

## Mapping QTLs Associated with Salt Tolerance Related Traits in Seedling Stage of Wheat (*Triticum aestivum* L.)

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### ABSTRACT

Salinity stress is a major limitation in wheat production. The lack of economically viable methods for screening salinity tolerance in the field is an obstacle to breeders. In this study, a population of 254 recombinant inbred lines (RILs), derived from a cross between two bread wheat cultivars, namely, Roshan × Sabalan, was assessed in glasshouse during the seedling phase in order to identify quantitative trait loci (QTLs) for salinity-tolerance related traits. A genetic linkage map was constructed from 239 markers, namely, 225 Diversity Arrays Technology markers (DARTs) and 14 simple sequence repeats (SSRs) which spanned a total of 1,099.7 cM. A total of 31 QTLs for salinity tolerance were identified on 13 chromosomes, contributing more than 50% of the total phenotypic variation. The frequency of Roshan and Sabalan alleles were high at loci in different homeologous groups. Most of the detected QTLs were located on chromosomes 3B and 5B, among the 13 chromosomes. Two QTLs related to fresh weight and height of shoot were detected on 1A and 3A, which explained 18% and 12.9% of the total phenotypic variation, respectively. Roshan (salt tolerance) alleles were associated with an increase in all traits under both control and stress conditions. SSR markers *gwm626* and *gwm540* (on chromosomes 6B and 5B, respectively) were tightly linked with different QTLs under control and stress conditions, and explained 21.1% and 8.1% of the total phenotypic variance, respectively. Some of these QTLs mapped to genomic regions previously associated with salt tolerance in wheat.

**Keywords:** QTL, RILs, Salinity, Seedling.

### INTRODUCTION

Wheat is one of the most important food crops in the world, and it's a part of daily diet of over 70% of the population of the world (Tang *et al.*, 2011). Salinity is one of the most important factors that limit crop production in irrigated and rain-fed

environments around the world. Salinity stress influences seedling establishment at early growth stages of crops and severely decrease yield (Bahrani *et al.*, 2012). Development of salt-tolerant crops is important on salt-affected soils, therefore, we should use strategies that reduce salt accumulation, such as improved agronomic

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practices and landscape management (Zhou *et al.*, 2011). From genetic and physiological point of view, salt tolerance is complex. Tolerance often shows the characteristics of a multigenic trait (Munns and Tester, 2008), with quantitative trait loci (QTLs) associated with tolerance identified in different plants (Flowers, 2004). Screening method based on hydroponics or supported hydroponics has become the preferred method for most researchers, because it gives a high degree of control and reproducibility (Genc *et al.*, 2007). Different screening methods have been reported by Munns and James (2003), however, it is necessary to test these methods in the field (El-Hendawy *et al.*, 2009). Hydroponic method was carried out at early growth stage because of limitations of space and time (Munns and James, 2003; Flowers, 2004; Xue *et al.*, 2009; Farshadfar *et al.*, 2008). Several studies about the genetics of salt tolerance have been conducted on wheat (Dashti *et al.*, 2010; Genc *et al.*, 2010a; Ma *et al.*, 2007). A gene, designated *NAX1*, for  $\text{Na}^+$  exclusion was mapped to the long arm of chromosome 2A using a QTL approach with AFLP, RFLP and microsatellite markers (Lindsay *et al.*, 2004). This locus had a LOD score of 7.5 and accounted for 38% of phenotypic variation. Ma *et al.* (2007) used 114 recombinant inbred lines (RILs) at the germination and seedling stages to identify QTLs for different agronomic traits. They detected 47 QTLs on all wheat chromosomes, except 1B, 1D, 4B, 5D, and 7D. El-Hendawy *et al.* (2007, 2009) reported that both physiological and agronomic traits had well-established screening criteria for salt tolerance under field and controlled conditions. Genc *et al.* (2010a) evaluated a population of 152 doubled haploids (DHs) wheat and detected 40 QTLs for different agronomical and physiological traits under salinity stress. They also found a QTL for  $\text{Na}^+$  exclusion named *HKT1; 4* that was located on 2A chromosome. This QTL coincided with locus *Nax1* in durum wheat found by Lindsay *et al.* (2004). In addition, a QTL for  $\text{Na}^+$  exclusion was identified that

associated with seedling biomass trait (Genc *et al.*, 2010a). Agronomic traits such as leaf area, shoot dry weight and physiological traits such as  $\text{Na}^+$  and  $\text{Cl}^-$  exclusion, leaf water relation, and photosynthesis (Chlorophyll content) have been studied for salinity tolerance (El-Hendawy *et al.*, 2007; 2009). High  $\text{K}^+/\text{Na}^+$  ratio in leaves of bread wheat correlates with higher salt tolerance and a locus named *Knal*, which is located on chromosome 4D, controls this parameter (Dubcovsky *et al.*, 1996). Salt tolerance was assessed as shoot dry matter after 4 weeks of salt treatment and, in general, the genotypes with the lowest  $\text{Na}^+$  concentrations produced the greatest dry matter (Benderradji *et al.*, 2011). The long arm of chromosome 4D of wheat contains a gene (or genes) which controls relative concentrations of  $\text{Na}^+$  and  $\text{K}^+$  in the shoot (Gorham *et al.*, 1990). Zhang *et al.* (2010) studied 114 recombinant inbred lines under hydroponic condition and detected 27 QTLs for leaf chlorophyll fluorescence traits. Tolerance to high saline concentration in bread wheat related to reduced accumulation of  $\text{Na}^+$ , to maintain adequate levels of  $\text{K}^+$  and to enhance capacity of osmotic adjustment (Benderradji *et al.*, 2011). In the current study, we used Diversity Arrays Technology (DARts) and simple sequence repeats (SSRs) markers to construct a genetic map, and screened recombinant inbred lines under non-saline and saline conditions in order to identify QTL associated with salinity tolerance traits and their contribution to salinity tolerance.

## MATERIALS AND METHODS

### Plant Materials

A population of 254  $F_7$  recombinant inbred lines (RILs), derived from a cross between two bread wheat cultivars, namely, Roshan  $\times$  Sabalan, by single seed descent were used in current study. Roshan is a native variety of Iran that is relatively tolerant to salinity stress (Poustini *et al.*, 2004). Sabalan was originally introduced

from a hybrid i.e. (908×FnA12) ×1-32-438<sup>2</sup>, produced in Iran, and is generally considered as susceptible to salinity.

### Phenotyping

RILs and their parents were evaluated for salt tolerance at two salt treatments (0 and 150 mM NaCl) with hydroponic in a greenhouse. The trial was arranged in randomized complete block design with three replications. The experiment was conducted with 16/8 day/night photoperiod, 27/20<sup>o</sup>C day/night temperature and relative humidity of about 60%. Seeds of parents and RILs were sterilized in 1% hypochlorite for 15 min and washed with distilled water, and germinated in petri dishes according to Munns and James (2003). After two days, germinated seeds were transferred to holes made in sheets of 2 cm styrofoam, which were floated on tap water (Azadi *et al.*, 2011). Two days after transplanting, half-strength Hoagland solution was applied for three days. Then, full-strength Hoagland solution was used. No salt was applied at the germination stage to ensure that all the lines germinated evenly. Ten days after transplanting, salt treatment started. NaCl was added to the solution 50 mM daily over 3 days to final concentration of 150 mM, with supplemental calcium as CaCl<sub>2</sub>.2H<sub>2</sub>O. Supplemental calcium was added to the salt treatment giving a Na<sup>+</sup>:Ca<sup>2+</sup> ratio of 15:1. This ratio was identified by Genc *et al.* (2007, 2010b) as optimum for growth under saline conditions. The nutrient solution was changed once a week. The pH was monitored daily with pH-meter. The pH of solution was maintained at 5.6-5.8 and adjusted using either HCl or NaOH every day. After three weeks of treatment with 150 mM NaCl, the chlorophyll content of base, middle, and tip (Munns and James, 2003; Munns *et al.*, 2003) of leaves was measured using a SPAD-502 chlorophyll-meter. Shoots were separately harvested and rinsed with distilled water. Shoot height and fresh weight were recorded, and then the materials

were oven-dried (48 h, 72 °C) for dry weight measurement. Salt tolerance was assessed by scores for leaf chlorosis. Low scores were assigned to lines with little or no signs of stress, while high scores were assigned to plants with severe chlorosis. The control experiment was conducted in the same way without adding salt. In the control conditions, symptoms of leaf chlorosis weren't observed. Pearson's correlation coefficients were calculated between traits under conditions of saline and control, separately, using SPSS 16. Analyses of the frequency distribution for traits among the 254 recombinant inbred lines in the salinity treatment were performed using SPSS 16.

### DNA Extraction and Marker Analysis

DNA was extracted from freeze dried leaves of 254 RILs and their parents using corporation protocol of the Diversity Array Technology Pty. Ltd. available ([http://www.diversityarrays.com/genotyping\\_serv.html](http://www.diversityarrays.com/genotyping_serv.html)); Jing *et al.*, 2009; Akbari *et al.*, 2006). A total of 232 SSR markers were tested for the existence of polymorphisms between parents. Sequence information of SSR markers was obtained: BARC (Song *et al.*, 2005), CFA and CFD (Guyomarc'h *et al.*, 2002), GWM (Röder *et al.*, 1998), WMC (Gupta *et al.*, 2002; Somers *et al.*, 2004) and the GrainGene database (<http://wheat.pw.usda.gov>). The PCR program for SSR primers was an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 50-65 °C (depending on the primer annealing) for 30 s, 72 °C for 1 min, and a final extension of 5 min at 72 °C before cooling to 4 °C. The PCR products were separated on 6% denatured polyacrylamide gel and visualized by silver staining. The banding patterns on one locus were scored as genotypes and represented by A, B, and “-” for the heterozygote genotype. In addition to the 232 SSR markers, 2112 DArT markers were provided by Diversity Array Technology Pty. Ltd. evaluated for genotyping of total



population. DArT markers are referred to using the prefixes “wPt” and “tpt”, followed by numbers.

### Linkage and QTL Analysis

Map Maker/EXP ver.3 (Lander *et al.*, 1987) was used to construct the linkage map using Kosambi mapping function. The logarithm of odds (LOD) threshold of higher than 3 was used. Segregation ratios of the two genotypes classes (Roshan and Sabalan alleles) at each locus were tested using the chi-square test ( $P < 0.01$ ). The segregation ratio at a locus deviating from the expected ratio of 1:1 indicated distorted segregation. These markers were excluded from QTL analysis (Collard *et al.*, 2005; Doerge *et al.*, 1997). Marker order analyses were conducted with LOD threshold of 0.1 and a REC threshold value of 0.45. The linkage mapping was compared with previous maps (Jing *et al.*, 2009; Akbari *et al.*, 2006; Semagn *et al.*, 2006; Genc *et al.*, 2010a). First, to identify putative QTLs and markers significantly associated with each trait, single marker analysis using the linear regression method option of QTL Cartographer v2.5 was performed. QTL analysis was performed by the Composite Interval Mapping (CIM) method using QTL Cartographer v2.5. Automatic cofactor selection was determined from a forward/backward regression using Windows QTL-Cartographer version 2.5. The walking speed chosen for all QTLs was 1 cM and the LOD threshold were calculated by 1000 permutation and  $P = 0.05$ , ranging from 2.8 for chlorophyll content under control condition to 3.1 for shoot dry weight under control condition. Two LOD support intervals around each QTL were established by taking the two positions, left and right of the peak, that had LOD values of two less than the maximum (Zhou *et al.*, 2011). The percentage of explained phenotypic variance was estimated for each QTL ( $R^2$ ). Graphical linkage groups were generated using Mapchart 2.2 (Voorrips, 2002). Epistatic

effects were calculated using QTLNetwork version 2.1 and  $P=0.05$  was used as the threshold for detecting epistatic QTLs.

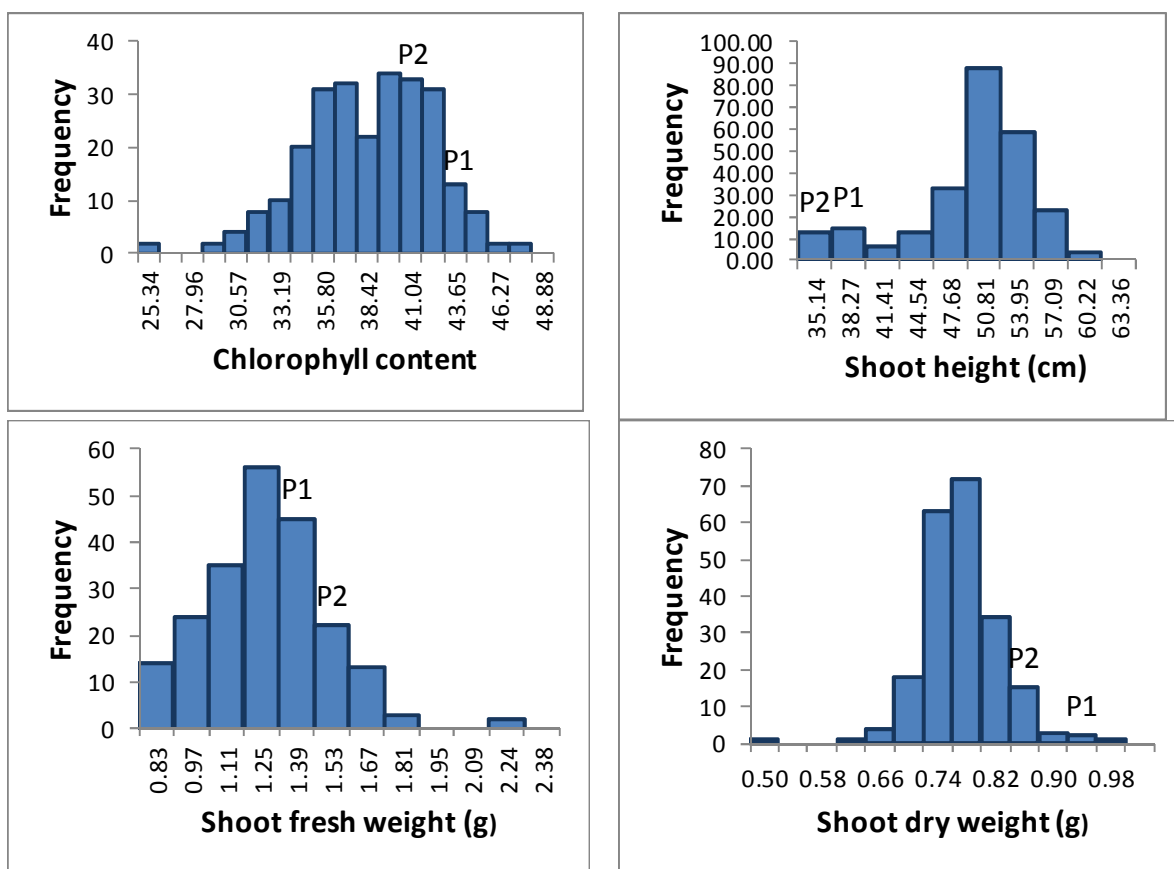
## RESULTS

### Statistical Analysis of the Phenotypic Assessments

Ten days after salt stress, the lower leaves of the susceptible lines started to show chlorosis that was a sign of salt damage, even leaves of some susceptible lines died under stress condition, while tolerant lines survived. RILs showed no symptoms of leaf chlorosis or wilting in the control condition. In contrast, they showed significant differences in the salinity stress damage (leaf chlorosis and wilting). Frequency distribution of population in this experiment showed approximately normal distribution for all traits with kurtosis and skewness values of population less than 1.0 (Figure 1). The grand mean and range of parents and 254 RILs are shown in Table 1. As shown in this Table, the two parents differed for all estimated traits. The salt-tolerant parent, Roshan, had a higher value than the salt-sensitive parent, Sabalan, for all traits under salinity stress. In addition, the mean value of RILs decreased under saline condition, except for chlorophyll content (Table 1). Simple correlation coefficients among all traits under conditions of control and stress are listed in Table 2. Significant correlations were obtained among traits in both conditions. Of these, the strongest correlation was between fresh and dry weight of shoots in both conditions ( $r = 0.65^{**}$  and  $r = 0.77^{**}$ , respectively). Fresh and dry weight of shoots was positively and significantly correlated with shoot height. Correlations were negative for shoot height versus chlorophyll content.

### Linkage Map of RIL Population

Out of 2344 DArT and SSR markers that were screened using DNA of the population



**Figure 1.** Frequency distribution of four traits (chlorophyll content, shoot height, shoot fresh weight and shoot dry weight) in saline conditions among 254 Recombinant Inbred lines originating from 'Roshan × Sabalan' cross.

**Table 1.** Mean ( $\pm$ SD) and range of studied traits in control and salt-stress condition for parents and 254 RILs derived from 'Roshan × Sabalan' cross.

Traits	Treatment	Parents			RILs			
		Roshan	Sabalan	Difference of Parents	Mean	Range	Skewness	Kurtosis
Leaf symptom	S <sup>a</sup>	4.37 $\pm$ 2.2	4.22 $\pm$ 1.9	0.15	4.05 $\pm$ 1.70	1-9	-----	-----
Shoot height (cm)	C <sup>b</sup>	51.01 $\pm$ 5.2	48.39 $\pm$ 6.5	2.62	50.23 $\pm$ 2.9	30.43-46.20	0.31	0.07
Shoot fresh weight (g)	S	37.09 $\pm$ 7.2	35.55 $\pm$ 4.4	1.54	37.65 $\pm$ 3.2	35.28-60.20	0.009	0.24
	C	3.98 $\pm$ 1.7	3.1 $\pm$ 8.8	0.88	3.25 $\pm$ 0.60	1.72-5.06	0.32	-0.14
Shoot dry weight (g)	S	1.50 $\pm$ 0.8	1.32 $\pm$ 2.9	0.18	1.2 $\pm$ 0.20	0.69-2.24	0.52	0.87
	C	1.01 $\pm$ 0.3	0.95 $\pm$ 1.9	0.06	0.9 $\pm$ 0.07	0.73-1.18	0.61	0.86
Shoot chlorophyll content	S	0.94 $\pm$ 0.5	0.86 $\pm$ 0.5	0.08	0.75 $\pm$ 0.05	0.47-0.98	0.05	0.92
	C	28.02 $\pm$ 5.1	30.55 $\pm$ 5.2	2.53	29.98 $\pm$ 2.80	21.62-37.32	-0.25	0.13
	S	42.67 $\pm$ 7.0	40.03 $\pm$ 6.7		37.94 $\pm$ 3.70	24.03-47.58	-0.41	0.53

<sup>a</sup> Salt-stress <sup>b</sup> Control, \*, \*\*, Significant at P<0.05, P<0.01, respectively

**Table 2.** Simple correlation coefficients between mean of traits measured on the Roshan × Sabalan mapping population in Salt stress (beneath part of diagonal) and Control conditions (upper part of diagonal)

Traits	Shoot height	Shoot fresh weight	Shoot dry weight	Shoot chlorophyll content
Shoot height	1	0.25**	0.23**	-0.16
Shoot fresh weight	0.24**	1	0.77**	-0.22**
Shoot dry weight	0.22**	0.65**	1	-0.15**
Shoot chlorophyll content	-0.56	0.34**	0.26**	1

\*\* significant at  $P < 0.01$ 

and parents i.e. Roshan and Sabalan, 501 polymorphic markers were found. Segregation distortion at each locus was tested using chi-square test. Sixteen SSR and 142 DArT markers that showed deviation from Mendelian ratio were excluded from further analysis. Finally, out of 343 remaining markers, 239 markers (14 SSR and 225 DArT markers) were used for a linkage map construction, which covered a length of 1,099.7 cM, while 104 markers remained unlinked. DArT and SSR markers were mapped on 21 linkage groups on 19 chromosomes (all, except 4D and 5D) with individual chromosome sizes ranging from 3.6 cM (6D chromosome) to 175.5 cM (1A chromosome) (Figure 2) with an average interval of 4.7 cM. The A, B and D genomes had 7, 7 and 5 chromosomes, covering lengths of 411.8, 620.4 and 67.5 cM, respectively. Maximum and minimum distances between two markers were 0 (in 3D) and 37.9 cM (in 6A). The numbers of markers per chromosome ranged from 38 markers on chromosome 1A to 4 markers on 2A. The DArT-SSR markers positions on each chromosome were compared with previously integrated DArT-SSR linkage maps (Jing *et al.*, 2009; Akbari *et al.*, 2006; Semagn *et al.*, 2006; Genc *et al.*, 2010a). Locations and the orders of the markers in our map were generally in agreement with previous studies and published reports in Grain Genes 2 (<http://wheat.pw.usda.gov/GG2/index.shtml>)

### Single Marker Analysis

To identify molecular markers significantly associated with each trait, single marker analysis using the linear regression method was performed. The significantly associated markers are indicated by asterisks in Table 3. Only markers with  $P \leq 0.01$  were considered and are shown in this table. *Gwm540* on chromosome 5B was tightly linked to shoot height in normal and fresh weight of shoot under stress conditions. *Gwm626* on chromosome 6B was significantly associated with fresh and dry weight of shoot and chlorophyll content under stress conditions. *gwm 88* on chromosome 6B was significantly associated with dry weight of shoot under stress conditions.

### QTL Mapping Analysis

#### Shoot Height

Eight QTLs (*Qsh3A*, *Qsh5B*, *Qsh3B*, *Qsh1D* and *Qsh2B*) were detected for shoot height on chromosomes 3A, 5B, 3B, 1D and 2B under saline and control conditions (Table 3). Roshan alleles were associated with increased shoot height for the QTLs on chromosomes 3A and 5B. The alleles for the QTLs on chromosomes 3B, 2B, 1D came from Sabalan and decreased shoot height. Two QTLs on 3A were expressed under both sets of conditions. These eight QTLs

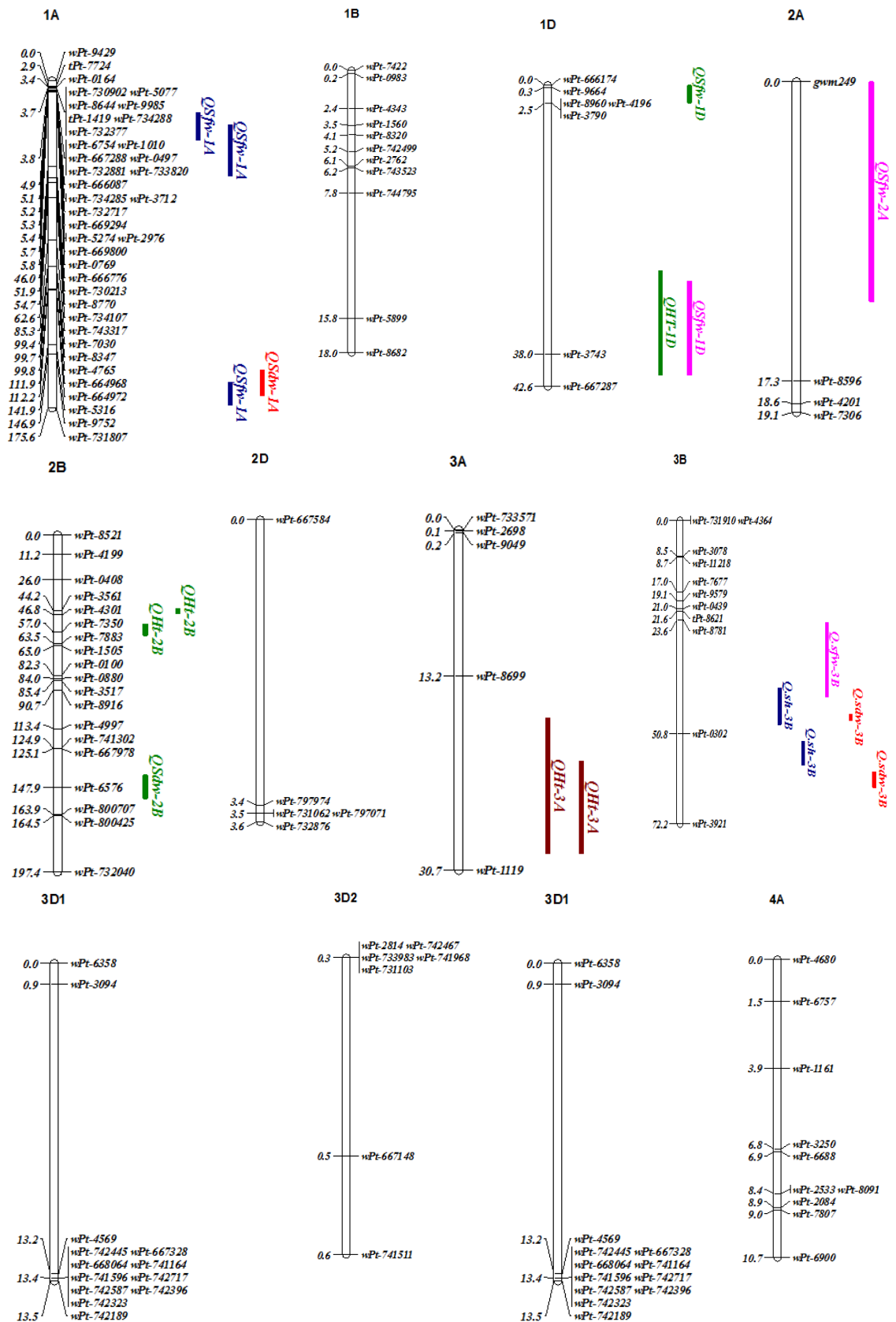
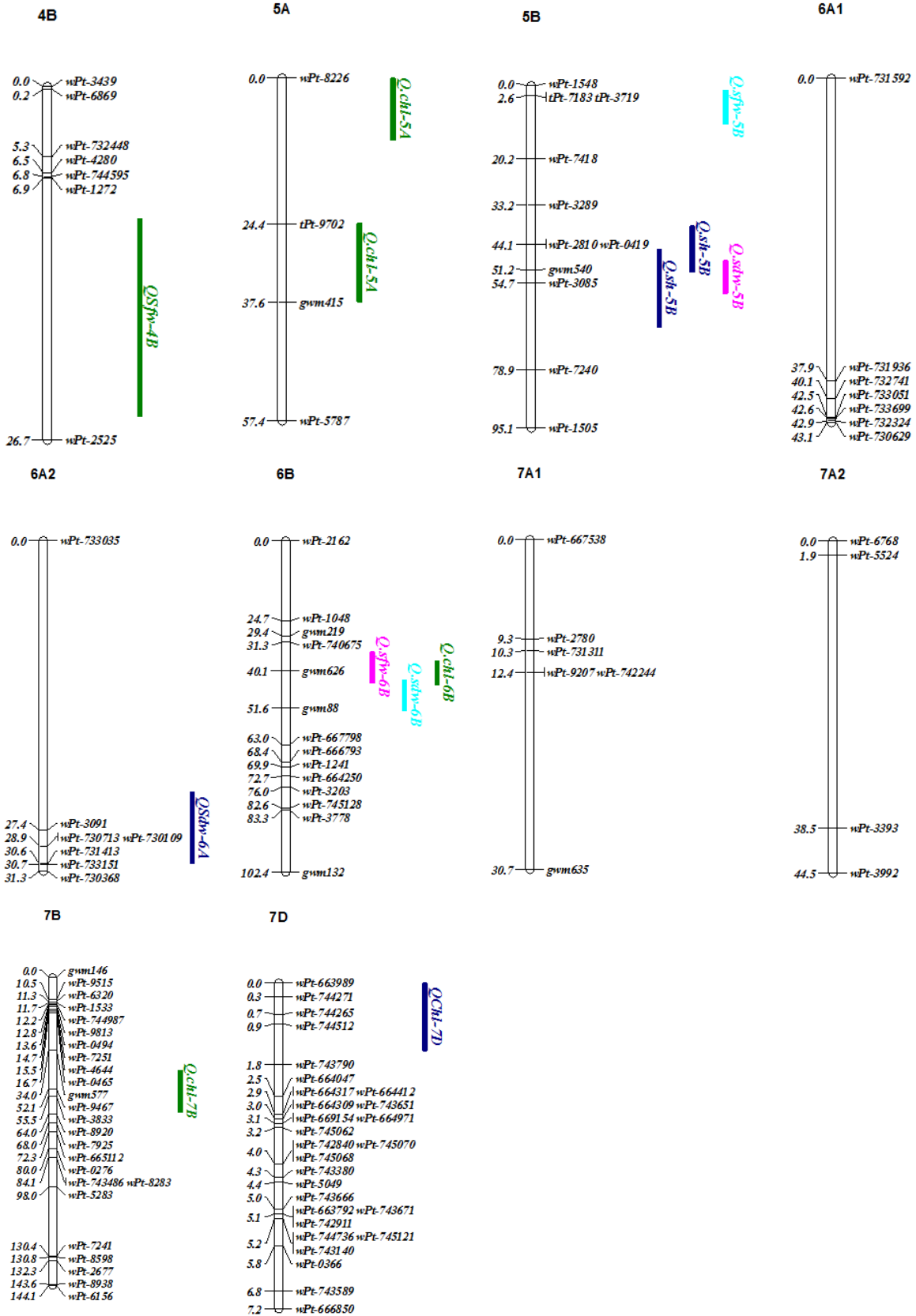


Figure 2. QTLs associated with traits measured in the Roshan × Sabalan mapping population. (continued)



Continued of Figure2



explained between 3.5%- 12.9% of phenotypic variation. The strongest QTL for shoot height was on chromosome 3A (*wpt8699-wpt119*), with a LOD score of 5.3 and  $R^2$  value of 12.9%, was identified in stress condition. This QTL had the largest effect on shoot height (1.15 cm). In general, the effects of QTLs were greater in saline than control conditions. Two QTLs were detected on chromosome 5B and 3A at approximately the same positions in both control and stress conditions (close to the SSR marker *gwm540*).

### Shoot Fresh Weight

Eleven QTLs (*Q.sfw1D*, *Q.sfw1A*, *Q.sfw4B*, *Q.sfw3B*, *Q.sfw6B*, *Q.sfw2A* and *Q.sfw5B*) were identified for shoot fresh weight on chromosomes 1D, 1A, 4B, 3B, 6B, 2A and 5B under both saline and control conditions (Table 3). Two QTLs on 1A and 1D were expressed under both conditions. Three QTLs on chromosomes 1A (under control and stress conditions) and 3B (control conditions) had the largest effects for this trait. Two QTLs on 1D (*wPt3743-wPt667287* interval) and 5B (*gwm540-wPt3085* interval) for fresh weight coincided with QTL for height. On chromosome 5B, the Roshan allele associated with higher shoot fresh weight and height under stress conditions. The QTLs for this trait explained 34.2% and 48.1% of the phenotypic variation under control and stress conditions, respectively. A QTL on 3B (*wPt0302-gwm533*), with a LOD score of 4.06 and  $R^2$  value of 9%, was identified in control conditions. The Roshan alleles for this QTL had association with increased fresh weight of shoot by 0.23 g.

### Shoot Dry Weight

Five QTLs (*Q.sdw3B*, *Q.sdw6A*, *Q.sdw6B* and *Q.sdw1A*) were found for shoot dry weight on chromosomes 3B, 6A, 6B, and 1A (Table 3). The alleles for the QTLs on chromosomes 6A, 3B came from Roshan and increased

shoot dry weight. The general contribution of additive effect of the five QTLs accounted for 40% of the phenotypic variation. A QTL in the interval between *gwm626* and *gwm88* on chromosome 6B, with a LOD=3.5 and  $R^2=5.7%$ , was associated with shoot dry weight. The numbers of QTLs were greater under saline than non-saline conditions. The most significant QTL for dry weight of shoot belonged to chromosome 1A (*wPt9752-wPt831807* interval), with a LOD score of 3.2, which explained 18% of the phenotypic variation. The QTL on 3B (*wPt8621-wPt0439* interval) co-located with QTL for shoot height. *Q.sdw1A* (*wPt9752-wPt731807* interval) coincided with the QTL for shoot fresh weight.

### Chlorophyll Content

Seven QTLs (*Q.chl2B*, *Q.chl6B*, *Q.chl7B*, *Q.chl7D* and *Q.chl5A*) were detected for chlorophyll content on chromosomes 2B, 6B, 7B, 5A and 7D (Table 3). The Roshan alleles for 2B, 5A and 7D were associated with an increase in chlorophyll content. QTLs on 6B and 5A had the largest effect on chlorophyll content under stress conditions. A QTL on chromosome 6B (*wPt740675-gwm626* interval) was co-located with the one for shoot fresh weight, and the Sabalan alleles on 6B were associated with decrease in shoot fresh weight and chlorophyll content. The numbers of QTLs were greater under non-saline than under saline conditions. Two QTLs were detected at approximately the same region on chromosome 5A under both conditions (close to the SSR marker *gwm415*). Under normal and stress conditions, QTLs for this trait explained 17.7% and 17% of phenotypic variation, respectively.

### Epistatic QTLs

Epistatic interactions were detected for only shoot fresh weight trait. Six QTLs were involved in epistatic QQ interactions (Table 4). Of these, four QTLs involved in QQ and QQE interactions had no main effects (Table 3, 4). Two QQE interactions (two QTLs) were observed (Table 4).

**Table 3.** QTL associated with evaluated traits in Roshan × Sabalan mapping population.

Traits	Condition	QTL	Position (cM)	Marker Interval	LOD	Additive effect <sup>a</sup>	R <sup>2</sup> -% <sup>b</sup>
Shoot height	S	<i>QHr-3A</i>	23.2	<i>wPr86998-wPr1119</i> ***	5.3	1.15	12.9
		<i>QHr-3B</i>	47.2	<i>tpi8621-wPr0439</i>	3	-0.639	3.9
		<i>QHr-3B</i>	52.8	<i>wPr17677-wPr11218</i>	2.9	-0.654	3.9
		<i>QHr-5B</i>	53.2	<i>gwm540-wPr3085</i>	4.1	0.837	5.9
		<i>QHr-2B</i>	25	<i>gwm526-wPr7350</i>	3.5	-0.842	6.4
Shoot fresh weight	C	<i>QHr-3A</i>	28.2	<i>wPr8699-wPr1119</i> **	3.8	0.817	6.9
		<i>QHr-5B</i>	44.1	<i>wPr0419-gwm540</i> **	2.9	0.62	3.5
		<i>QHr-1D</i>	40	<i>wPr1743-wPr1667287</i>	2.8	-0.6	3.6
		<i>QJfw-5B</i>	4.7	<i>tpi3719-wPr7418</i> **	4.9	0.077	8
		<i>QJfw-5B</i>	52.2	<i>gwm540-wPr3085</i> **	3.2	0.058	4.6
Shoot dry weight	S	<i>QJfw-2A</i>	2	<i>gwm249-wPr8596</i>	3.2	0.06	5
		<i>QJfw-1A</i>	45.9	<i>wPr0769-wPr1666776</i>	3.1	0.063	3.7
		<i>QJfw-1A</i>	171.9	<i>wPr19752-wPr1731807</i>	3	-0.265	16
		<i>QJfw-1D</i>	40	<i>wPr3743-wPr1667287</i>	3.1	-0.05	3
		<i>QJfw-6B</i>	38.3	<i>wPr740675-gwm626</i> **	3.5	-0.074	7.8
	C	<i>QJfw-3B</i>	27.6	<i>wPr0302-gwm533</i>	4.06	0.23	9
		<i>QJfw-1D</i>	0.3	<i>wPr9664</i>	2.9	-0.137	4
		<i>QJfw-1A</i>	20.9	<i>wPr0769-wPr1666776</i>	2.8	0.237	13.6
		<i>QJfw-4B</i>	19.9	<i>wPr1272-wPr2525</i>	3.8	0.18	7.6
		<i>QJfw-3B</i>	47.2	<i>wPr8621-wPr0439</i>	4.7	0.018	6.3
Shoot dry weight	S	<i>QJfw-3B</i>	60.1	<i>wPr11218-wPr3078</i>	3.8	-0.016	5
		<i>QJfw-6A</i>	28.4	<i>wPr3091-wPr730711</i>	4.3	0.014	5
		<i>QJfw-6B</i>	50.1	<i>gwm626-gwm88</i> **	3.5	-0.014	5.7
		<i>QJfw-1A</i>	62.9	<i>wPr9752-wPr1731807</i>	3.2	-0.07	18
		<i>QJfw-1D</i>	0.9	<i>wPr744512</i> **	3.2	0.8	4
Chlorophyll content	S	<i>QCht-6B</i>	40.1	<i>wPr740675-gwm626</i> **	3.4	-1.09	7.6
		<i>QCht-5A</i>	2	<i>wPr8226-tpi9702</i>	3.4	-0.99	6.1
		<i>QCht-7B</i>	55.1	<i>wPr9467-wPr3833</i>	2.9	-0.67	3
		<i>QCht-2B</i>	75.6	<i>gwm55</i>	2.9	0.597	3.9
		<i>QCht-2B</i>	162.3	<i>wPr0880</i> **	3.9	-0.693	5
		<i>QCht-5A</i>	31.4	<i>tpi9702-gwm415</i>	2.8	0.719	5.1

C, Control; S, Salt treatment, \*\*, \*\*\*, and \*\*\*\* present significant levels at P<0.01, 0.001, and 0.0001, respectively. <sup>a</sup> QTL with positive and negative additive effect contributed by Roshan and Sabalan, respectively. <sup>b</sup> Per-cent of phenotypic variation explained by each QTL

**Table 4.** QTL interactions involving (Q × Q or Q × E) for shoot fresh weight trait in Roshan × Sabalan mapping population.

QTL	Marker Interval	Position (cM)	QTL	Marker Interval	Position (cM)	AA	AAE1	AAE2
<i>QJfw.abrii-4B</i>	<i>wPr1272-wPr2525</i>	14.9	<i>QJfw.abrii-5B</i>	<i>wPr7418-wPr3289</i>	31.2	0.08	----	----
<i>QJfw.abrii-1A</i>	<i>wPr664972-wPr5316</i>	122.2	<i>QJfw.abrii-7D</i>	<i>wPr0366-wPr743589</i>	5.8	0.12	0.09	-0.09
<i>QJfw.abrii-3D.2</i>	<i>wPr667148-wPr741511</i>	0.5	<i>QJfw.abrii-7A.2</i>	<i>wPr3393-wPr3992</i>	42.5	-0.08	----	----

AA additive effect; AAE1 and AAE2 epistasis associated with environments of control and stress, respectively.

## DISCUSSION

### Linkage Mapping

Akbari *et al.* (2006) reported that Diversity Arrays Technology (DArT) performs well for the hexaploid genome of bread wheat. Different factors caused segregation distortion (Peleg *et al.*, 2008; Genc *et al.*, 2010a); which may lead to a biased estimate of marker-trait association (Gupta *et al.*, 2002; Genc *et al.*, 2010a). As a result, markers with distorted segregation were not used in current study. The total length of map was 1,099.7 cM, with an average distance of 4.7 cM between adjacent markers. Genome coverage and marker densities were different in the previous DArT-SSR integrated map in wheat (Semagn *et al.*, 2006; Singh *et al.*, 2005; Jing *et al.*, 2009; Ding *et al.*, 2011; Genc *et al.*, 2010a; Akbari *et al.*, 2006). Differences in lengths and marker densities for linkage maps depended on differences in mapping population, the types and error-rates the genetic marker systems used, and mapping function type used (Kosambi's and Haldane's) (Jing *et al.*, 2009; Singh *et al.*, 2007; Semagn *et al.*, 2006). The total length and the number of markers mapped to the D genome were much lower than the A and B genomes in the present map. The low level of polymorphism in the D genome has been reported (Chalmer *et al.*, 2001; Röder *et al.*, 1998; Semagn 2006). There was variation in the number of markers, map length and density according to the type of genome. Marker number, density, and map length were the greatest in B genome. Furthermore, the distribution of markers among the genomes and homeologous groups was not uniform. This might be attributed to lack of a sufficient number of polymorphic markers in the gaps of the chromosomes.

### Distribution of Additive Effect

The effects of all identified QTLs were additive for all traits. The alleles frequency of Roshan parents were high at loci on

chromosomes 3A, 5B, 1A, 4B, 3B, 6A, 2B, 7D, 5A, 2A (a total of 15 loci). The alleles frequency of Sabalan parents were higher than Roshan at loci on chromosomes 3B, 1D, 2B, 1A, 5A, 6B, 7B (a total of 16 loci). In general, additive effects were higher in the intervals *wPt8699-wPt119* (chromosome 3A), *gwm540-wPt3085* (chromosome 5B), *gwm526-wPt7350* (chromosome 2B), *wPt740675-gwm626* (chromosome 6B), and *wPt8226-tp19702* (chromosome 5A). The magnitude of the QTLs effects detected in the primary mapping population is important for further QTL fine mapping and cloning (Zhang *et al.*, 2008).

### QTL Analysis

In this research, thirty-one QTLs were associated with salinity-related traits in the RILs population derived from a cross between Roshan × Sabalan. A large number of QTLs for salt tolerance have been identified in important crop species, including rice and other cereal, using a range of traits under stress conditions (Genc *et al.*, 2010a; Koyama *et al.*, 2001; Lindsay *et al.*, 2004; Ma *et al.*, 2007; Mohammadi-Nejad *et al.*, 2008; Tang *et al.*, 2011; Yang *et al.*, 2009; Zhang *et al.*, 2010; Zhou *et al.*, 2011). In our study, the D genome had the fewest QTLs among the three genomes. This might be attributed to length and marker density of D genome (Quarrie *et al.*, 2005). Chromosome 3B with lengths of 68.6 and 5 QTLs had the largest number of QTLs. Adequate marker distribution may cause the identification of numerous QTLs on the chromosomes. Some of QTLs found only in normal or stress conditions for all traits. Nineteen out of thirty-one detected QTLs were associated with stress conditions. It is difficult to compare this study results with previous studies. In previous studies, researchers seldom mapped QTLs for salinity-related traits with identical markers. One of the reasons is that the linkage maps have been constructed using different



populations, which are hard to be compared with each other (Ding *et al.*, 2011). These linkage maps were constructed by Ding *et al.* (2011) using 2 associated RIL populations with a common parent for yield related traits, but only nine QTLs and two chromosomal regions were identical in the two populations. Five QTLs were associated with shoot height and shoot fresh weight on chromosomes 5B and 4B under salt stress. McCartney *et al.* (2005) reported QTLs for shoot fresh and dry weight on chromosomes 2D, 4B, 4D, 5B, 7A and 7B. Two co-located QTLs for height and fresh weight of shoot were detected on 5B and 1D chromosomes. Moreover, a positive and significant correlation was found between shoot height and fresh weight (Table 2). This suggested that pleiotropy is the possible cause of the correlations among these traits. The QTLs for fresh weight and height of shoot were present under both control and saline conditions (Table 3), indicating that genes responsible for these traits should be constitutively expressed and not specific to salinity stress. In general, most of the QTLs for traits were found on genome B. The QTL *Q.sfw5B* contributed to the shoot fresh weight of 5.8% of total phenotypic variability and mapped in interval of *gwm540-wPt3085*. Genc *et al.* (2010a) detected that a QTL in the interval between *gwm213* and *wPt0103* on chromosome 5B was associated with wheat seedling biomass, and SSR marker of *gwm540* was very close to *gwm213* (3 cM in mapping of Genc *et al.*, 2010a). Several QTLs were detected on chromosomes 2A, 3B and 1A for shoot dry and fresh weight. Ma *et al.* (2007) found QTLs on chromosomes 2A, 3B, 1A, 2B, 3D and 4A for these traits. Chlorophyll content and shoot fresh weight related QTLs on stress condition were located on chromosome 6B. This QTL co-located to the same interval *wpt740675-gwm626* on chromosome 6B. Significant correlations and coincident QTL were observed between Chlorophyll content and fresh weight by Genc *et al.* (2010a). The coincident QTLs are useful for marker assisted selection

technique and high-resolution mapping leading to map-based cloning of QTLs for agronomically important traits (Kumar *et al.*, 2007). Three QTLs explaining most of the variation were located on chromosomes 1A: 18% of total phenotypic variation, related to shoot dry weight under control conditions and 16% of total phenotypic variation for fresh weight, under stress conditions, 3A: 12.9% of total phenotypic variation, related to height under stress conditions. Moreover, most of the markers were located on chromosome 1A of the linkage map. One of QTLs identified for shoot chlorophyll content was located on chromosome 7D. Zhang *et al.* (2010) identified QTLs for leaf chlorophyll fluorescence traits on chromosome 7D. The QTL *Q.chl5A* explained 11 % of phenotypic variance (*wPt8226-tpt9702-gwm415* interval) under saline and control conditions. Genc *et al.* (2010a) found a QTL on chromosome 5A for chlorophyll content (but no similar marker interval). Three SSR markers, namely, *gwm626*, *gwm540*, and *gwm88* were significantly associated with different QTLs and they account for 21.1%, 8.1%, and 5.7% of phenotypic variance, respectively. These markers could be used for wheat breeding program by MAS. We identified QTLs on chromosomes 3A (*Q.sh3A*) and 3B (*Q.sh3B*, *Q.sdw3B*) for shoot height. Effective QTLs for plant height were similarly mapped on the same chromosomes 3A and 3B by Ma *et al.* (2007). In our study, QTLs related to salt tolerance were located on different homeologous groups, but we have no results supporting the hypothesis that these QTLs are effective at the later growth stages. Therefore, further experiments for salinity tolerance with plants at developed stages of growth will be required in future studies. In addition, QTLs on chromosomes 7D (linked to chlorophyll content) and 6A (linked to dry weight) were the only loci identified under salt stress. These regions could be considered in future studies for salinity tolerance in wheat. In our study, four QTLs involved in QQ/QQE interactions had no main effects QTLs. QTLs with no main

effects actuate their effect through interaction with other QTLs (Kumar *et al.*, 2007). In general, the coverage of markers on the map was relatively low and gaps between markers still remained. Thus, it is necessary to increase marker density especially on chromosomes with important QTLs which become a source for candidate genes or can be used for marker-assisted selection (MAS).

### CONCLUSION

In a population of 254 F<sub>7</sub> RILs derived from a cross between Roshan and Sabalan, thirty-one QTLs in total were mapped on 13 chromosomes of wheat for traits such as shoot height, fresh and dry weight of shoot, and chlorophyll content. Co-location QTLs on chromosomes 5B, 6B, and 1D could be attributed to pleiotropic effects. Some QTLs were detected under both saline and control conditions. Chromosomal regions containing co-located QTLs are useful in marker assisted selection.

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## نقشه یابی QTL های صفات مرتبط با تحمل به شوری در گیاهچه گندم (*Triticum aestivum* L.)

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### چکیده

تنش شوری یکی از محدودیت های مهم تولید گندم است. یکی از مشکلات بهنژادگران برای غربال تحمل به شوری در شرایط مزرعه فقدان روش های غربال مقرون به صرفه است. در این مطالعه به منظور شناسایی QTL های صفات مرتبط با تحمل به شوری، ۲۵۴ لاین اینبرد نوترکیب حاصل از تلاقی دو واریته گندم روشن × سبلان در مرحله گیاهچه در شرایط گلخانه مورد ارزیابی قرار گرفتند. نقشه پیوستگی ژنتیکی از ۲۳۹ نشانگر شامل ۲۲۵ نشانگر DArT و ۱۴ نشانگر SSR ترسیم شد که کل طول نقشه ۱۰۹۹.۷ سانتی مورگان بود. بطور کلی ۳۱ QTL بر روی ۱۳ کروموزوم برای تحمل به شوری شناسایی شدند که بیش از ۵۰ درصد از تغییرات فنوتیپی را توجیه کردند. در گروه های همپولوگی مختلف فراوانی آللهای روشن و سبلان در مکان ها بالا بودند. در بین ۱۳ کروموزوم، بیشترین تعداد QTL بر روی کروموزوم های ۳B و ۵B مکان یابی شدند. دو QTL مرتبط با وزن تر و ارتفاع اندام هوایی بر روی کروموزوم های ۳A و ۱A شناسایی شدند که به ترتیب ۱۸ و ۱۲.۹ درصد تغییرات فنوتیپی را توجیه کردند. تحت هر دو شرایط نرمال و تنش، آللهای روشن (والد متحمل به شوری) با افزایش همه صفات همبسته بودند. نشانگرهای SSR، *gwm626* و *gwm14* (به ترتیب بر روی کروموزوم های ۶B و ۵B) بطور محکمی با QTL های مختلف تحت هر دو شرایط نرمال و تنش پیوسته بودند و به ترتیب ۲۱.۱ و ۸.۱ درصد از تغییرات فنوتیپی را توجیه کردند. بعضی از این QTL ها در مناطق ژنومیکی نقشه یابی شدند که در تحقیقات پیشین با تحمل به شوری گندم همبسته بودند.