Identification of microsatellite alleles for salt tolerance at seedling stage in wheat (*Triticum aestivum* L.)

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Abstract: A diverse collection of wheat genotypes, consisting of 21 bread wheat genotypes with varying levels of salt tolerance were evaluated under salt stress at seedling stage. Subsequently in order to assess the allele diversity of *QTLs* attributed to salt tolerance, the genotypes were genotyping on the basis of seedling traits using a set of 16 microsatellite markers. In total, 107 marker-trait associations significant *QTLs* for seedling traits were identified (21, 35, 32 and 19) at 0, 150, 250 and 350 mM NaCl, respectively. More *QTLs* were located on D, B and A genomes (45, 34 and 28), respectively. Association analysis of SSR markers showed 7 markers i.e. *Xgwm485, Xgwm261, Xgwm389, Xgwm165, Xgwm408, Xgwm190* and *Xgwm631* on chromosomes 1D, 2D, 3B, 4D, 5B, 5D and 7A, had significant association with most of seedling traits. Detection of *QTLs* for seedling traits at different chromosomes indicated that these characters are controlled by multiple loci. A higher R^2 values were obtained for most traits and ranged from 0.369* to 0.773** for root fresh weight at 250 and 350 mM NaCl, respectively. Genetic analysis identified the best microsatellite markers attributed to salt tolerance and they can be informative for improvement of salt tolerance through marker-assisted selection programs. Breeders can use this information to design crosses that assemble new, potentially durable combinations of salt tolerance genes to improve wheat genotypes.
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1. Introduction

Bread wheat is the most important and strategic cereal crop and widely cultivated in most of countries of the world which suffer saline soils, and therefore increasing salt tolerance in wheat is necessary (Tuna *et al*., 2008). For this reason, the development of salt tolerance wheat genotypes is important (Flowers *et al*., 1997, Ma *et al*., 2007 and Diaz De Leon *et al*., 2011). In bread wheat germplasm, salt is one of the major abiotic stress factor reducing plant growth and crop productivity (Diaz De Leon *et al*., 2000). To obtain better yield from saline soils and saline irrigation waters on a sustained basis, it is imperative that along with improved agronomic practices. The genetic resources should be exploited with the help of modern molecular techniques, such as *QTLs* mapping (Ma *et al*., 2007, Salem *et al*., 2007 and Sardouie-Nasab *et al*., 2013), marker assisted breeding (Eagles *et al*., 2001 and Sorrells, 2007) map based cloning (Huang *et al*., 2003 and Uaug *et al*., 2006) and gene transformation (Hu *et al*., 2003 and Harwood 2012) to develop high yielding salt tolerant wheat genotypes. Marker-assisted selection (MAS) is helpful in identifying qualitatively and quantitatively inherited desirable traits (Ogbonnaya *et al*., 2001, Lange and Whittaker 2001, Kuchel *et al*., 2007 and William *et al*., 2007). DNA markers have great scope in the construction of linkage maps for a range of crop species. Linkage maps can be utilized for the identification of genes/ *QTLs* controlling simple or quantitative traits (Collard *et al*., 2005). DNA markers which are tightly linked to important genes may be used as molecular tools for marker–assisted selection in plant breeding (Ribaut and Hoisington, 1998). The genetics of salt tolerance in wheat has been unraveled for seedling traits, such as shoot dry weight, root dry weight, shoot height, root length and total dry weight under salt stress. All these analysis revealed that genetic variation of salt tolerance was associated with multiple genes (Ma *et al*., 2007 and Xu *et al*., 2013). A number of *QTL* mapping studies have analyzed the genetic control of salt tolerance. QTLs were detected under both control and salt stress conditions on chromosomes 2A, 5A, 6A, 7A, 1B, 4B, 3B, 6B, 7B and 6D (Ma *et al*., 2007, Genc *et al*., 2010, Diaz De Leon *et al*., 2011 and Xu *et al*., 2013). Most agronomically important traits in cereals are quantitatively inherited, making the genes underlying variation for these traits hard to detect.

Association mapping has more recently been used to identify marker- trait relationships in plants (Oraguzie *et al*., 2007 and Zhu *et al*., 2008). Unlike linkage analysis, where mapping populations are used to determine correlations between phenotype and genotype, association mapping relies on unrelated individuals to create marker- trait associations

(MTAs), (Jannink *et al*., 2001, Agrama *et al*., 2007 and Neumann *et al*., 2011). There are some examples for application of association mapping in cereal crops (Somers *et al*., 2003, Breseghello and Sorrells 2006, Agrama *et al*., 2007 and Neumann *et al*., 2011). Selection of *QTLs* for abiotic stress can be achieved via haplotype selection based on flanking markers (Chen *et al*., 2004). SSR markers and their allele diversity are useful to effectively distinguish wheat genotypes. This approach is now being used to differentiate rice germplasm with different sources of mineral elemental contents and phenotypic traits (Zeng *et al*., 2009). Also, this approach used to differentiate wheat germplasm with different sources of salt tolerance genes (Sardouie-Nasab *et al*., 2013), through the use of microsatellite markers. *QTLs* mapping of many important agronomic traits, a major goal in plant breeding, requires informative markers in an intra-specific context. A diverse collection of 30 extremes tolerant and sensitive genotypes was haplotyped for salt tolerance using microsatellite markers, a total of 30 different haplotypes were observed by 32 microsatellite markers (Sardouie-Nasab *et al*., 2013). The objectives of the present study were to i) validate microsatellite markers for salt tolerance by marker-trait association analysis on a diverse collection, ii) obtain informative on genetic variation for salt tolerance during seedling stage of wheat and iii) identify novel and potentially new sources of salt tolerance.

2. Materials and methods

Plant materials

A diverse collection of 21 Egyptian bread wheat (*Triticum aestivum* L.) genotypes were used for estimation of salt tolerance. All genotypes were supplied by Agricultural Research Center (ARC), Giza, Egypt. All the wheat genotypes used in the present study have been released in Egypt. A List of the wheat genotypes, their breeding program and pedigree is presented in Table 1.

Genomic DNA isolation

Total genomic DNA was extracted from leaf tissue per each genotype. Young leaves from eight weeks old plants were cut as tissue samples for DNA extraction. DNA was isolated from these genotypes as described by Plaschke *et al.,* (1995).

Microsatellite markers analysis

A total of sixteen wheat microsatellite markers were selected for genotyping as given in Table 2. All Gatersleben Wheat Microsatellites (*Xgwm*) used were dinucleotide repeats. SSR markers were chosen on the basis of their proximity to genome specificity and according to information available in the Grain Genes database (Matthews *et al*., 2003). Most of the marker positions within chromosomes were based on the consensus map wheat composite 2004 map (http://rye.pw.usda.gov/cmap). Microsatellite amplifications, polymerase chain reaction and fragment analysis for SSR markers were performed according to Röder *et al*., (1998). GWM designation, chromosomal location, motif, annealing Tm **ºC** and fragment size location in 'CS' (bp) of the amplified loci were reported by Röder *et al*., (1998).

Salt test

A diverse collection of 21 wheat genotypes were used to determine the variability in salt tolerance. Grains were surface sterilized with 0.2% Clorox for 10 minutes and rinsed thoroughly in sterilized distilled water. Then they were germinated in dark at 4ºC for 3 days. The most uniformly germinated individuals were sown in sheets of thin Styrofoam, which were floated over a solution of half-strength Hoagland solution. To avoid salt shock, NaCl was added in equal daily increments over 3 days to a final concentration of (0, 150, 250 and 350 mM NaCl). Each treatment was represented by three replicates (ten seedlings per replicate). The experiment was conducted in a growth chamber with a 16 h light/ 8 h dark photoperiod, 20 ºC day/ 18 ° C nights mean temperature. The culture solution was renewed weekly and the pH was maintained at 6.5 and adjusted every day. After the plants had kept under treatments for four weeks, the measurements of seedling traits were recorded.

Evaluation of seedling traits:

In total, 11 traits were scored in each treatment. The symbolization of *QTLs* follows the rules of MacIntosh *et al*., (2003) (Table 3). Seedlings of each genotype from each replicate were selected randomly for measurements. The following traits were recorded i.e. coleoptile length (*Cl*), seedling length up to second leaf ($Sl2^{nd}l$), seedling length (*Sl*), root length (*Rl*), root number (*Rn*), seedling fresh weight (*Sfw*), shoot fresh weight (*Shfw*), root fresh weight (*Rfw*), seedling dry weight (*Sdw*), shoot dry weight (*Shdw*) and root dry weight (*Rdw*) of six seedlings per genotype were measured after 28 days. The same seedlings were weighted and oven dried at 70°C for 2 days and dry weight for both shoot and root were recorded.

Statistical analysis

Data on each of the 11 seedling traits were separately correlated on each of the 16 polymorphic microsatellite markers. Given that some genotype showed its heterozygosity at a certain SSR locus, the molecular weight for that SSR marker in that accession was represented by the mean of two allele size. Correlation was determined by applying Pearson's method. Statistical significance was defined as $P<0.05$. The coefficient of determination

(R^2) was estimated for each of SSR markers using SPSS 10.5 software (SPSS, Inc., Chicago, USA).

3. Results

Association analysis

Significant association was observed for 15 of the 16 polymorphic microsatellite markers with at least one of the 11 seedling traits and 15 markers identified with R^2 >10 % for traits (explained more than 10% of the phenotypic variation for each trait) (Table 3). In total, 107 marker-trait associations significant *QTLs* for seedling growth traits were identified 21, 35, 32 and 19 at 0, 150, 250 and 350 mM NaCl, respectively. More *QTLs* (45, 34 and 28), were located on D, B and A genomes, respectively. The *QTLs* were distributed across 6 chromosomes, ranging from 3 mapping to chromosomes 1D, 5A and 7A under 350 mM NaCl to 7 located on chromosome 7A under 250 mM NaCl. Seedling vigor *QTL*s were present on chromosomes 1B, 1D, 2D, 3A, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 7A, 7B and 7D of which 1D, 2D, 3B, 4D, 5B, 5D and 7A were expressed in both stress and non-stress conditions. In this study, *Xgwm458, Xgwm261, Xgwm389, Xgwm165, Xgwm408* and *Xgwm631* were appropriate MTAs to improve seedling traits because most of seedling related traits such as *Cl*, *Sl2nd l*, *Sl*, *Rl*, *Rn*, *Sfw*, *Shfw*, *Rfw*, *Sdw*, *Shdw* and *Rdw* were significant with these microsatellite markers. A higher R^2 values were obtained for most seedling traits and ranged from 0.369* to 0.773** for root fresh weight at 250 and 350 mM NaCl, respectively.

Marker-traits association analysis (MTAs)

Coleoptile length (*Cl*)

Correlation analysis indicated that there was a significant correlation only in 7 *QTLs* (r = 0.438* to -0.554**) of 64 pair traits between microsatellite allele size and *Cl* under stress and non-stress conditions (Table 3). *Cl* showed a significant correlation with the allele size of *Xgwm458*-1D, *Xgwm389-3*B, *Xgwm513*-4B and *Xgwm437*-7D. *Clc* at 0 mM NaCl displayed a significant correlation with the allele size of *Xgwm458*-1D. Furthermore, *Cls* at 150 mM NaCl had a significant correlation with the allele size of both *Xgwm458*-1D and *Xgwm389*-3B. While at 250 mM NaCl, *Cls* displayed a significant correlation with the allele size of *Xgwm458*-1D, *Xgwm513*-4B and *Xgwm437*-7D and at 350 mM NaCl *Cls* showed a significant marker-trait association with *Xgwm458*-1D. The *Xgwm458*-1D marker was linked with *Cl* under salt and non-salt stress.

Seedling length up to second leaf (*Sl2nd l*)

For *Sl2nd l*, the correlation analysis indicated that there was a significant correlation in 5 *QTLs* (r = 0.446* to 0.549**) of 64 pair traits between microsatellite allele size and *Sl2nd l* (Table 3). *Sl2nd lc* had no correlation with any of the allele size of wheat microsatellite markers. While, at 150 mM NaCl, *Sl2nd ls* had a significant correlation with the allele size of *Xgwm261, Xgwm389* and *Xgwm160* on chromosomes 2D, 3B and 4A, respectively. In addition, at 250 and 350 mM NaCl, *Sl2nd ls* showed a significant correlation with the allele size of *Xgwm261*-2D and *Xgwm408*-5B, respectively.

Seedling length (*Sl*)

From MTAs, there was a significant association with 14 *QTLs* (r = 0.386* to 0.591**) of 64 pair traits between microsatellite allele size and *Sl* (Table 3). *Slc* showed a significant association with one allele size (*Xgwm458*-1D). Whilst, *Sls* under salt stress had a significant correlation with a number of allele size (*Xgwm18*-1B, *Xgwm261*-2D, *Xgwm389*-3B and *Xgwm631*-7A) at 150 mM NaCl, (*Xgwm261*-2D, *Xgwm389*-3B, *Xgwm513*-4B *and Xgwm631*-7A) at 250 mM NaCl and (*Xgwm458*-1D, *Xgwm261*-2D, *Xgwm389*-3B, *Xgwm408*-5B and *Xgwm631*-7A) at 350 mM NaCl.

Root length (*Rl*)

As for *Rl*, there was a significant correlation in 11 *QTLs* (r = 0.377* to 0.663**) of 64 pair traits between microsatellites allele size and *Rl* (Table 3). *Rlc* showed a significant correlation with some allele size of *Xgwm155, Xgwm408* and *Xgwm631* on chromosomes 3A, 5B and 7A, respectively. Further, *Rls* had a significant correlation with the allele size of *Xgwm261* and *Xgwm389* on chromosomes 2D and 3B, respectively at 150 mM NaCl, while at 250 mM NaCl, *Rls* showed a significant correlation with the allele size of *Xgwm458, Xgwm261, Xgwm389, Xgwm513* and *Xgwm46* on chromosomes 1D, 2D, 3B, 4B and 7B. Also at 350 mM NaCl, *Rls* showed a significant correlation with *Xgwm458* marker on chromosome 1D.

Root number (*Rn*)

A significant MTAs in 6 *QTLs* (r = -0.380* to 0.669**) of 64 pair traits was obtained between microsatellites allele size and *Rn* (Table 3). *Rnc* showed a significant correlation with the allele size of *Xgwm261*-2D, *Xgwm165*-4D and *Xgwm631*-7A. However, under salt stress, *Rns* had a significant correlation with allele size of *Xgwm186*-5A at 150 mM NaCl, *Xgwm631*-7A at 250 mM NaCl and *Xgwm631*-7A at 350 mM NaCl.

Seedling fresh weight (*Sfw*)

Concerning the *Sfw,* there was a significant correlation in 10 *QTLs* (r = 0.407* to 0.686**) of 64 pair traits between microsatellites allele size and *Sfw* (Table 3). *Sfwc* showed a significant correlation with allele size of *Xgwm513*-4B, *Xgwm165*-4D and *Xgwm46*-7B. Also, under salt stress at 150 mM NaCl, *Sfws* had a significant correlation with the allele size

of *Xgwm165*-4D and *Xgwm46*-7B. Moreover, at 250 mM NaCl, *Sfw* showed a significant correlation with allele size of *Xgwm513*-4B, *Xgwm165*-4D and *Xgwm6317*A. However, *Sfw* at 350 mM NaCl, showed a significant correlation with the allele size of *Xgwm186*-5A and *Xgwm190*-5D.

Shoot fresh weight (*Shfw*)

There was a significant correlation in 7 *QTLs* (r = 0.376* to 0.585**) of 64 pair traits between microsatellites allele size and *Shfw* (Table 3). *Shfwc* showed a significant correlation with the *Xgwm165*-4D allele size. Moreover, under salt stress, *Shfws* had a significant correlation with the allele size of (*Xgwm408*-5B), (*Xgwm3*-3D and *Xgwm408*-5B) and (*Xgwm155*-3A, *Xgwm160*-4A and *Xgwm186*-5A) at 150, 250 and 350 mM NaCl, respectively.

Root fresh weight (*Rfw*)

In case of *Rfw,* there was a significant correlation in 15 *QTLs* (r = 0.369* to 0.773**) of 64 pair traits between microsatellites allele size and *Rfw* (Table 3). *Rfwc* showed a significant correlation with the allele size of *Xgwm458*-1D, *Xgwm513*-4B, *Xgwm165*-4D and *Xgwm46*-7D. However, *Rfw* at 150 mM NaCl, had a significant correlation with allele size of *Xgwm458*-1D, *Xgwm155*-3A, *Xgwm513*-4B, *Xgwm165*-4D and *Xgwm46*-7B. *Rfws* at 250 mM NaCl showed a significant correlation with allele size of *Xgwm513*-4B, *Xgwm165*-4B, *Xgwm190*-4D and *Xgwm631*-7A. *Rfws* at 350 mM NaCl indicated a significant correlation with the allele size of *Xgwm186*-5A and *Xgwm190*-5D.

Seedling dry weight (*Sdw*)

With regard to *Sdw,* there was a significant correlation only in 12 *QTLs* (r = 0.372* to 0.559**) of 64 pair traits microsatellites allele size and *Sdw* under stress and non-stress conditions (Table 3). *Sdwc* was indicated a significant correlation with the allele size of *Xgwm389*-3B and *Xgwm165*-4D. For salt stress 150 mM NaCl, *Sdws* appeared a significant correlation with the allele size of *Xgwm458*-1D, *Xgwm513*-4B, *Xgwm165*-4D, *Xgwm186*-5A and *Xgwm46*-7B. While, *Sdws* at 250 mM NaCl showed a significant correlation with the allele size of *Xgwm458*-1D, *Xgwm513*-4B, *Xgwm165*-4D, *Xgwm186*-5A and *Xgwm631*-7A and at 350 mM NaCl had no correlation with any of the 16 SSR markers.

Table (1). List of bread wheat cultivars used in the present study

No	Varieties	Pedigree
1	Giza 139	Hindi 90/ Kenya B256
2	Giza 144	Rgent/2* Giza 139
3	Giza 155	Regent/2* Giza 139//Mida-Cadit /2* Hindi 62
4	Giza 157	Giza 155//Pit 62 /LR 64/3/Tzpp/Knott
5	Sakha 8	Indus/Norteno “s”
6	Sakha 61	Inia/RL 4220//7C/Yr “s”
7	Sakha 69	Inia/RL 4220//7C/Yr “s”
8	Giza 160	Chenab70/Giza 155
9	Giza 162	Vcm//Cno67/7C/3/Kal/BbCM8399-D-4M-3Y-1M-1Y-1M-0Y
10	Giza 163	*T.aestivum*/Bon//Cno/7C CM33009-F-15M-4Y-2M-1M-1M-1Y-0M
11	Giza 164	Kvz/Buha “s”//Kal/Bb CM33027-F-15M-500y-0M
12	Gemmieza 1	Maya 74/On//1160.147/3/Bb/Gall/4/Chat”s” CM58924-1GM-OGM
13	Giza 167	Au/Up301//Gll/Sx/Pew”s”/4/Mai”s”/May”s”//Pew”s”CM67245-C-1M-2Y-1M-7Y-1M-0M
14	Sids 1	HD 2172/Pavon”s”//1158.57/Maya 74 “s” SD46-4SD46-4Sd-2SD-1SD-0SD
15	Sids 4	Maya "s"/Mon "S"/CM H74.A592/3/Giza 157*2
16	Sids 7	Maya "s"/Mon "S"/CM H74.A592/3/Sakha 8*2 SD10002-8SD-1SD-1SD-0SD
17	Sids 8	Maya "s"/Mon "S"/CM H74.A592/3/Sakha 8*2 SD10002-14SD-3SD-1SD-0SD
18	Gemmieza 3	Bb/7C*2//Y50/Kal*3//Sakha8/4/Prv/WW/5/3/Bg”s”//OnCGM.4024-1GM13GM2GM0GM
19	Gemmieza 5	Vee”s”/SWM 6525 CGM.4017-1GM-6 GM-3 GM-0GM
20	Gemmiza 7	CMH74 A. 630/5x//Seri 82/3/Agent CGM.4611-2GM-3GM-1GM-0GM
21	Gemmiza 9	Ald”s”/Huac”\s”//CMH74A.630/5x CGM.4583-5GM-1GM-0GM

Shoot dry weight (*Sdw*)

There was a significant correlation in 9 *QTLs* (r = 0.386* to 0.547**) of 64 pair traits between microsatellites allele size and *Sdw* (Table 3). *Sdwc* indicated a significant correlation with the allele size of *Xgwm190*-5D. Moreover, *Sdws* under salt stress had a significant correlation with the allele size of *Xgwm513*-5B, *Xgwm165*-4D and *Xgwm46*-7B at 150 mM NaCl, *Xgwm458*-1D, *Xgwm165*-4D and *Xgwm631*-7A at 250 mM NaCl, and *Xgwm155*-3A and *Xgwm186*-5A at 350 mM NaCl.

Root dry weight (*Rdw*)

As for *Rdw,* a significant correlation only in 11 *QTLs* (r = -0.441* to 0.514**) of 64 pair traits was

obtained between microsatellites allele size and *Rdw* under salt and non- salt stresses (Table 3). *Rdwc* had a significant correlation with the allele size of *Xgwm389*-3B and *Xgwm165*-4D. Under salt stress, at 150 mM NaCl, *Rdws* elucidated a significant association with the allele size of *Xgwm186*-5A, *Xgwm190*-5D, *Xgwm631*-7A, *Xgwm46*-7B and *Xgwm437*-7D. Otherwise, *Rdws* showed a significant correlation with the allele size of (*Xgwm190*-5D, *Xgwm631*-7A and *Xgwm437*-7D) and (*Xgwm631*-7A) at 250 and 350 mM NaCl, respectively.

Table 2: SSR markers, chromosomal location, motive, annealing temperature (ºC) and expected fragment size in Chinese Spring.

No	SSR markers	Chromosomal location	Motif	Annealing Tm (ºC)	Fragment size in CS (bp)
1	*Xgwm3*	3D	(CA)18	55	79
2	*Xgwm18*	1B	(CA)17GA(TA)4	55	183
3	*Xgwm 46*	7B	(GA)3GC(GA)33	60	179
4	*Xgwm 95*	2A	(AC)16	60	179
5	*Xgwm155*	3A	(CT)19	60	144
6	*Xgwm160*	4A	(GA)21	60	182
7	*Xgwm165*	4A	(GA)20	60	190
8	*Xgwm186*	5A	(GA)26	60	135
9	*Xgwm190*	5D	(CT)22	60	209
10	*Xgwm261*	2D	(CT)21	55	189
11	*Xgwm389*	3B	(CT)14 (GT)16	60	129
12	*Xgwm408*	5B	(CA)>22(TA)(CA)7(TA)9	55	176
13	*Xgwm437*	7D	(CT)24	50	107
14	*Xgwm458*	1D	(CA)13	60	113
15	*Xgwm513*	4B	(CA)12	60	140
16	*Xgwm631*	7A	(GT)23	60	196

Table 3: Association of microsatellite markers with seedling traits

Trait	*QTL symbol*	Chromosome	*Marker*	R-value
Coleoptile length control (*Clc*)(0mM)	***QClc.1D(c).1***	**1D(c)**	***Xgwm458***	**-0.458***
Coleoptile length stress (Cls 150 mM)	***QCls.1D(c).1***	**1D(c)**	***Xgwm458***	**-0.511***
	QCls.3BS.2	**3BS**	***Xgwm389***	**-0.554****
Coleoptile length stress (Cls) (250 mM)	***QCls.1DS.1***	**1D(c)**	***Xgwm458***	**-0.465***
	QCls.3BS.2	**4BL**	***Xgwm513***	**-0.537***
	QCls.7Dl.3	**7DL**	***Xgwm437***	**0.438***
Coleoptile length stress (Cls) (350 Mm)	***QCls.1D(c).1***	**1D(c)**	***Xgwm458***	**-0.458***
Seedling length up to 2 leaf (Sl2ndls)(150 mM)	***QSl2ndls.2DS.1***	**2DS**	***Xgwm261***	**0.549****
	QSl2ndls.3BS.2	**3BS**	***Xgwm389***	**0.446***
	QSl2ndls.4Al.3	**4AL**	***Xgwm160***	**0.479***
Seedling length up to 2 leaf (Sl2ndls)(250 mM)	***QSl2ndls.2DS.1***	**2DS**	***Xgwm261***	**0.503***
Seedling length up to 2 leaf ((Sl2ndls)(350 mM)	***QSl2ndls.5BL.1***	**5BL**	***Xgwm408***	**0.525****
Seedling length control (Slc)(0 mM)	***QSlc.1D(c).1***	**1D(c)**	***Xgwm458***	**0.4**
Seedling length stress (Sls)(150 mM)	***QSls.1BS.1***	**1BS**	***Xgwm18***	**0.418***
	QSls.2DS.2	**2DS**	***Xgwm261***	**0.471***
	QSls.3BS.3	**3BS**	***Xgwm389***	**0.503***
	QSls.7AS.4	**7AS**	***Xgwm631***	**0.576****
Seedling length stress (Sls1)(250 mM)	***QSls.2DS.1***	**2DS**	***Xgwm261***	**0.591****
	QSls.3BS.2	**3BS**	***Xgwm389***	**0.538****
	QSls.4BL.3	**4BL**	***Xgwm513***	**0.461***
	QSls.7AS.4	**7AS**	***Xgwm631***	**0.515****
Seedling length stress (Sls)(350 mM)	***QSls.1D(c).1***	**1D(c)**	***Xgwm458***	**0.529****

	QSls.2DS.2	2DS	*Xgwm261*	0.386*
	QSls.3BS.3	3BS	*Xgwm389*	0.399*
	QSls.5BL.4	5BL	*Xgwm408*	0.429*
	QSls.7AS.5	7AS	*Xgwm631*	0.389*
Root length control (Rlc)(0 mM)	*QRlc.3AL.1*	3AL	*Xgwm155*	0.576**
	QRlc.5BL.2	5BL	*Xgwm408*	0.377*
	QRlc.7AS.3	7AS	*Xgwm631*	0.388*
Root length stress (Rls)(150 mM)	*QRls.2DS.1*	2DS	*Xgwm261*	0.472*
	QRls.3BS.2	3BS	*Xgwm389*	0.436*
Root length stress (Rls)(250 mM)	*QRls.1D(c).1*	1D(c)	*Xgwm458*	0.595**
	QRls.2DS.2	2DS	*Xgwm261*	0.439*
	QRls.3BS.3	3BS	*Xgwm389*	0.464*
	QRls.4BL.4	4BL	*Xgwm513*	0.505**
	QRls.7B(c).5	7B(c)	*Xgwm46*	-0.445*
Root length stress (Rls)(350 mM)	*QRls.1D(c).1*	1D(c)	*Xgwm458*	0.663**
Root number control (Rnc)(0 mM)	*QRnc.*2DS.*1*	2DS	*Xgwm261*	-0.380*
	*QRnc.*4DL.*2*	4DL	*Xgwm165*	0.500*
	*QRnc.*7AS.*3*	7AS	*Xgwm631*	0.463*
Root number stress (Rns)(150 mM)	*QRns.*5AL.*1*	5AL	*Xgwm186*	-0.466*
Root number stress (Rns)(250mM)	*QRns.*7AS.*1*	7AS	*Xgwm631*	0.669**
Root number stress (Rns)(350 mM)	*QRns.*7AS.*1*	7AS	*Xgwm631*	0.422*
Seedling fresh weight control (Sfwc)(0 mM)	*QSfwc.*4BL.*1*	4BL	*Xgwm513*	0.463*
	*QSfwc.*4DL.*2*	4DL	*Xgwm165*	0.557**
	*QSfwc.*7B(c).*3*	7B(c)	*Xgwm46*	0.407*
Seedling fresh weight stress (Sfws)(150 mM)	*QSfws.*4DL.*1*	4DL	*Xgwm165*	0.531**
	*QSfws.*7B(c).*2*	7B(c)	*Xgwm46*	0.464*
Seedling fresh weight stress (Sfws)(250 mM)	*QSfws.*4BL.*1*	4BL	*Xgwm513*	0.493*
	*QSfws.*4DL.*2*	4DL	*Xgwm165*	0.620**
	*QSfws.*7AS.*3*	7AS	*Xgwm631*	0.467*
Seedling fresh weight stress (Sfws)(350)	*QSfws.*5AL.*1*	5AL	*Xgwm186*	0.515**
	*QSfws.*5DS.*2*	5DS	*Xgwm190*	0.686**
Shoot fresh weight control (Shfwc)(0 mM)	*Q*Shfwc.4DL.*1*	4DL	*Xgwm165*	0.376*
Shoot fresh weight stress (Shfws)(150 mM)	*Q*Shfws.5BL.*1*	5BL	*Xgwm408*	-0.487*
Shoot fresh weight stress (Shfws)(250 mM)	*Q*Shfws.3DL.*1*	3DL	*Xgwm003*	-0.407*
	*Q*Shfws.5BL.*2*	5BL	*Xgwm408*	-0.560**
Shoot fresh weight stress (Shfws)(350 mM)	*Q*Shfws.3AL.*1*	3AL	*Xgwm155*	-0.585**
	*Q*Shfws.4AL.*2*	4AL	*Xgwm160*	-0.419*
	*Q*Shfws.5AL.*3*	5AL	*Xgwm186*	0.476*
Root fresh weight control (Rfwc)(0 mM)	*QRfwc.1DS.1*	1D(c)	*Xgwm458*	0.387*
	*QRfwc.*4BL.*2*	4BL	*Xgwm513*	0.534**
	*QRfwc.*4DL.*3*	4DL	*Xgwm165*	0.590**
	*QRfwc.*7B(c).*4*	7B(c)	*Xgwm46*	0.415*
Root fresh weight stress (Rfws)(150 mM)	*QRfws.1DS.1*	1D(c)	*Xgwm458*	0.393*
	*QRfws.*3AL.*2*	3AL	*Xgwm155*	0.385*
	*QRfws.*4BL.*3*	4BL	*Xgwm513*	0.370*
	*QRfws.*4DL.*4*	4DL	*Xgwm165*	0.506**
	*QRfws.*7B(c).*5*	7B(c)	*Xgwm46*	0.428*
Root fresh weight stress (Rfws)(250 mM)	*QRfws.*4BL.*1*	4BL	*Xgwm513*	0.586**
	*QRfws.*4DL.*2*	4DL	*Xgwm165*	0.680**
	*QRfws.*5DS.*3*	5DS	*Xgwm190*	0.369*
	*QRfws.*7AS.*4*	7AS	*Xgwm631*	0.475*
Root fresh weight stress (Rfws)(350 mM)	*QRfws.* 5AL.*1*	5AL	*Xgwm186*	0.409*

	QRfws. 5DS.2	**5DS**	***Xgwm190***	**0.773****
Seedling dry weight control (Sdwc)(0 mM)	***QSdwc.3BS.1***	**3BS**	***Xgwm389***	**0.429***
	QSdwc.4DL.2	**4DL**	***Xgwm165***	**0.488***
Seedling dry weight stress (Sdws)(150 mM)	***QSdws.1D(c).1***	**1D(c)**	***Xgwm458***	**0.372***
	QSdws. 4BL.2	**4BL**	***Xgwm513***	**0.402***
	QSdws. 4DL.3	**4DL**	***Xgwm165***	**0.503***
	QSdws. 5AL.4	**5AL**	***Xgwm186***	**0.428***
	QSdws.7B(c).5	**7B(c)**	***Xgwm046***	**0.433***
Seedling dry weight stress (Sdws)(250 mM)	***QSdws.1D(c).1***	**1D(c)**	***Xgwm458***	**0.423***
	QSdws.1D(c).2	**1D(c)**	***Xgwm513***	**0.373***
	QSdws.1D(c).3	**1D(c)**	***Xgwm165***	**0.559****
	QSdws.1D(c).4	**1D(c)**	***Xgwm186***	**0.415***
	QSdws.1D(c).5	**1D(c)**	***Xgwm631***	**0.468***
Shoot dry weight control (Shdwc)(0 mM)	***QShdwc.5DS.1***	**5DS**	***Xgwm190***	**0.432***
Shoot dry weight stress (Shdws)(150 mM)	***QShdws.4BL.1***	**4BL**	***Xgwm513***	**0.418***
	QShdws.4BL.2	**4DL**	***Xgwm165***	**0.512****
	QShdws. 7B(c).3	**7B(c)**	***Xgwm046***	**0.386***
Shoot dry weight stress (Shdws)(250 mM)	***QShdws. 1D(c).1***	**1D(c)**	***Xgwm458***	**0.473***
	QShdws. 4DL.2	**4DL**	***Xgwm165***	**0.543****
	QShdws. 7AS.3	**7AS**	***Xgwm631***	**0.396***
Shoot dry weight stress (Shdws)(350 mM)	***QShdws. 3AL.1***	**3AL**	***Xgwm155***	**-0.377***
	QShdws. 5AL.1	**5AL**	***Xgwm186***	**0.547****
Root dry weight control (Rdwc)(0 mM)	***QRdwc. 3BS.1***	**3BS**	***Xgwm389***	**0.450***
	QRdwc.4DL.2	**4DL**	***Xgwm165***	**0.442***
Root dry weight stress (Rdws)(150 mM)	***QRdws. 5AL.1***	**5AL**	***Xgwm186***	**0.476***
	QRdws. 5DS.2	**5DS**	***Xgwm190***	**0.514****
	QRdws. 7AS.3	**7AS**	***Xgwm631***	**0.475***
	QRdws. 7B(c).4	**7B(c)**	***Xgwm046***	**0.441***
	QRdws. 7DL.5	**7DL**	***Xgwm437***	**-0.441***
Root dry weight stress (Rdws)(250 mM)	***QRdws. 5DS.1***	**5DS**	***Xgwm190***	**0.478***
	QRdws. 7AS.2	**7AS**	***Xgwm631***	**0.491***
	QRdws. 7DL.3	**7DL**	***Xgwm437***	**-0.448***
Root dry weight stress (Rdws)(350 mM)	***QRdws. 7AS.1***	**7AS**	***Xgwm631***	**-0.496***

The statistics shown refer to the coefficient of determination (R^2), Only SSR markers with significant marker-trait association are given. *, ** Indicate significance at the probability levels of 0.05 and 0.01, significance respectively. *Cl* coleoptile length, *Sl2nd l* seedling length up to second leaf, *Sl* seedling length, *Rl* root length, *Rn* root number, *Sfw* seedling fresh weight, *Shfw* shoot fresh weight, *Rfw* root fresh weight, *Sdw* seedling dry weight, *Shdw* shoot dry weight and *Rdw* root dry weight

4. Discussion

Genotypes isolation through phenotypic selection under stress conditions has resulted in major progress (Banziger and Araus, 2007), but it is time-consuming and laborious. DNA markers linked with genes/QTL for traits of interest are being routinely developed in several crops using different mapping populations such as $F_{2:3}$, RILs, DHLs and BC. Hopefully, some of these DNA markers will be used for MAS in future wheat breeding programs (William *et al*., 2007). However, non-availability of mapping populations and substantial time needed to develop such populations are sometimes major limitations in the identification of DNA markers for some traits. Another limitation is the absence of tight linkage observed in these studies. To overcome these limitations markers for traits of interest have been identified through association studies conducted using germplasm collections (Gupta *et al*., 2005). In bread wheat, further studies are required to identify *QTLs* associated with salt tolerance traits. Marker-trait association has been examined to identify *QTLs* controlling trait in most plants. When creates such a MTAs, indirect selection can be done via study the presence or absence of informative DNA marker, that can reduced cost and time of breeding programs (Yin *et al*., 2003). Once MTAs has been detected for trait of interest, it can be used as a marker-assisted selection to obtain an indirect response in the trait. In the present study, a total of 15 microsatellite markers

were associated significantly with eleven seedling traits under 0, 150, 250, 350 mM NaCl. Therefore, these DNA markers can be used as beneficial markers to increase salt tolerance in wheat using marker-assisted selection programs. The present study showed significant MTAs under stress and non-stress conditions. Also, the current study showed that MTAs are an effective means of relating genotypes to complex quantitative phenotypes, which illustrates the utility of microsatellite markers to identify genotypes likely carrying the same salt tolerance *QTLs* and potentially novel tolerance. The majority of microsatellite markers were significantly associated with more than one trait, particularly in *Xgwm458*, *Xgwm261, Xgwm389, Xgwm165, Xgwm408, Xgwm190* and *Xgwm631*. Such MTAs may arise due to pleiotropic effects of the linked *QTLs* on different seedling traits (Miller and Rawlings 1967, Meredith and Bridge 1971 and Culp *et al*., 1979). Closely linked *QTLs* affecting different phenotypic traits may be due to a single marker association with multiple traits which would be reflected in correlations between such traits. This study has identified one highly reliable microsatellite marker *Xgwm631* on chromosome 7A associated with seedling traits including *Sl, Rl, Rn, Sfw, Rfw, Sdw, Sdw* and *Rdw* which can be utilized for indirect selection to increased seedling traits under stress conditions. The present study demonstrated that MTAs analysis in wheat genotypes can enhance the information from genes/*QTLs* studies toward the implementation of marker-assisted selection. The finding of significant markers on mentioned chromosomes agrees with previous investigations that suggested existence of salt tolerance genes/*QTLs* on the wheat chromosomes (Ma *et al*., 2007 and Garcia-Suarez *et al*., 2010). Genes/*QTLs* that are found to be associated with complex traits such as salt tolerance are highly useful and can be exploited to improve our knowledge of mechanisms that wheats employ to deal with the stress. This knowledge in turn will be useful not only for designing marker-assisted selection strategies but also for optimizing traditional wheat breeding programs. Identification of microsatellite alleles for salt tolerant *QTLs* might provide useful information for predicting novel *QTLs* (Yu *et al*., 2006). The ability to characterize genetic diversity in *QTL*s intervals associated with salt tolerance of wheats will be an important strategy for identifying novel salt alleles that confer better salt tolerance. This study indicated the MTAs linked to salt tolerant *QTLs* across a diverse collection of bread wheat genotypes. Our target was to identify alleles for the number of seedling traits. Twenty-one genotypes had different alleles by 16 microsatellite markers. Wheat genotypes with an allelic pattern in common with a salt tolerance might have a similar salt tolerance genes, moreover can helping to choose genotypes for subsequent analysis. Presence or absence of *QTLs* in such wheat genotypes would provide additional genetic evidence of *QTLs* location on the chromosome. This information about the different microsatellite alleles can be used to design optimum strategies for the pyramiding of salt tolerance into wheats. In the current study, the microsatellite markers linked to salt tolerance *QTLs*, were analyzed and amplified various microsatellite allele sizes in the genotypes. Based on MTAs results, it is concluded that *Xgwm*458, *Xgwm*165 and *Xgwm*631 markers on chromosomes 1D, 4D and 7A are useful markers for molecular breeding and identifying salt tolerance genotypes. The present study had identified potentially novel sources for further genetic analysis on salt stress. Zeng *et al*. (2009) studied the correlation between microsatellites allele sizes and phenotypic variations in rice landraces, they found 182 significant correlation using 20 microsatellite markers. Mohammadi-Nejad *et al*. (2010) identified 16 haplotype groups with 30 genotypes controlling salt tolerance in rice. Islam *et al*. (2012) reported seven haplotypes among 115 rice genotypes when used 3 SSR markers to compare the haplotypes. Also, McCartney *et al*., (2004) analyzed haplotype diversity for fusarium head blight resistance *QTLs* in wheat; they found 76 haplotypes using 41 microsatellite markers. Sardouie-Nasab *et al*. (2013) reported 30 haplotype groups with 30 genotypes for salt tolerance in wheat. At present study, the wheat microsatellite markers, *Xgwm458*, *Xgwm165* and *Xgwm631* markers on chromosomes 1D, 4D and 7A showed higher MTAs. In other hand these markers were introduced as beneficial marker for salt tolerance. It seems these markers have strong and positive association with salt tolerance genes/*QTLs* at their regions. MTAs allow the identification source of salt tolerant allele that can greatly increase the success of gene/*QTLs* postulation based on marker allele size (Zeng *et al*., 2009 and Sardouie-Nasab *et al*., 2013).

MTA is new approach in cereal genetics and particularly in wheat. In contrast to conventional bi-parental mapping, which can only analyze allelic differences between two parents, association mapping attempts to scan genetic variation across a wide spectrum of genotypes. The present study underlines the value of genetic basis of seedling traits even with a relatively small collection of genotypes. A substantial number of MTAs for a whole set of seedling traits were detected. Many loci were detected that coincide with known major genes or *QTLs*, indicating the power of association mapping.

Additionally, potential novel loci were identified that may help to better understand the architecture of complex genetic traits. Based on marker approach, the novel loci provide opportunities for further improvement of wheat,. Breeders can use this information to design crosses that assemble new, potentially durable combinations of salt tolerance genes/*QTLs* to improve wheat genotypes.

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