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Quantitative trait loci (QTL) mapping for physiological and biochemical attributes in a Pasban90/Frontana recombinant inbred lines (RILs) population of wheat (*Triticum aestivum*) under salt stress condition

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ABSTRACT

Salt stress causes nutritional imbalance and ion toxicity which affects wheat growth and production. A population of recombinant inbred lines (RILs) were developed by crossing Pasban90 (salt tolerant) and Frontana (salt suceptible) for identification of quantitative trait loci (QTLs) for physiological traits including relative water content, membrane stability index, water potential, osmotic potential, total chlorophyll content, chlorophyll a, chlorophyll b and biochemical traits including proline contents, superoxide dismutase, sodium content, potassium content, chloride content and sodium/potassium ratio by tagging 202 polymorphic simple sequence repeats (SSR) markers. Linkage map of RILs comprised of 21 linkage group covering A, B and D genome for tagging and maped a total of 60 QTLs with major and minor effect. B genome contributed to the highest number of QTLs under salt stress condition. Xgwm70 and Xbarc361 mapped on chromosome 6B was linked with Total chlorophyll, water potential and sodium content. The increasing allele for all these QTLs were advanced from parent Pasban90. Current study showed that Genome B and D had more potentially active genes conferring plant tolerance against salinity stress which may be exploited for marker assisted selection to breed salinity tolerant high yielding wheat varieties.

1. Introduction

Wheat (*Triticum aestivum* L.) is main food commodity in many countries and used as a staple food in Pakistan (Braun et al., 2010; Zeb et al., 2013). Crop plants growth and productivity show significant reduction in water shortage, high salt levels in soil, high temperature and low temperature stress (Azadi et al., 2015). High salt levels in soil is main abiotic stress which impose negative impact on wheat plant growth and yield (Manzoor et al., 2015).

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Salt stress affecting around 800 million hectares of cultivated land worldwide (FAO, 2016) and in Pakistan, India and Egypt about 10% cultivated land affected by high salt levels (Shahzad et al., 2012). Total area under cultivation in Pakistan is 20.36 Mha and high salt in soil severely damaged 6.67 Mha land (Ashraf, 2014).

Various scientists finding suggest that, high salt levels in soil inflict negative influence on crop plant through osmotic and ionic stress (Ghavami et al., 2004; El-Hendawy et al., 2005). Brini and Masmoudi (2012) findings suggest that salt stress is main source in wheat genotypes to cause hormonal imbalance, fluctuation in nutrient uptake and overproduction of oxidizing agent. Wheat genotypes grown in lowest salt level produced more dry matter as compared to wheat genotypes in higher salt levels (Asgari et al., 2012). Furthermore, wheat genotypes physiological processes show considerable variation in high salt level, primarily photosynthetic processes, plant water relation and cell membrane stability (Ashraf and Harris, 2013). Salt stress predominant impact on wheat genotypes morphological and physiological parameter leads to drastic reduction in quality and quantity of wheat plant yield.

Salinity tolerance mechanism of the crop is very complex and polygenic trait, and is recognized by the ability of the crop to withstand saline conditions. Multiple salt tolerance mechanism is adapted by crop plant at physiological level such as selective ion uptake, sodium ion compartmentation at organ & organelle level and ion exclusion & excretion (Arraouadi et al., 2012). To maintain sodium low level inside plant organ and cell is not a tranquil technique. Quantitative inheritance can be examined by identifying morphological and physiological characters of crops for salt tolerance using molecular markers (Azadi et al., 2015). Quantitative trait loci (QTLs) identification helps to recognize natural variation in salt tolerance ability of the crops. Most of already identified QTLs are associated with yield and yield components but few studies are related to physiological attributes. So, wheat plant water relation, chlorophyll content, membrane stability, sodium & potassium ion distribution in shoot & root and Na^+/K^+ ratio have been great interest to study for QTLs. Compartmentalization of salt ions at organ level and physiological attributes might be playing a role in salt tolerance in wheat mapping population. This study was carried out to categorize new QTLs associated with physiological and biochemical attributes for salt tolerance in wheat Pasban90 × Frontana RILs mapping population. We map many QTLs related to physiological attributes show variation between 0 and 150 mM NaCl at vegetative stage

2. Materials and methods

A study of phenotypic traits was carried out at the National Institute of Genomics and Advanced Biotechnology (NIGAB), the National Agribusiness Research Center, Islamabad. The genotypic studies were conducted at the Department of Biology at the University of Florida, USA. The cartographic population consisted of recombinant inbred lines, which were obtained by crossing between Frontana and Pasban 90. The developed RILs population has been used for QTLs mapping for morphological traits at germination stage (Batool et al., 2018). Among the both varieties, Frontana was moderately salt sensitive and Pasban90 resistance to salts. Experiments were performed in a completely randomized block design with three replicates per treatment. Parents and RILs were examined for both treatments controls and 150 mM NaCl stress. Sand was used for seed germination. After 10 days, the seedlings were transferred to a 200 L steel tank containing Hoagland nutrient solution (Hoagland and Arnon, 1950). A 150 mM salinity level was maintained by adding 50 m M NaCl per day.

2.1. Phenotyping

Phenotypic data was recorded after 30 days of stress. The parameters that were observed included physiological and biochemical traits. physiological traits including relative water content (RWC), membrane stability index (MSI), water potential (WP), osmotic potential (OP), total chlorophyll content (TChl), chlorophyll a (Chl a), chlorophyll b (Chl b) and biochemical traits including proline contents (Pro), superoxide dismutase (SOD), sodium content (Na), potassium content (K), chloride content (Cl) and sodium/potassium (Na/K). The compartmentalization of sodium, potassium and chloride contents at the organ level was measured using a flame emission and atomic absorption spectrophotometer (Sulian et al., 2012). Relative water content and the membrane stability index was measured following the methodology of Manzoor et al. (2015). Scholander pressure chamber was used to record leaf water potential (Scholander et al., 1965). Capell and Doerffling (1993) method was used to determine leaf osmotic potential by osmometer. Chlorophyll conten t was determined by using method of Manzoor et al. (2015). Proline content was estimated by using method of Bates et al. (1973). Superoxid e dismutase was determined with the help of spectrophotometer (Giannopolitis and Reis, 1997).

2.2. Genotyping

Sharp et al. (1988) method was used for DNA extraction from young leaves of RILs and parent plants. Mapping population RILs (AR04) was genotyped with 202 SSR markers. These SSR markers were selected (WMC, BARC, GWM, CFA and CFD) on a perennially published wheat map (graingenedatabase http://wheat.pw.usda.gov) (Gupta et al., 2001; Guyomarćh et al., 2002; Sourdille et al., 2003; Roder et al., 1998; Xue et al., 2008). The link map was constructed using the mapping function Kosambi by the worm mapping. 3.0 after linkage map construction, QDF data file generated by using excel sheets in which phenotypic and genotypic data was combined. QDF data files were subjected to QGene ver. 4.0 to identify QTLs using composite interval mapping and logarithm of odds (LOD) 2.5 thresholds. QTLs clustering were analyzed on chromosomes by using map chart ver. 2.0. Data of all parameters was subjected to Statistix 8.1 v for analysis of variance (ANOVA) and correlation was calculated among all traits under control l and salt stress conditions.

3. Results

3.1. Phenotypic description

The results of ANOVA showed all the traits showed significant differences between RILs, treatments and RILs × treatment interaction (Tables 1 and 2). RILs and treatment interaction was highly significant for ($p \leq 0.01$) for RWC, MSI, WP, TChlC, Chl a, Chl b, Pro, SOD, Na^+, K^+ and significant for ($p \leq 0.05$) OP, Cl- and Na^+/K^+ ratio. Correlation coefficients among physiological traits (Table 3) and among biochemical traits (Table 4) is given under control and salt stress conditions. All recorded traits show high and low trangressive segregation in mapping population under salt stress. Frequency distribution is given for parents positions among RILs and RWC, MSI, WP, OP (Fig. 1), TChlC, Chl a, Chl b, Pro, SOD (Fig. 2) and K^+, Cl^-, Na^+/K^+ and Na^+ (Fig. 3) under control and salt stress condition. Parental position is clearer from population range. Parents (Pasban90 and Frontana) were highly polymorphic under salt stress conditions. Pasban90 show higher values for all traits as compared to Frontana under salt stress environment.

Table 1
Analysis of variance of physiological traits under hydroponics culture for parents and recombinant inbred lines (RILs) under control and salt stress.

	df	RWC	MSI	WP	OP	TChl	Chla	Chlb
Lines	86	4.8**	3.51*	2.32*	1.8**	2.053**	0.88*	0.630**
Treatment	1	32939*	59125*	3127*	1830*	4088.4**	1441.2*	2053.5*
L*T	86	4.65**	3.29**	1.9**	1.6*	1.914**	0.980**	0.663**
Error	348	0.000	0.000	0.000	0.000	0.013	0.002	0.005

Significance levels: *P = 0.05, ** P = 0.01, nsnon-significant; RWC (Relative Water Content), MSI (Membrane Stability Index), WP (Water Potential), OP (Osmotic Potential), TChl (Total Chlorophyll Content), Chl-a (Chlorophyll-a), Chl-b (Chlorophyll-b).

Table 2
Analysis of variance of biochemical traits under hydroponics culture for parents and recombinant inbred lines (RILs) under control and salt stress.

	df	P	SOD	Na	K	Cl	K/Na Ratio
Lines	86	9.715**	0.534*	1.411*	1.174*	5.881*	4.503*
Treatment	1	475618.9**	4133.7*	7247.6*	6713.4*	8942.4*	3002.1*
L*T	86	15.291**	0.572**	1.231**	0.921**	3.010*	4.137*
Error	348	0.103	0.000	0.110	0.156	0.151	0.134

Significance levels: *P = 0.05, **P = 0.01, nsnon-significant; P (Proline), SOD (Super Oxide Dismutase), Na (Sodium), K (Potassium), Cl (Chloride).

Table 3
Correlation coefficients for physiological traits under hydroponic culture for recombinant inbred lines (RILs) population of Pasban 90 × Frontana in control and salt stress.

		RWC	MSI	WP	OP	TChl	Chl-a	Chl-b
RWC	C	1						
	S	1						
MSI	C	0.10ns	1					
	S	0.26*	1					
WP	C	−0.10ns	0.21*	1				
	S	0.28*	0.34**	1				
OP	C	−0.15ns	0.25*	0.24*	1			
	S	0. 34*	0.27*	0.21*	1			
TChl	C	0.12ns	0.23*	0.19ns	0.12ns	1		
	S	0.27*	0.26*	−0.04ns	−0.26*	1		
Chla	C	0.09ns	0.21*	0.13ns	0.10ns	0.18*	1	
	S	0.31**	0.19*	−0.20*	−0.32**	0.19*	1	
Chlb	C	0.19*	−0.10ns	−0.02ns	−0.019ns	0.24*	0.11ns	1
	S	0.053ns	0.21*	−0.11ns	−0.108ns	0.21*	0.13ns	1

Significance levels: *P = 0.05, **P = 0.01, nsnon-significant, RWC = relative water contents, MSI = membrane stability index, WP = water potential, OP = osmotic potential, TChl = total chlorophyll, Chl-a = chlorophyll-a, Chl-b = chlorophyll-b.

Table 4
Correlation coefficients for biochemical traits under hydroponic culture for recombinant inbred lines (RILs) population of Pasban 90 × Frontana in control and salt stress.

		Pro	SOD	Na	K	Cl	K/Na Ratio
Pro	C	1					
	S	1					
SOD	C	0.22*	1				
	S	0.21*	1				
Na	C	0.10ns	0.11ns	1			
	S	0.23*	0.28*	1			
K	C	0.11ns	0.14ns	0.13ns	1		
	S	0.29*	0.31*	−0.19*	1		
Cl	C	0.12ns	0.09ns	0.13ns	0.149ns	1	
	S	0.21*	0.14ns	0.28*	−0.23*	1	
K/Na Ratio	C	0.11ns	0.11ns	0.062ns	0gy.091ns	0.13ns	1
	S	0.19*	0.29*	−0. 21*	0.20*	−0.11ns	1

Significance levels: *P = 0.05, **P = 0.01, nsnon-significan, Pro = proline, SOD = super oxide dismutase, Na = Sodium, Cl = Chloride, K/Na ratio = Potassium/Sodium ratio.

3.2. QTL mapping

A total 280 SSRs were used to screen parents and out of which 202 were identified as polymorphic which were used to develop linkage maps. Linkage map covered the distance of 2721.5 cM on 30 linkage groups. Out of 30 linkage groups, 21 were assigned to chromosomes and with an average length of 200 cM. Linkage map covered the A, B and D of genome *Triticum aestivum* with average length of 1058.3 cM, 901.6 cM and 761.6 cM respectively. A total of 60 major and minor QTLs were mapped (Table 5).

3.3. Quantitative trait loci for physiological attributes

QTLs for all physiological traits were associated with all three sets of chromosomes: genome A ranging from 2A to 7A (Fig. 4), genome B ranging from 1B to 7B (Fig. 5) and 1D to 7D (Fig. 6) QTLs for RWC, MSI, WP, OP, TChl and Chl b were localized on chromosome 2A, 3A, 4A, 7A (Fig. 4), 2B, 5B, 6B, 7B (Fig. 5), 1D, 3D, 6D and (Fig. 6) but no QTL were identified for Chl a under control condition. *q*TChl.3D.CH and *q*TChl.6D.CH were associated with D genome and highest phenotypic variation ranged from 19% and 26%,

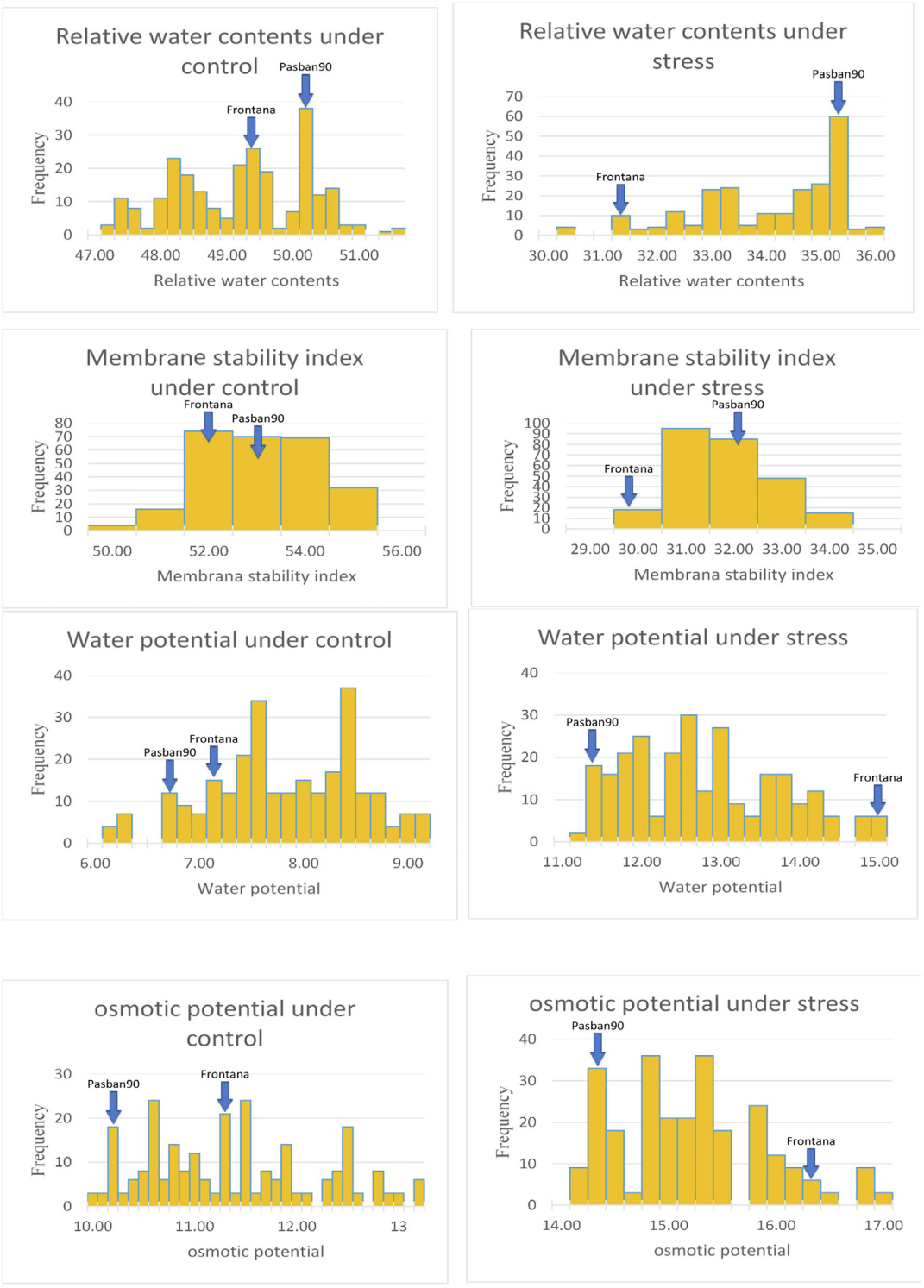

Fig. 1. Frequency distribution of relative water contents (RWC), membrane stability index (MSI), water potential (WP) & osmotic potential (OP) and parents positions among recombinant inbred lines (RIL) under control and stress i.e. NaCl (150 mM).

respectively. *q*OP.5D.CH was reported first time on 5D chromosome of mapping population contributes 16% of phenotypic variation. *q*WP.5D.CH is new QTL related to water potential contributes 15% of phenotypic variations (Fig. 6). *q*Chlb.1D.CH was associated with D genome of RILs explained 21% R2 *q*RWC.4A.CH, QTLs (*q*MSI.4A.CH and *q*MSI.3D.CH linked to A genome and explained 15 to 16 percentages of phenotypic variation. QTL for chlorophyll a *q*Chla.7D.SH was identified on 7D of mapping population with 21% of phenotypic variation. RWC, MSI, WP, OP, TChl, Chl a and Chl b QTLs were associated with 1B, 2A, 3A, 3B, 4A 5B, 6A, 6B, 7A 7B and 7D chromosome of RILs under salt stress (Figs. 4–6). *q*Chl b.7B.SH is a new QTL mapped on 7B and contribute 17% phenotypic variation. *q*OP.7A.SH for osmotic potential was flanked in Xgwm63-Xbarc49 contributes 13% of phenotypic variation. QTL for membrane stability index was localized on 3A and contributes 15 percent of phenotypic variation.

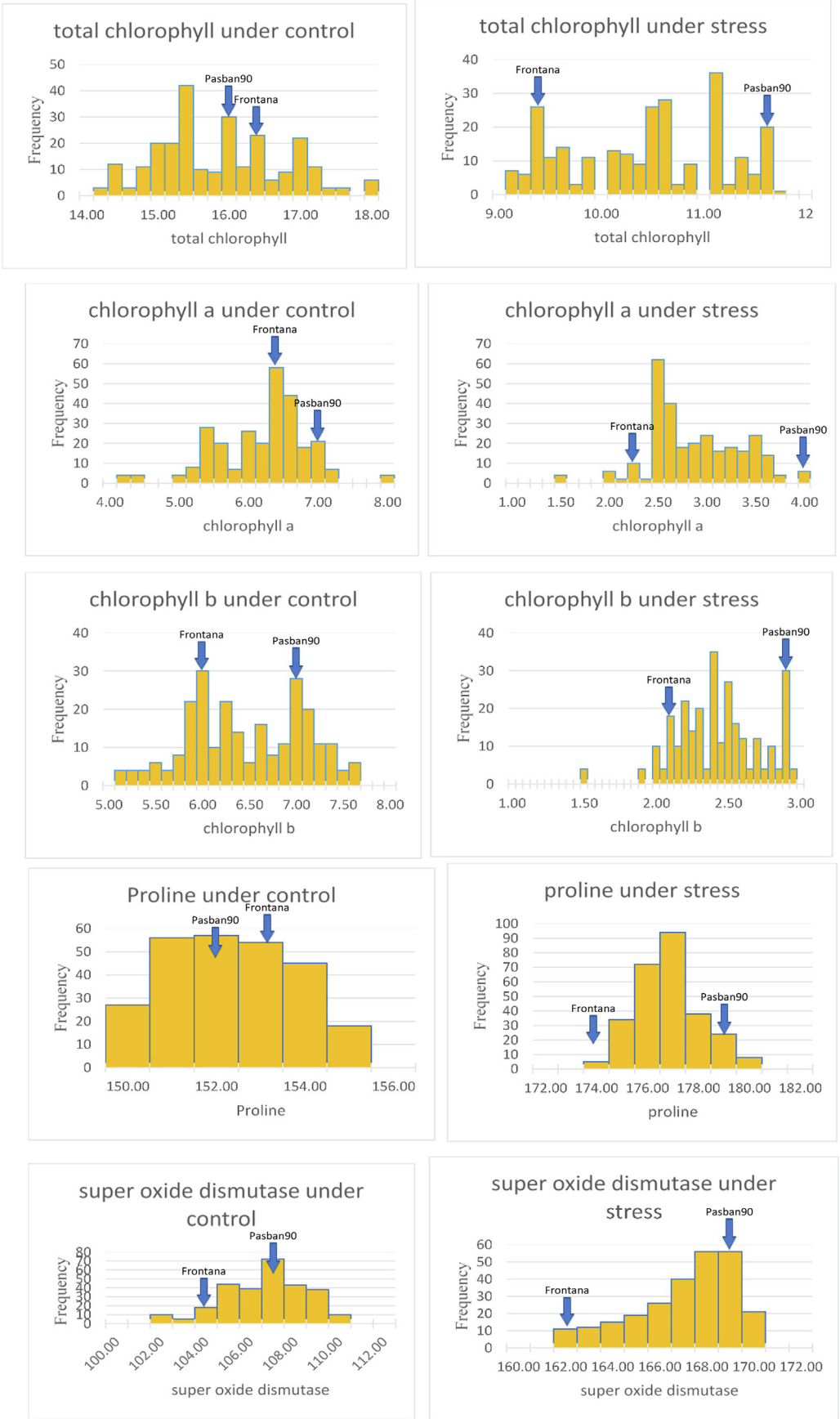

Fig. 2. Frequency distribution of total chlorophyll (TChl), chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), proline (PC) & super oxide dismutase (SOD) and parents positions among recombinant inbred lines (RIL) under control and stress i.e. NaCl (150 mM).

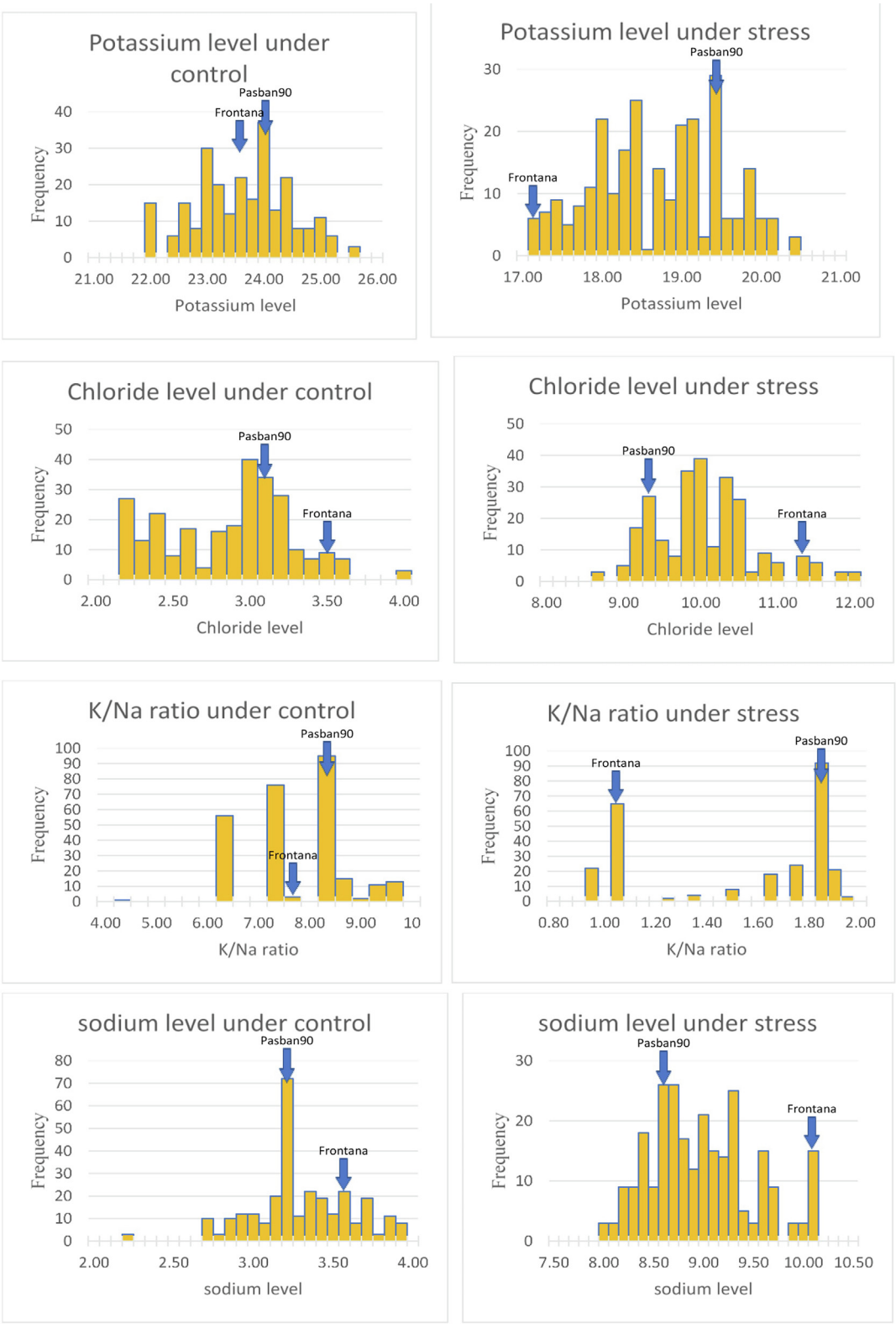

Fig. 3. Frequency distribution of K^+, Cl^- & K^+/Na^+ ratio, Na^+ and parents positions among recombinant inbred lines (RIL) under control and stress i.e. NaCl (150 mM).

3.4. Quantitative trait loci for biochemical attributes

Na^+ compartmentalization in root and stem related QTLs associated with 1D, 3B and 6B chromosome in control however in salt stress QTL were localized on 2A and 2B chromosome of mapping population (Figs. 4–6). *q*Na.6B.CH was mapped on 6B of RILs mapping population and explained 14% of phenotypic variation. K+ content QTL was associated with 2B chromosome of RILs in salt stress condition and contributes 17% in phenotypic variation. Three QTLs were mapped on A genome (4A, 5A & 6A) for potassium content compartmentation in control condition. Four QTL for chloride content in saline environment were mapped on 1D, 2B, 3B and 7A

Table 5
Quotative Trait Loci (QTLs) List for Physiological and Biochemical Traits.

S. No	QTL	Flanking Markers	LOD	R^2	Add.	Trait	C.N.
1	*q*RWC	Xwmc232-Xcfa2147	2.6	13	−0.44	RWC	4A
2	*q*RWC	Xwmc695-xbarc174	2.5	13	0.77	RWC	7A
3	*q*RWC	Wmc407-wmc179	2.5	13	0.1	RWC	2A
4	*q*RWC	Xwmc161-Xwmc232	2.9	15	−0.28	RWC	4A
5	*q*RWC	Xgwm131-xbarc182	3	15	0.1	RWC	7B
6	*q*MSI	xbarc78-xgwm60	3.3	16	−2	MSI	4A
7	*q*MSI	Xgwm674-Xbarc314	3	15	0.34	MSI	3A
8	*q*MSI	xgdm149-xbarc11	2.6	13	0.19	MSI	5B
9	*q*MSI	Xgwm233-xgwm130	2.4	12	0.4	MSI	7B
10	*q*MSI	xgdm142-xbarc352	2.7	13	−0.01	MSI	7D
11	*q*MSI	xgwm71-xgwm341	3.1	15	−0.2	MSI	3D
12	*q*WP	xgwm174-xwmc357	3.2	16	0.5	WP	5D
13	*q*WP	Xwmc177-Xbarc199	3.5	17.01	0.35	WP	2A
14	*q*WP	Xgdm146-xgwm213	2.7	13	0.095	WP	5B
15	*q*WP	Xgwm70-Xbarc361	2.6	13	0.2	WP	6B
16	*q*WP	Xcfd190-Xwmc1	2.5	13	−1	WP	6A
17	*q*OP	Xgwm47-Xgdm87	2.6	13	−0.62	OP	2B
18	*q*OP	Xbarc20-Xwmc10	2.5	13	−0.6	OP	7B
19	*q*OP	xgwm174-xwmc357	3.2	16	−0.4	OP	5D
20	*q*OP	XGWM63-Xbarc49	2.7	13	−0.5	OP	7A
21	*q*TChl	Xgdm8-xgwm314	3.8	19	−0.37	TChl	3D
22	*q*TChl	Xbarc59-xgdm8	2.8	14	−0.19	TChl	5B
23	*q*TChl	Xgwm70-Xbarc361	2.6	13	−0.15	TChl	6B
24	*q*TChl	Xcfd13-xgwm132	5.6	26	−0.08	TChl	6D
25	*q*TChl	xcfd21-xwmc630	2.8	14.01	−0.43	TChl	7D
26	*q*TChl	xwmc416-xbarc174	2.5	13	−0.53	TChl	1B
27	*q*TChl	Xbarc23-wmc179	4.4	21	0.26	TChl	6A
28	*q*Chl a	xgdm142-xbarc352	4.4	21	1.1	Chl a	7D
29	*q*Chl b	Xbarc324-xbarc19	3	15	−0.17	Chl b	3A
30	*q*Chl b	xgwm60-xgwm130	2.3	12	0.22	Chl b	7A
31	*q*Chl a	xgwm233-xgwm130	2.5	13	0.88	Chl a	7B
32	*q*Chl b	xbarc346-xgdm111	4	21	0.78	Chl b	1D
33	*q*Chl b	Xwmc1-Xbarc358	2.7	14	0.19	Chl b	3B
34	*q*Chl b	xgwm165-xbarc184	2.5	13	−0.1	Chl b	4A
35	*q*Chl b	xgdm108-xwmc149	3	15	0.3	Chl b	6B
36	*q*Chl b	xwmc399-xgwm302	3.4	17.01	0.2	Chl b	7B
37	*q*Pro	xgdm33-xwmc416	2.5	13	−0.28	Pro	1B
38	*q*Pro	xgwm314-xgwm538	4	21	−0.2	Pro	4B
39	*q*Pro	XGWM63-Xbarc49	2.7	14	0.15	Pro	7A
40	*q*SOD	xgdm33-xcfd21	2.4	12	0.46	SOD	1D
41	*q*SOD	Xbarc5-Xwmc407	2.8	14	0.3	SOD	2A
42	*q*SOD	xcfd15-xgwm264	3.2	16	10	SOD	1B
43	*q*SOD	xgdm108-xbarc23	3.4	17.01	0.22	SOD	6D
44	*q*Na	xcfd21-xbarc240	2.6	13	−0.31	Na	1D
45	qNa	xwmc695-xgwm108	2.6	13	−0.5	Na	3B
46	*q*Na	Xgwm70-Xbarc361	2.8	14.01	−0.5	Na	6B
47	*q*Na	wmc179-wmc149	2.8	14.01	−0.3	Na	2A
48	*q*NA	wmc35-wmc179	2.5	13	0.7	Na	2B
49	*q*K	Xwmc630-xgwm304	3.1	15	0.41	K	5A
50	*q*K	xgwm350-xbarc78	2.5	13	0.13	K	4A
51	*q*K	xgwm132-xcfd190	2.6	13	0.65	K	6A
52	*q*K	wmc35-wmc179	3.5	17	0.2	K	2B
53	*q*Cl	xbarc346-xgdm111	2.5	13	0.2	Cl	1D
54	*q*Cl	xwmc179-xbarc101	3	15	−0.48	Cl	2B
55	*q*Cl	xwmc695-xgwm108	3.2	16	0.15	Cl	3B
56	*q*Cl	wmc179-Xgwm356	2.5	13	0.16	Cl	7A
57	*q*Na/K	xcfd21-xbarc240	2.5	13	−0.1	Na/K	1D
58	*q*Na/K	xwmc111-xcfd36	2.5	13	0.25	Na/K	2D
59	*q*Na/K	Xbarc314-Xgwm247	2.7	13	0.08	Na/K	3A
60	*q*Na/K	xgwm165-xcfd193	2.6	13	0.23	Na/K	4D

but no QTL identified in control condition. *q*Cl.3B.SH was mapped for RILs mapping population contributes 16% phenotypic variation. A and D (1D, 2D, 3A and 4D) Genome contribute QTLs for potassium sodium ratio in salt stress environment. Three QTLs for proline content associated with 1B, 4B and 7A chromosome of RILs in high salt levels while no QTL for proline content was identified in control condition. *q*Pro.4B.SH flanked in interval of xgwm314-xgwm538 contributes 21% of Phenotypic Variation. *q*SOD.2A.CH was mapped on 2A chromosome of RILs in control condition while in NaCl (150 mM) level, two main QTLs for SOD were mapped on 1B and 6D chromosome. D Genome (6D) mapped with major QTL (*q*SOD.6D.SH) was mapped on 6D chromosome of recombinant inbred lines mapping population and it contributes 17% of phenotypic variation.

4. Discussion

Salt stress imposed negative effects on plant morphology and physiological processes. Genetic studies for salt tolerance could be useful if both conventional and non-conventuoial techniques are applied. RILs populations have been succefully used for QTLs analysis related to different traits in wheat such as yield traits (Ren et al., 2018), flag leaf related trait, for micronutirent

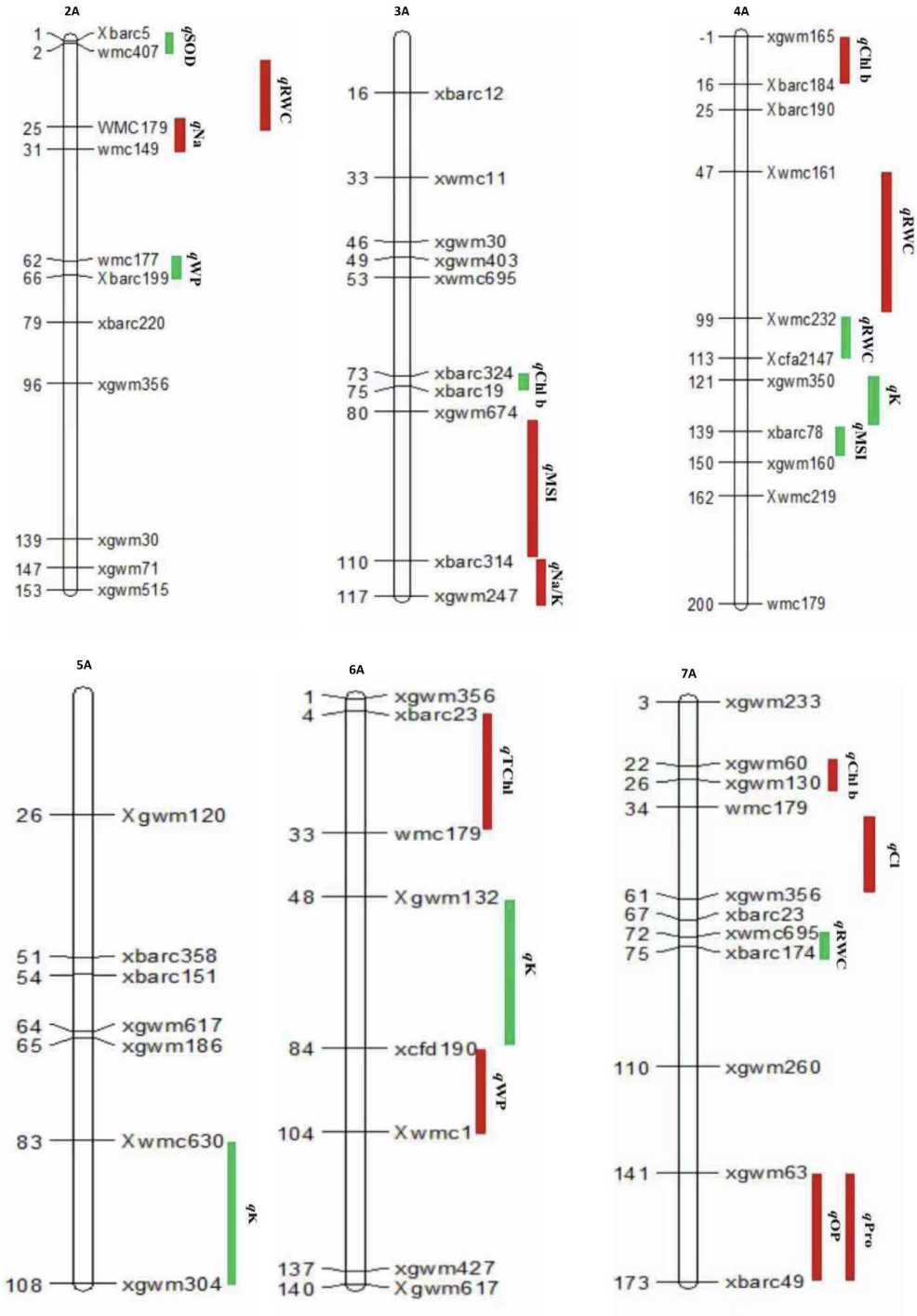

Fig. 4. Quantitative trait loci (QTL) mapping for physiological and biochemical traits using recombinant inbred lines (RILs) Pasban90/Frontana in genome A of wheat (*Triticum aestivum*).

(Liu et al., 2019) and quality traits (Goal et al., 2019). In this study the RILs population derived from parents Frontana and Pasban 90 is used for QTLs mapping for physiological and biochemical traits under normal and salt stress conditions. The same RILs population has been used to identify QTLs related to morphological traits earlier (Batool et al., 2018). The traits analysed in this study are considered as good indicator of salt stress tolerance. The QTLs mapping for the pyhysiological traits such relative water content of the leaves, water potential and total chlorophyll content would provide genomic insight of the traits. The QTLs related to potassium content and K/Na ratio would also be useful for salt tolerance breeding for wheat. Gurmani et al. (2014) identified potassium sodium ratio, chlorophyll content, SOD and proline content as salt stress indicators for screening of wheat lines under salt stress. Sodium and chloride accumulations interfere with metabolic process leading to reduction in plant growth and development due to decreased water potential (Harris et al., 2001).

Another important trait affected under salt stress is chlorophyll content (Ghogdi et al., 2012). For chlorophyll content a single recessive gene *clm1* has been reported on the 7AL chromosome by using mutant of diploid wheat (Ansari et al., 2013a). In this study by using RILs populations, QTLs for cholorophyll has been mapped on 7A and 7B chromosome. The plasma membrane of cells is the first organelle affected by salt stress and its integrity damage

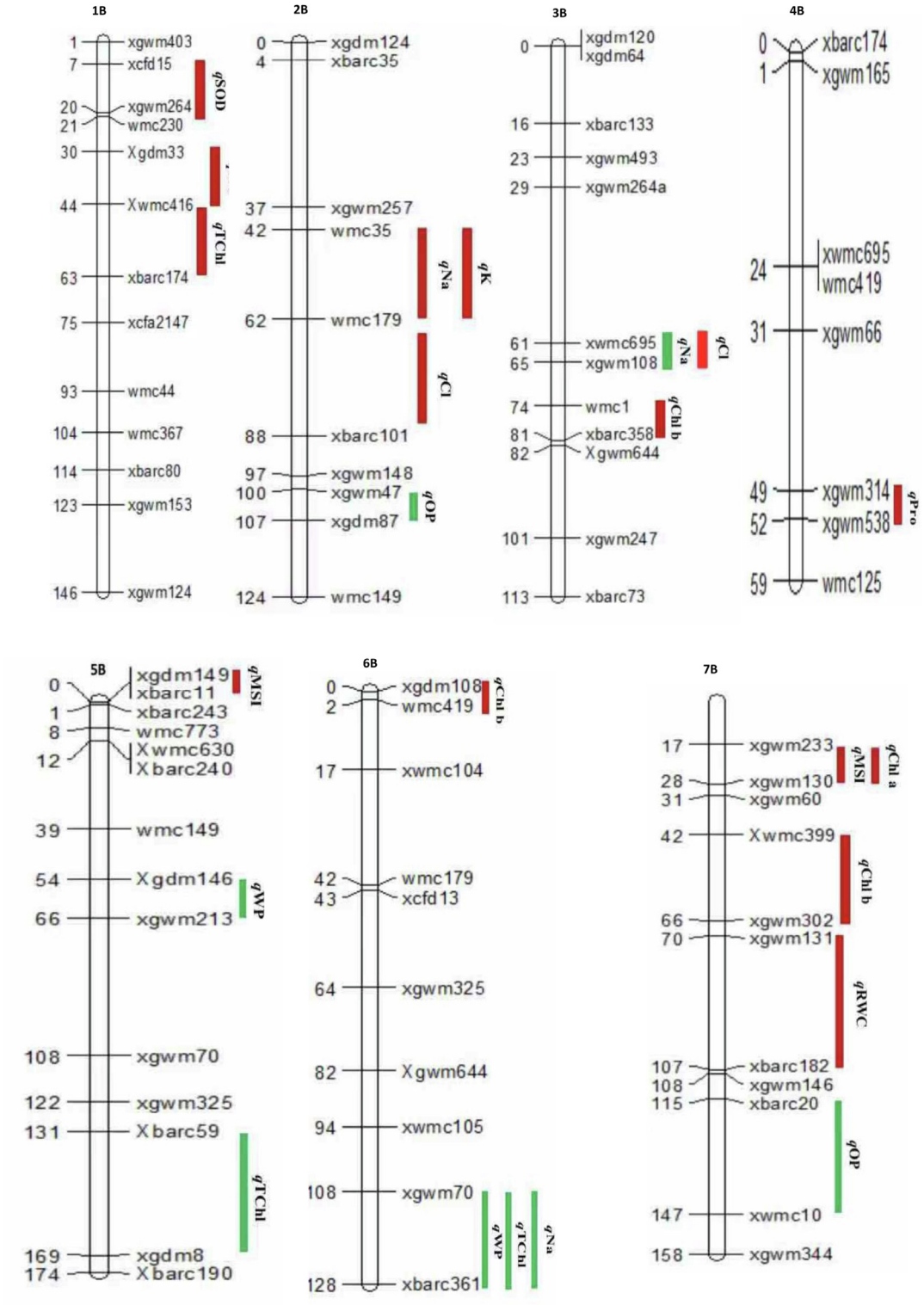

Fig. 5. Quantitative trait loci (QTL) mapping for physiological and biochemical traits using recombinant inbred lines (RILs) Pasban90/Frontana in genome B of wheat (*Triticum aestivum*).

due to lipids destroyed by each oxidation, due to increased cell membrane permeability (Radi et al., 2013). Malik et al. (2015), reported two QTL on 2A chromosome for cell membrane stability. We identified QTLs for membrane stability index on chromosome 2A, 3A and 3D. These QTLs may be used in further breeding programs for cell membrane stability.

A number of researchers constructed linkage map for the related traits (Ansari et al., 2012; Ansari et al., 2013b; Ansari et al., 2014). Shamaya (2014) developed linkage map by using 87 DArT and 500 SSR markers. These markers were used to construct linkage map of 3643.2 cM, total 30 linkage groups were assigned to 21 chromosomes and with an average length of chromosome RILs

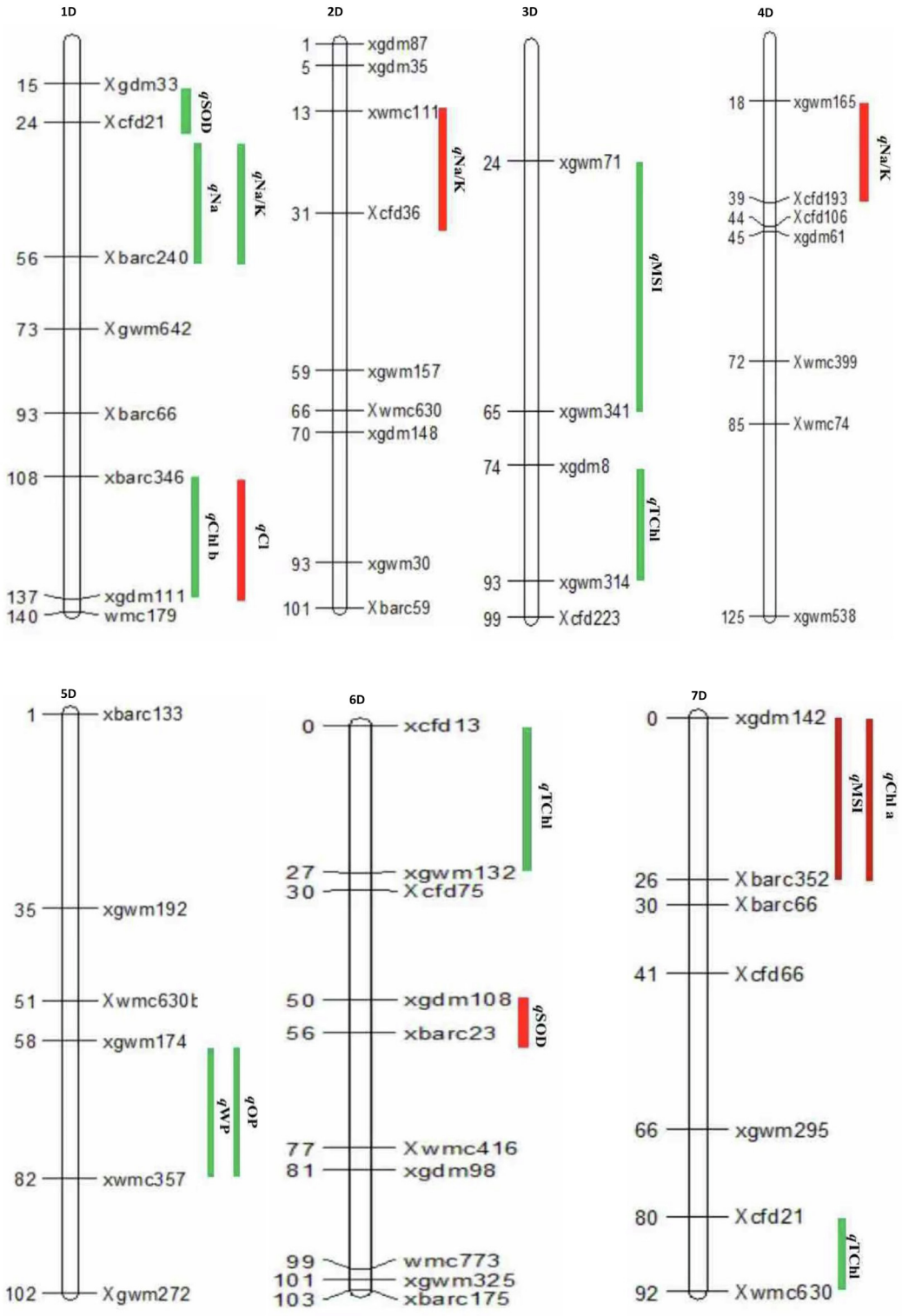

Fig. 6. Quantitative trait loci (QTL) mapping for physiological and biochemical traits using recombinant inbred lines (RILs) Pasban90/Frontana in genome D of wheat (*Triticum aestivum*).

was 200 cM. Genc et al. (2010) constructed linkage using 557 SSR-DArT on 21 linkage group with an average of 25 markers per chromosome with 6 cM spacing. Azadi et al. (2015) reported that 451 markers were used to construct linkage map of length 1390.3 cM with an average 3.08 cM and twenty-seven linkage groups were identified. Cui et al., 2015 developed a genetic linkage map of recombinant inbred lines (RILs) using 591 loci including g-SSR, DArT, e-SSR, STS, SRAP and ISSR. Srinivasachary et al. (2009) constructed a link map using 468 DArT, and 54 SSR markers were drawn on all chromosomes except 3D and 6D. Tian et al. (2015) identified eight root length QTLs 2A, 2D, 3B, 4D, 6B and 7B under stress and salt control. Ghaedrahmati et al. (2014) reported 5

shoot-length QTLs on 3A, 2B, 3B and 5B under control and 3 QTL for saline chromosomes 3A, 5B and 1D. 2A, 2D, 4D, 5A, 5D, 6A and 7A for the height of the plant in various salt affected areas. Genc et al. (2010) reported QTLs for the number of columns of 1A, 4B, 5A, 5B and 5D under controlled and salt stress. Fresh weight QTLs were identified at 1A, 1D, 2A, 3B, 4B, 5B and 6B under control and in saline (Ghaedrahmati et al., 2014). The first large QTL for relative water content (QRWC.7B.SH) accounted for 15% of the phenotypic variation with the LOD 3 value was localized on 2A chromosome (Malik et al., 2015). In this study we have identifies QTLs for relative water contents for leaves on chromosome 2A, 4A, 7A and 7B. The comprehensive linkage map developed in this study could be useful for further studies as well as in breeding programs for salt tolerance using physiological traits.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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