

## QUANTITATIVE TRAIT LOCI (QTLs) MAPPING FOR SALT STRESS TOLERANCE IN WHEAT AT GERMINATION STAGE

Nazima Batool<sup>1</sup>, Noshin Ilyas<sup>1,\*</sup>, Armghan Shahzad<sup>2,\*</sup>, Bernard A. Hauser<sup>3</sup> and Muhammad Arshad<sup>1</sup>

<sup>1</sup>Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan; <sup>2</sup>National Institute for Genomics and Advanced Biotechnology, NARC, Islamabad, Pakistan; <sup>3</sup>Department of Biology, College of Arts and Science, University of Florida, USA.

\*Corresponding author's e-mail: noshinilyas@yahoo.com

Identification of genetic factors responsible for controlling germination traits under salinity will allow developing tolerant cultivars. Here we have studied quantitative trait loci (QTL) linked to seed germination and early seedling growth traits using recombinant inbred lines generated from crossing of wheat cultivars, Pasban 90 (salt tolerant) and Frontana (salt susceptible). QTL analysis were done using composite interval mapping (CIM) for germination percentage (G%), germination index (GI), seedling vigor index (SVI), shoot length (SL), root length (RL), fresh weight (FW) & dry weight (DW). In this QTLs analysis total 44 QTLs were identified, 26 QTLs out of 44 were identified in 150 mM salt stressed and eighteen QTLs under control environment. Eleven main quantitative trait loci (QTLs) were localized on 1B, 2B, 3B, 5B, 6B and 7B chromosome of mapping population in stressed environment. QTLs mapped on 6A, 3B, 6B and 6D chromosome for root and shoot length, fresh and dry weight were recognized as a major QTLs under control treatment. QTLs for root and shoot length were mapped on 2D same position in xgdm36-xwmc111. Root length and dry weight QTLs was flanked in interval of xgwm70-xgwm325 markers on 5B chromosomes. The result of this study revealed that QTLs play main role in salt tolerance at seed germination and early seedling growth stage.

**Keywords:** Salinity, germination attributes, QTLs, shoot length, 5B chromosomes.

### INTRODUCTION

Various abiotic stress factors impose negative effect on plant growth and productivity (Shahzad *et al.*, 2012), among them salt stress is one of major factor that drastically reduce crop plant growth and development (Azadi *et al.*, 2015). Salinity affects 800 mh land area in arid and semi-arid environment (Ahmad *et al.*, 2013). Wheat (*Triticum aestivum*) is staple food and primary crop plant in Pakistan (Batool *et al.*, 2016). Farmers in Pakistan grow wheat in an area nine thousand hectares and 3.1% contribution in an annual GDP. Although enhancement of wheat tolerance for salt stress is main concern but, salt tolerance through physiological, biochemical and genetic mechanism is complex trait (Genc *et al.*, 2010). Wheat plant exhibits many QTLs that enhance salt tolerance in this species (Ma *et al.*, 2007; Munns and Tester, 2008). Genetic markers development revealed genetic architecture of salt tolerance trait. This help to analyzed individual QTLs playing role in salt tolerance (Ghaedrahmati *et al.*, 2014). Number of scientist mapped QTLs related to salt tolerance mainly linked with morphological and yield trait (El-Hendawy *et al.*, 2009; Genc *et al.*, 2010; Amin and Ayman, 2013; Mahdinejad *et al.*, 2014; Azadi *et al.*, 2015). Present study differs from above mention studies because it identifies QTLs in wheat mapping population connected to salt

tolerance at germination and early seedling growth stage. Shahzad *et al.* (2012) describes that seed germination and seedling growth is utmost vulnerable phase to salinity and germination ability of wheat plant & is controlled by many genes (Song *et al.*, 2005). In order to develop salt tolerant line it is very important to identify salt tolerance mechanism at seedling stage. Simple sequence repeats (SSR) are being used in present study to categorize QTLs linked to salinity tolerance at seed germination and early seedling growth stage.

### MATERIALS AND METHODS

The study was conducted at National Agriculture Research Center (NARC), Islamabad Pakistan and Department of Biology, University of Florida, USA. Pasban90 and Frontana were used to generate a mapping population mixture of salt tolerant and salt susceptible recombinant inbred lines (RILs). Mapping population comprised of eighty seven (F7) RILs and evaluated at germination and seedling stge. Pasban-90 shows tolerance toward salt stress but Frontana is salt susceptible (Ahmad *et al.*, 2013). Complete randomized design with three replications was used for this trial. Surface sterilization of RILs and parental seeds were done using 1% sodium hypochlorite solution for 10 minute and distilled water used for washing. Ten seeds were germinated in petri plates using

method describe by Munns and James (2003). During seed germination and propagation, photoperiod 18/10 day/night, temperature 26/19°C day/night & relative humidity 65 percent was maintained. All seeds of RILs and parentes were evaluated against control growth and salt stress (supplemented with 150 mM NaCl). For salt stress induction, apply 3ml of sodium chloride was applied to all petri-plates on second day for 10 days. RILs and parental seeds were considered germinated when a seedling length was 5 mm (Nichols *et al.*, 2009).

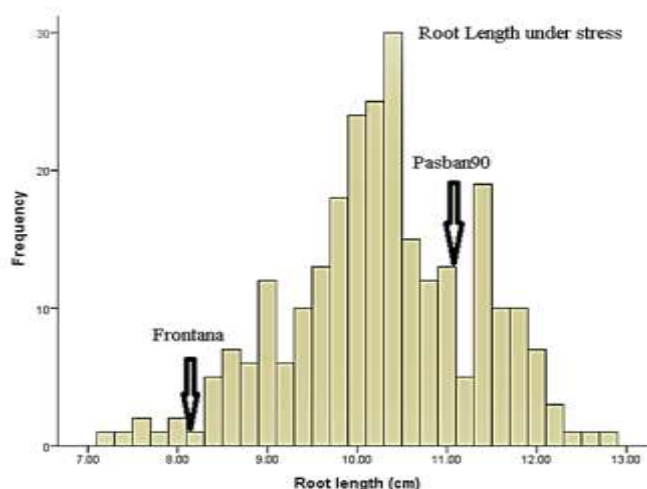
Data for following germination attributes such as germination percentage, germination index and seedling vigor index were measured. Mirza *et al.* (2015) method was used to calculate germination percentage and germination index was calculated by using ISTA (2005). Seedling vigor index was recorded by Abdulbaki and Anderson (1973) method. For early seedling growth following parameter was recorded such as shoot and root length, fresh and dry weight (Mirza *et al.*, 2015). Sharp *et al.* (1988) describe a procedure to extract DNA from young leaves of RILs and Parents was used. Two hundred and eighty SSR markers were used to check polymorphism among parents, 202 markers out of 280 show polymorphism with parents were applied on mapping population. SSR markers sequence record was used from GrainGene database (<http://wheat.pw.usda.gov>) WMC (wheat microsatellite consortium) (Guyomarc'h *et al.*, 2002 (CFA & CFD); Somers *et al.*, 2004), BARC (Beltsville Agricultural Research Center) (Song *et al.*, 2005) and GWM (Gatersleben wheat microsatellites) (Roder *et al.*, 1998). Polymerase chain reaction (PCR) used for DNA amplification reaction using SSR markers, initial denaturation at 94°C for 4 min, 35-40 cycles was carried out at 94°C for 45 sec, for primer annealing 45 sec at 55 to 60°C and 45 sec for DNA amplification at 72°C, 10 min for DNA final extension at 72°C and 2% agarose gel was used to separate PCR and ethidium bromide was used for visualization.

After gel band scoring, word data files were generated in which locus A, B and - were scored as a genotype. Linkage map was constructed using Kosambi mapping function by map maker ver. 3.0. Twenty one linkage groups were identified by comparing earlier maps in literature (Somar *et al.*, 2004). 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 and statistical evaluation was done by using STATISTIX.

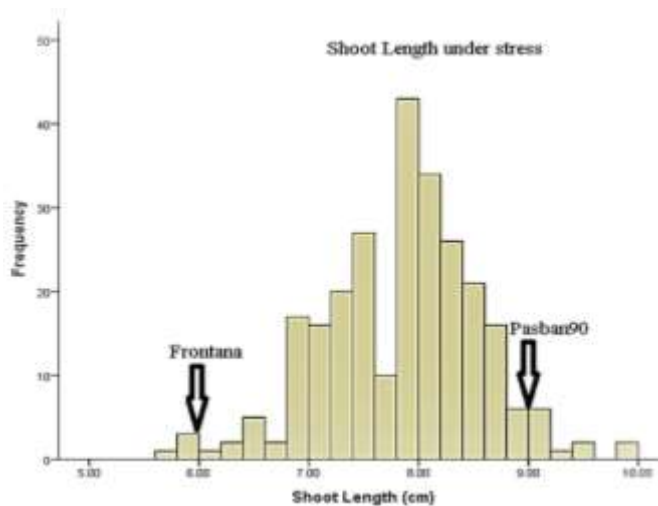
## RESULTS

**Phenotypic variation:** Seed germination and seedling growth phase is most important stage in wheat plant growth and

development. Salt stress imposed negative impact on wheat seed germination and seedling growth. Recombinant inbred lines studied traits show normal distribution under salt stress condition. RILs were significantly ( $p \leq 0.05$ ) different for each trait under 150 mM salt stress. RILs distribution show high and low transgressive segregation for all recorded data. There was a closer distribution of mapping population for germination percentage and germination index under salt stress (Fig. 1-2).



**Figure 1.** Frequency distribution of traits and parent position among RILs under salt stress.



**Figure 2.** Frequency distribution of traits and parent position among RILs under salt stress.

Parents (Pasban 90 and Frontana) were lies far from each other in stress condition. The phenotypic variation in seedling vigor index (SVI), root length (RL), shoot length (SL), fresh weight (FW) and dry weight (DW) show wide distribution among mapping population under salt stress was observed

(Fig 3-7). Pasban 90 have shown higher value for all traits as compared to Frontana under salt stress environment. Correlation co-efficient analysis was recorded for all studied traits from values of non-stressed and salt stress conditions (Table 1). A significant correlation was measured between root length and shoot length under control and salt stress condition. Positive correlation was recorded under salt stress environment but most significant correlation among root length, fresh weight and dry weight ( $p \leq 0.05$ ).

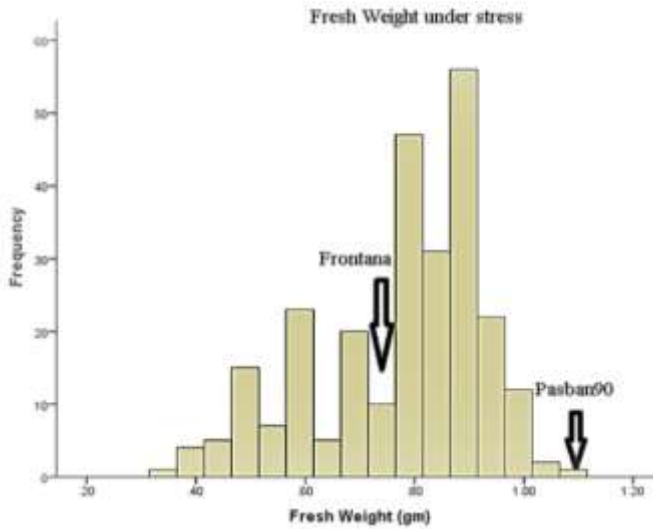


Figure 3. Frequency distribution of traits and parent position among RILs under salt stress.

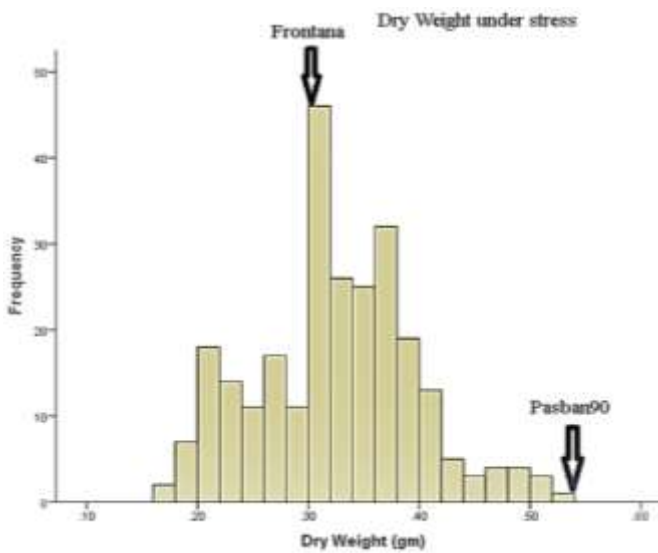


Figure 4. Frequency distribution of traits and parent position among RILs under salt stress.

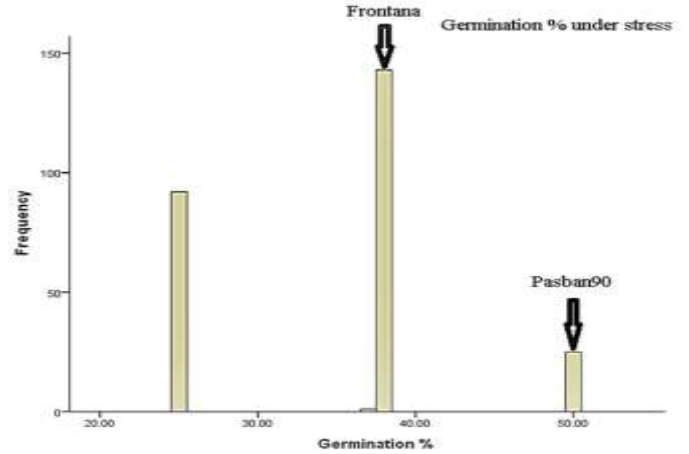


Figure 5. Frequency distribution of traits and parent position among RILs under salt stress.

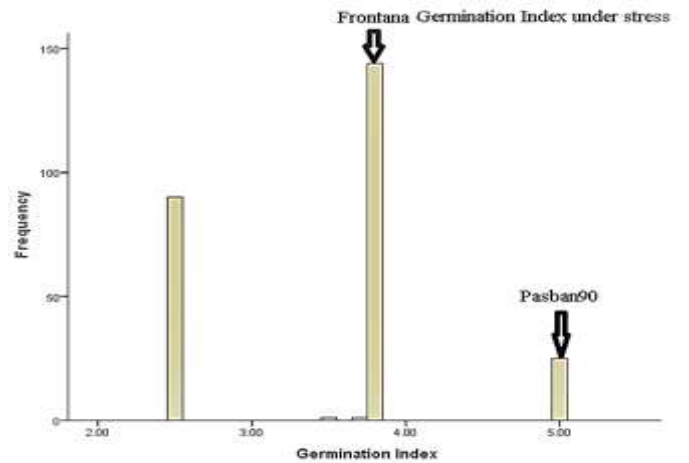


Figure 6. Frequency distribution of traits and parent position among RILs under salt stress.

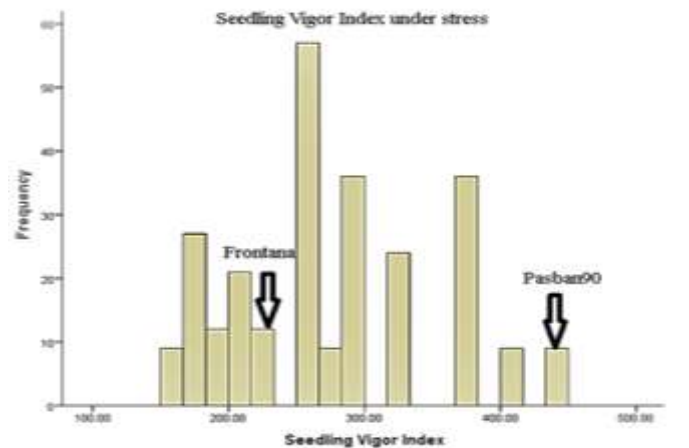


Figure 7. Frequency distribution of traits and parent position among RILs under salt stress.

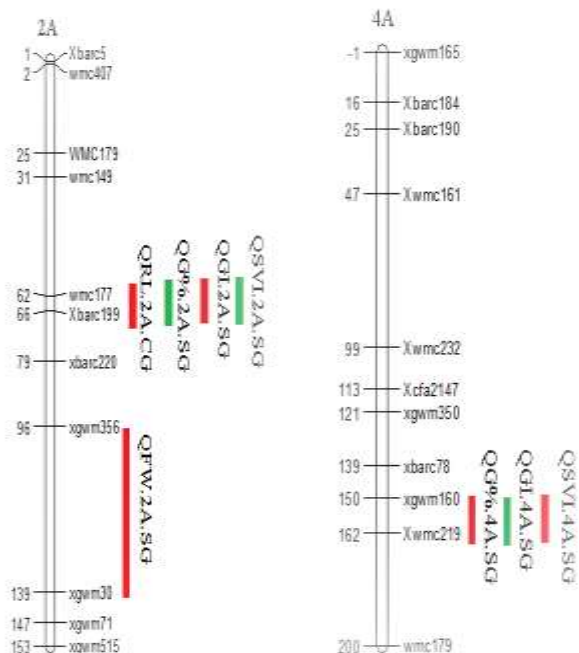
**Table 1. Correlation coefficients between traits measured on the Parents and RILs under control and salt stress.**

		RL	SL	FW	DW	G%	GI	SVI
Root	Con	1.00						
Length	Str	1.00						
Shoot	Con	0.61**	1.000					
Length	Str	0.57*	1.000					
Fresh	Con	0.35*	0.340*	1.000				
Weight	Str	0.33**	0.510*	1.000				
Dry	Con	0.35*	0.390	0.510**	1.000			
Weight	Str	0.46*	0.290*	0.280*	1.000			
Germination	Con	0.39*	0.029 <sup>ns</sup>	0.010 <sup>ns</sup>	0.029*	1.00		
Percentage	Str	0.41*	-0.014 <sup>ns</sup>	0.029 <sup>ns</sup>	0.390*	1.00		
Germination	Con	0.55*	0.041 <sup>ns</sup>	-0.010 <sup>ns</sup>	0.030 <sup>ns</sup>	0.91*	1.00	
Index	Str	0.29*	-0.019 <sup>ns</sup>	0.023 <sup>ns</sup>	0.340*	0.92*	1.00	
Seedling	Con	0.54*	0.089 <sup>ns</sup>	-0.010 <sup>ns</sup>	0.031	0.99*	1.00**	1.00
Vigor Index	Str	0.32*	-0.031 <sup>ns</sup>	0.012 <sup>ns</sup>	0.330*	0.90**	0.95**	1.00

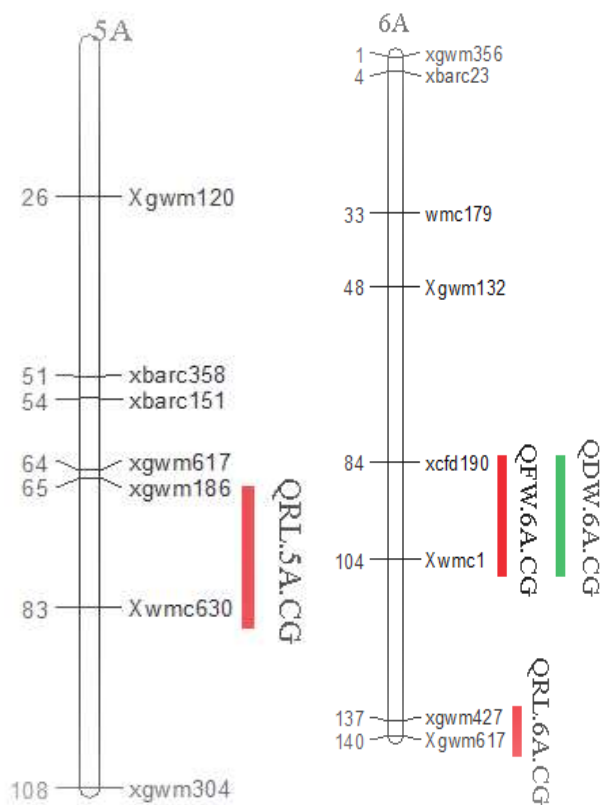
Significance levels: <sup>ns</sup>non-significant, \*P=0.05, \*\*P=0.01.

**Composite interval mapping:** In total 280 SSR markers were applied to mapping population and out of 280 SSR markers 202 were polymorphic. Chi- square test was used to confirm 1:1 ratio and twenty one linkage groups were identified. Total forty four QTLs were mapped on 16 chromosomes under control and salt stress condition. QTLs were recorded using composite interval mapping (CIM) on sixteen out of twenty one linkage groups for all traits under control and 150 mM stressed environment with logarithm of odds (LOD) value ranging from 2.5 to 4.1 (Fig. 8-15).

QTLs were mapped in control and stressed environment which might be permitting the difference between constitutive and adaptive reactions. Mostly QTLs were recorded in stressed environment and mainly contributes for seedling growth trait.



**Figure 8. QTL identified on 2A and 4A chromosome of recombinant inbred lines in non-stressed and stressed conditions.**



**Figure 9. QTL identified on 5A and 6A chromosome of recombinant inbred lines in non-stressed and stressed conditions.**

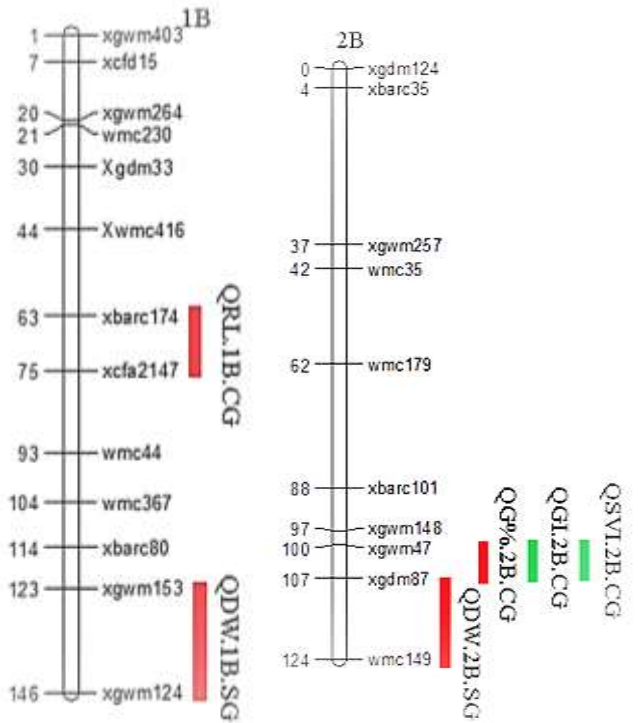


Figure 10. QTL identified on 1B and 2B chromosome of recombinant inbred lines in non-stressed and stressed conditions.

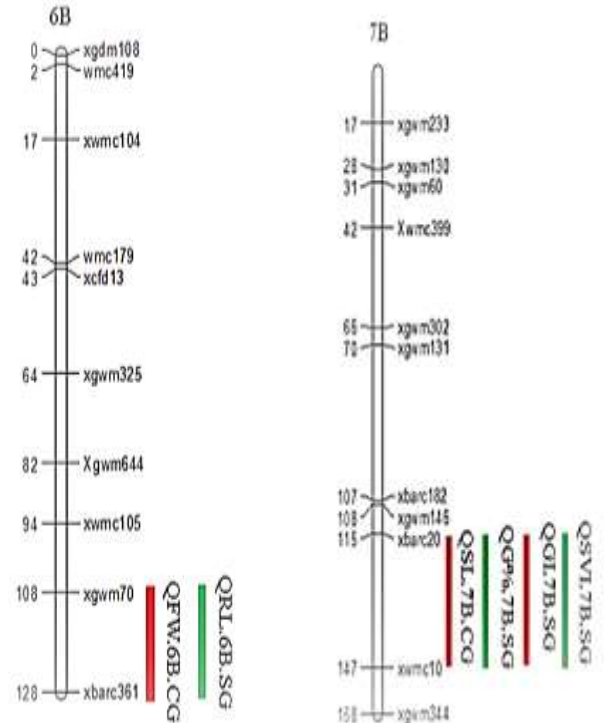


Figure 12. QTL identified on 6B and 7B chromosome of recombinant inbred lines in non-stressed and stressed conditions.

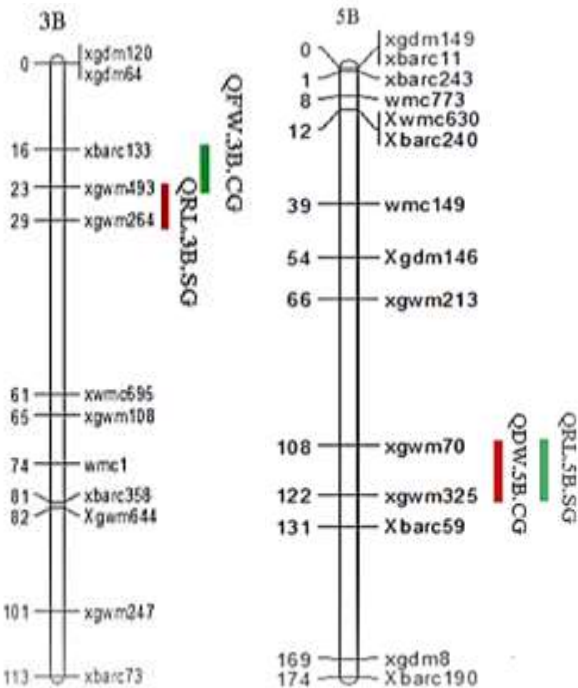


Figure 11. QTL identified on 3B and 5B chromosome of recombinant inbred lines in non-stressed and stressed conditions.

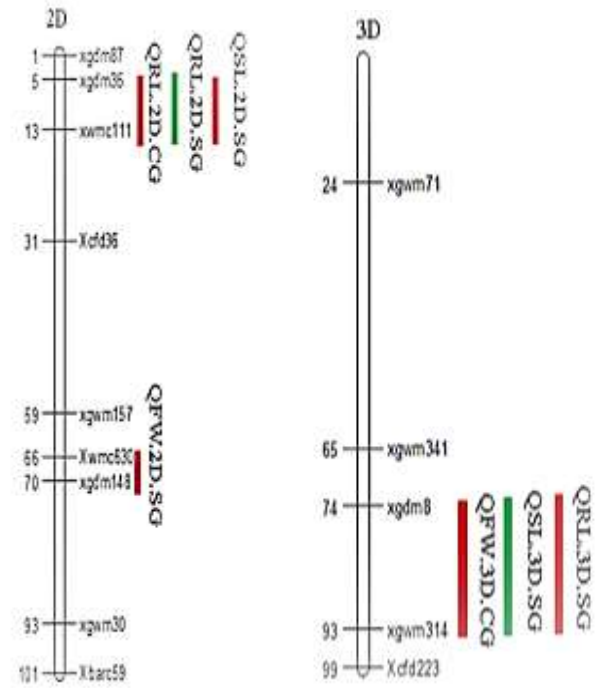


Figure 13. QTL identified on 2D and 3D chromosome of recombinant inbred lines in non-stressed and stressed conditions.



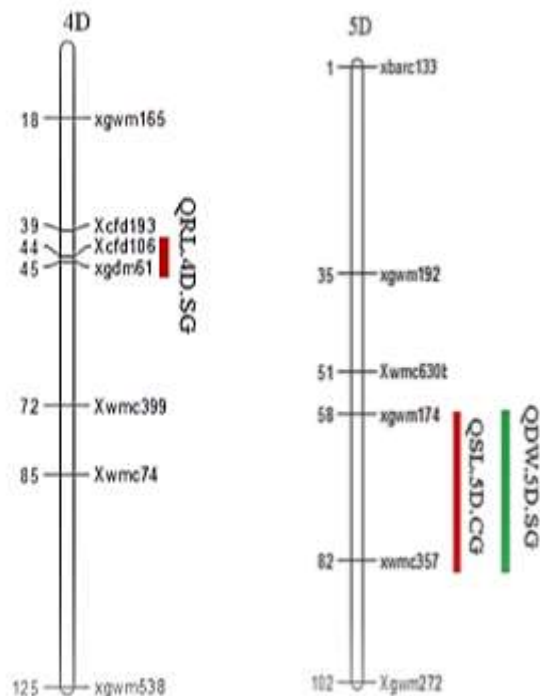


Figure 14. QTL identified on 4D and 5D chromosome of recombinant inbred lines in non-stressed and stressed conditions.

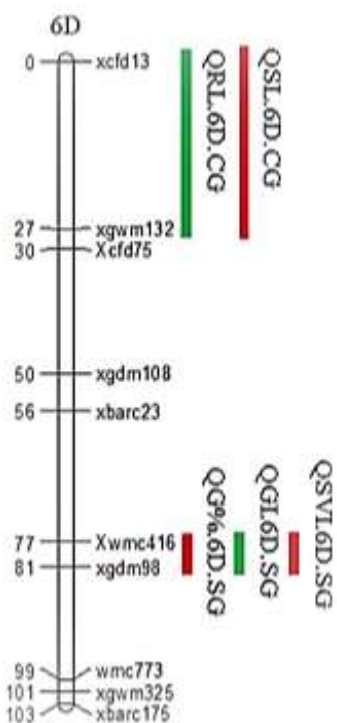


Figure 15. QTL identified on 6D chromosome of recombinant inbred lines in non-stressed and stressed conditions.

**QTL for germination attributes:** QTL (QG%.2B.CG) for germination percentage was localized on 2B chromosome of recombinant inbred lines under control condition and contributes 13% of phenotypic variation. In salt stress treatment (150 mM), QTLs for germination percentage (G%) were mapped on 2A, 4A, 7B and 6D chromosomes (Table 2). One major QTL (QG%.4A.SG) for germination percentage was mapped on 4A chromosomes having 14 percent phenotypic variation.

Table 2. Quantitative trait loci (QTL) was associated with traits of RILs mapping population.

Quantitative trait loci (QTL)	Markers Interval	LOD	Phenotypic variation	Add. Eff.
QG%.2B.CG	xgwm47-Xgdm87	2.6	13.00	1.500
QG%.2A.SG	wmc177-xbarc199	2.8	14.01	-3.370
QG%.4A.SG	xgwm160-xwmc219	2.9	14.01	1.900
QG%.6D.SG	xwmc416-xgdm98	2.6	13.00	3.100
QGL.2B.CG	xgwm47-Xgdm87	2.6	13.00	3.000
QGL.2A.SG	wmc177-xbarc199	2.7	13.00	-0.340
QGL.4A.SG	xgwm160-xwmc219	3.1	15.00	-0.100
QGL.6D.SG	xwmc416-xgdm98	2.6	13.00	0.310
QGL.7B.SG	Xbarc20-Xwmc10	2.5	12.00	0.300
QSVI.2B.CG	xgwm47-Xgdm87	2.6	13.00	0.010
QSVI.2A.SG	wmc177-xbarc199	2.7	13.00	0.340
QSVI.4A.SG	xgwm160-xwmc219	3.1	15.00	-0.100
QSVI.6D.SG	xwmc416-xgdm98	2.6	13.00	0.030
QSVI.7B.SG	Xbarc20-Xwmc10	2.6	12.00	0.020
QRI.1B.CG	xbarc174-xcfa2147	2.8	14.01	0.100
QRI.2A.CG	Wmc177-xbarc199	2.6	13.00	0.100
QRI.2D.CG	xgdm35-xwmc111	2.6	12.00	-0.900
QRI.6A.CG	xgwm427	3.1	15.00	-0.200
QRI.5A.CG	Xgwm186-Xwmc630	2.6	13.00	-0.730
QRI.6D.CG	Xcfd13-xgwm132	3.6	17.00	-0.420
QRI.2D.SG	xgdm35-xwmc111	3.9	19.00	-2.810
QRI.3B.SG	Xgwm493-Xgwm264	3.4	16.00	0.190
QRI.5B.SG	xgwm70	2.7	13.00	0.260
QRI.6B.SG	Xgwm70-Xbarc361	3.2	16.00	0.200
QRI.3D.SG	xgdm8-xgwm314	3.2	16.00	0.050
QRI.4D.SG	xcfd106-xgdm61	2.6	13.00	0.010
QSI.6D.CG	Xcfd13-xgwm132	3.3	16.00	-0.370
QSI.7B.CG	Xbarc20-Xwmc10	2.8	14.01	0.620
QSI.5D.CG	Xgwm174-xwmc357	3.0	15.00	-0.100
QSI.2D.SG	xgdm35-xwmc111	2.6	13.00	0.010
QSI.3D.SG	xgdm8-xgwm314	2.9	14.01	-0.100
QFw.3B.CG	xbarc133-xgwm493	3.0	15.00	0.028
QFw.3D.CG	xgdm8-xgwm314	2.6	13.00	0.020
QFw.6A.CG	Xcfd190-xwmc1	2.6	13.00	-0.080
QFw.6B.CG	Xgwm70-Xbarc361	3.3	16.00	0.042
QFw.1D.SG	xbarc66-xbarc346	2.8	14.01	0.010
QFw.2A.SG	Xgwm356-xgwm30	2.6	13.00	0.014
QFw.2D.SG	xwmc630-xgdm148	3.1	15.00	0.010
QDw.5B.CG	Xgwm70-xgwm325	2.6	13.00	0.020
QDw.6A.CG	Xcfd190-xwmc1	4.1	20.00	-0.040
QDw.1B.SG	Xgwm153-Xgwm124	2.6	13.00	-0.200
QDw.2B.SG	Xgdm87-xwmc149	2.6	13.00	0.010
QDw.5D.SG	Xgwm174-xwmc357	2.6	13.00	-0.050

One major and three minor QTLs were mapped on 2A, 4A, 7B and 6D chromosome of RILs for germination index (GI) in 150 mM salt level. Germination index QTLs with LOD value 2.5 to 3.1 contributes about 13 to 15% in phenotypic variation (Table 2). Positive alleles for these QTLs are from Pasban-90. In control condition one QTL for germination index (GI) was identified on 2B. QTLs for seedling vigor index were identified on 2A, 4A and 7B chromosome of recombinant inbred lines under salt stress. Seedling vigor index QTL (QSVI.4A.SG) was mapped on 4A with LOD 3.1 and contributes phenotypic variation fifteen percent (Table 2).

**QTL for seedling growth:** QTLs for root length were mapped on chromosome 3B, 5B, 6B, 2D, 3D and 4D of RILs in stressed treatment. Both parents donate positive alleles for these QTLs and contribute thirteen to nineteen percent phenotypic variation. One major QTL for root length (QRL.2D.SG) was localized on 2D chromosome and 3.9 LOD value. Pasban90 contributes positive allele for this QTL and explained 19% of phenotypic variation. In control condition, for root length six QTLs were mapped on 2A, 5A, 6A, 1B, 2D and 6D chromosome of RILs. QTL (QRL.6D.CG) was identified as major QTL for root length on 6D chromosome in control condition. This QTL was identified with 3.5 LOD value and 17% phenotypic variation (Table 2).

QTLs were mapped on chromosome 2D, 3D, and 4D of RILs for shoot length in stressed environment. For shoot length one major QTL was identified on 3D chromosome and having fourteen percentage of phenotypic variation. Three QTLs for shoot length are identified in control condition, out of three one major and two minor QTLs were mapped on 7B, 5D and 6D chromosomes of RILs. QTLs for shoot length showed 13 to 16% phenotypic variation. QTL (QSL.6D.CG) were mapped on 6D chromosome recognized as a major QTL for shoot length with LOD of 3.2 and are found in interval of Xcfd13-xgwm132.

QTLs for seedling fresh weight in stressed treatment were mapped on 2A, 1D and 2D chromosome of RILs. One major QTL for fresh weight having 16 percentage of phenotypic variation was localized on 2D chromosome and this QTL flanked between SSR markers xwmc630-xgdm148. QTLs for fresh weight were mapped on 6A, 3B, 6B and 3D chromosome of RILs in non-stressed environment. Fresh weight QTLs contributes 13 to 16% phenotypic variation. QTL for fresh weight was mapped on 6B was considered major QTL with 3.2 LOD. This QTL was found in interval of SSR markers Xgwm70-Xbarc361 and Pasban 90 and contributes positive allele for this QTL.

QTL for dry weight were mapped on 1B, 2B and 5D chromosomes of recombinant inbred lines in stressed environment (Table 2). One major QTL for fresh weight in salt stress was mapped on 2B and flanked between SSR markers Xgdm87-xwmc149. Dry weight QTL was mapped on 6A and 5B chromosome of RILs in non-stressed environment (Table 2). Dry weight QTL explained 13 to 20

percentages of phenotypic variation and dry weight QTL was identified on 6A chromosome of RILs considered a major QTL for dry weight. This QTL was identified with 4.1 LOD and 20% of phenotypic variation.

Germination attributes QTLs were segregated with QTLs of early seedling growth (Fig. 8-15). Germination percentage QTLs was co-segregated with germination index and seedling vigor index QTLs on 4A, 2B and 6D chromosome of RILs. Germination attributes QTLs were co-segregated with root length and shoot length QTL on 2A and 7B chromosome of RILs. QTL on 2A chromosome flanked in interval of SSR markers xwmc177-xbarc199. Fresh weight and dry weight QTL in control condition shared location on 6A chromosome in interval xcf190-xwmc1. QTLs of root length co segregated with dry weight, fresh weight and shoot length QTLs on 5B, 6B and 2D chromosome of RILs. Root length QTLs shared location on 2D chromosome under stressed and non-stressed condition. Shoot length QTLs co segregated with dry weight and root length on 5D and 6D of chromosome of recombinant inbred lines.

## DISCUSSION

Present study suggests a significant correlation between germination attributes and seedling vigor index. Our results are similar to Arraouadi *et al.* (2012) findings, who observed a significant correlation among germination percentage and seedling parameters (dry weight and root length). The significant transgressive segregation were recorded for all trait of RILs generated from cross between Pasban90 and Frontana. Ghaedrahmati *et al.* (2014) recorded similar trend in which wheat mapping population (RILs) display transgressive segregation for all observed traits in stressed environment. Parents lies far from each other in distribution under salt stress (Czyczyło-Mysza *et al.*, 2014; Azadi *et al.*, 2015).

Current study finding prove that wheat seed germination & seedling growth phase is polygenic nature and many genes involved. Germination percentage QTL in stressed condition was localized on 4A chromosome recognized as major QTL and QTL flanked in interval xgwm160-xwmc219. Similar findings by Czyczyło-Mysza *et al.* (2014), recorded germination percentage QTLs in non-stressed and stressed condition on chromosome 5A, 1B, 3B, 4B and 6B of mapping population (double haploid lines). Likewise, QTLs for germination percentage were mapped on 2A and 4B chromosome of recombinant inbred lines (Marouf and Mohammadi, 2015) and QTLs for germination percentage was identified in non-stressed and stressed environment (Wang *et al.*, 2011). QTLs for seedling vigor index and germination index were localized on chromosome 2A, 4A, 2B, 7B and 6D of RILs under control and salt stress. Germination attributes QTLs shared location in interval of SSR markers Xwmc177-Xbarc199. Similarly, Germination

rate index QTLs were mapped on chromosome 2A and 4B of mapping population (Marouf and Mohammadi, 2015). Wheat seed germination attributes QTLs were mapped on chromosome 1D, 5D and 7D (Landjeva *et al.*, 2014).

QTLs affecting seedling growth was mainly mapped on chromosome 1B, 2A, 2B, 2D, 3B, 3D, 4D, 5A, 5B, 5D, 6A, 6B, 6D and 7B of recombinant inbred lines under control and salt stress condition. QTLs for root length were mapped on 1B, 2A, 2D, 3B, 3D, 4D, 5A, 5B, 6A, 6B, and 6D chromosome of wheat RILs under non-stressed and stressed condition. Likewise, Czyczyło-Mysza *et al.* (2014) conducted study to identify QTLs for root length in wheat mapping population in non-stressed and stressed condition. They mapped root length QTLs on chromosome 1A, 2A, 2B, 5B, 7B, 2D, 4D and 6D of mapping population. In different salt levels, Salem and Magdy (2014) mapped QTLs for root length on 1D, 2D, 3A, 3B, 4B, 5B, 7A and 7B chromosome. Bai *et al.* (2013) finding suggests that QTLs localized on 6A, 3B, 5B and 4D chromosome for root length. QTLs for shoot length were identified on 1D, 2D, 3B, 3D, 4B, 5D, 6D and 7B chromosome of wheat recombinant inbred lines under normal and 150 mM salt stress. QTLs for shoot length was reported under non-stressed and stressed condition. Shoot length QTLs were mainly mapped on wheat D genome (Salem and Magdy, 2014). Similarly, another study conducted by Marjaei *et al.* (2014), their finding depict that shoot length QTLs were mapped on 2D, 3B and 5D chromosome of RILs. Fresh weight QTLs were mapped on 1D, 2A, 2D, 4B, 4D, 5D, 7A and 7D chromosome of RILs in both normal and stressed environment. QTL for fresh weight explained phenotypic variation 15 percentage and found between SSR markers xwmc630-xgdm148. Similar, Salem and Magdy (2014) conducted study to identify QTLs for fresh weight under control and salt stress condition. They mapped QTLs on chromosome 4D, 5A, 5D, 7A and 7D of mapping population. Dry weight QTLs were localized on chromosome 1B, 2B, 5B, 5D and 6A of recombinant inbred lines under non-stressed and stressed condition. Our findings are similar to Salem and Magdy (2014) study, in which they used different salt levels such as 15 mM, 250 mM and 350 mM. Dry weight QTLs were mapped on 1D, 3B, 4B, 4D, 5A, 7A and 7D chromosome of RILs. Czyczyło-Mysza *et al.* (2014) findings depict that dry weight QTLs were mapped on chromosome 2B, 3B, 6B, 5B, 5D, 6B, 6D and 7A of mapping population

**Conclusion:** Current study findings show that wheat seed germination and early seedling growth is poly genic trait. QTLs for seed germination and seedling growth are collocated at same in locus interval and this show a main mechanism for salt tolerance.

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