	5.10	BARC275	gwm233	22.31
	qSTI-A6	A6	93.24	43.24	7.23	CAAT4-A	CAAT4-A	17.28
	qSTI-A7b	A7	176.25	-30.70	5.56	ET12-28-B	ET12-28-B	8.71
YI	qYI-A7a	A7	12.33	30.55	3.21	BARC275	gwm233	18.80
	qYI-A7b	A7	177.63	-28.15	4.70	ET12-28-B	gwm332	15.97
	qYI-A6	A6	93.24	33.91	6.14	CAAT4-A	CAAT4-A	23.18
YSI	qYSI-B3	B3	15.29	0.07	3.17	Xwmc43-3B	Xwmc43-3B	43.12

locations were traced for germination percentage on chromosomes 2A and 4B of recombinant inbred lines (Marouf and Mohammadi, 2015) and QTLs were identified for germination percentage in non-stressed and stressed environments (Wang *et al*., 2011). Gene locations for seedling vigor index and germination index were identified on chromosomes 2A, 4A, 2B, 7B and 6D under control and salt stress conditions (Batool *et al*., 2018). qSVI-B3 was also identified in this research at 12 dS/m salinity for the seed vigor index. A large number of studies have been conducted to investigate the gene loci for sodium elimination, but only one gene locus related to this trait was located on chromosome 2AL (Munns *et al*., 2002; Lindsay *et al*., 2004).

Zebeau and Vos (1993) showed that correlated traits are often controlled by QTLs located in similar regions on chromosomes. In the present study, according to the results obtained from normal and salt stress conditions, it seems that there is a pleiotropy effect of genes controlling traits. In this study, a highly significant correlation was observed between ILVS and SVI under the 12 ds/m stress and between ILVS and LS under the 6 dS/m stress, which confirms this.

The observed results indicate the difference between the studied lines in terms of stress tolerance indices. These indices were able to distinguish sensitive and resistant lines from each other according to characteristics GR. The results of stress resistance indices showed that line 6 is considered as a resistant line and line 2 as a sensitive line in most of the indices.

Izaddoost *et al*. (2013) and Hosseini *et al*. (2012) in research studies on indices related to grain yield (SSI and STI), introduced these indices as the best for selecting stress-tolerant cultivars. Hosseini *et al*. (2012) calculated TOL, GMP, STI, SSI and MP indices based on seedling length and root dry weight in 65 rice genotypes and concluded that the three GMP, STI and MP are the best among the indices.

CONCLUSION

There are various abiotic stresses that have a negative effect on plant growth and productivity, among which salt stress can be mentioned, which is one of the important factors that severely reduces plant growth and development. Germination is one of the important stages in the life span of plants, and the presence of tension in this stage can be very damaging. Identification of genetic factors responsible for controlling germination traits in salt stress conditions allows the development of tolerant cultivars. For this reason, it is very important and vital to identify the loci that control salinity resistance trait. In this regard, we discovered 31 gene locations with different explanation for the investigated traits, in three different conditions (normal, 6 and 12 dS/m). Respectively, 7 QTLs for normal condition, 42 QTLs for 6 dS/m salt stress and 35 QTLs for 12 dS/m salt stress were detected, which can be used to construct suitable populations for breeding programs.

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