



RESEARCH PAPER

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Evaluation of QTL associated with salt tolerance on seedling stage in wheat

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Abstract

In order to map QTLs related to traits at seedling stage in bread wheat under both salinity stress and control conditions, shoot length (SL), root length (RL), root/shoot length ratio (RSR), shoot wet weight (SWW), root wet weight (RWW), shoot dry weight (SDW) and root dry weight (RDW) were evaluated in a mapping population of 102 F_{2:8} recombinant inbred lines derived from a cross between an Iranian local variety Roshan and European variety Superhead. Stress condition was provided applying 250 mM NaCl and QTL analyses were done on 45 SSR marker loci with using single marker analysis method. A total of 50 QTL major and minor effects on chromosomes 1A, 2A, 4A, 6A, 1B, 5B, 2D and 7D were identified in this study. Number 29 QTL stress and 21 QTL were found in the control condition. In the present study, the largest has identified QTL in the D-genome in the seedling stage under salinity stress. Among the traits most QTL were detected for root dry weight While trait QTL has accounted for the smallest proportion of the root seedling growth. All traits measured, significant and meaningful parent Superhead stress reduction and stress conditions relative to Roshan parent is shown.

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Introduction

Soil salinity is one of the major environmental problems affecting agricultural production in arid and semi-arid regions of the world, both in irrigated and dryland agriculture. Improving salinity tolerance of wheat is a key target for many wheat breeding programs worldwide. Amongst the synthetic hexaploid wheats (SHWs) ($2n=6x=42$, AABBDD) derived from crosses between *Triticum turgidum* L. ssp. *durum* ($2n=4x=28$, genome AABB) and *Aegilops tauschii* (syn *Ae. squarrose*, *T. tauschii*; $2n=2x=14$, genome DD), significant variation was observed for salinity tolerance. To investigate natural allelic variants contributing to quantitative variation for salinity tolerance in hexaploid wheat, a quantitative trait mapping approach was used to analyse a population of RILs synthetic-derived from crossing Roshan (Local varieties tolerant to salinity) and superhead (European and salt-sensitive varieties) to bread wheat (Ogbonnaya *et al.*, 2008). The RILs were screened for salinity tolerance based on the sodium exclusion mechanism. The RILs proved to be a promising tool to identify, characterize and introgress different salinity tolerance genes into adapted wheat genetic backgrounds. In order to learn more about the genetics of and genes controlling salinity tolerance (ST), two major studies need to be undertaken: firstly, screening and identification of the most appropriate accessions, lines or genotypes for further crossings; and secondly, identification of potential candidate genes using mapping populations and QTL analysis. Genetics, mapping and identification of candidate genes using QTL analysis for ST in wheat, has not yielded many significant outcomes, despite a long history of ST research. This probably reflects the complexity of the ST trait. Usually, QTL analysis in saline hydroponics reports several chromosomes associated with ST (eg. Ma *et al.* 2007), while QTLs on chromosomes 5B and 5D were identified in a field experiment with saline irrigation (Quarrie *et al.* 2005).

Materials and methods

Plant materials

A mapping population of 100 F_{2:8} recombinant inbred

lines derived from a cross between “Roshan” and “Superhead” was used in this study. Number of 100 RILs by Najy (2008), using a single seed progeny derived by successive selfing. Seeds lines and parents were sown in plastic pots. To do this in three replicates of five plants per pot was used for both the environment and stress control. Pots were in a controlled environment greenhouse in a completely randomized design. Zero solution (distilled water), and 250 mM were used for control and stress treatments.

At the end of the 8-leaf stage, traits such as number of leaves, seedling length (SL), root length (RL), the root seedling growth rate (SRR), seedling wet weight (SWW), root wet weight (RWW), dry weight seedlings (SDW), root dry weight (RDW) were measured for each treatment in both environments. To study the characteristics of 3 seedlings in each plot were selected and measured. High-precision digital scale ruler and measure the length and weight of the seedlings were used. Descriptive parameters, mean, variance analysis and comparison were performed with SPSS software. QTL analysis method Single marker analysis was performed using Windows QTL Cartographer software.

Results and discussion

The average distribution of phenotypic traits in this study proved the existence of a normal distribution in all traits. The mean of all the studied traits under conditions of Roshan parental control has lower performance and reduce stress while that parent Superhead in performance is striking and significant. In order to evaluate genetic and QTL localization of single marker analysis method was used. This method is based on a comparison of the mean phenotypic groups based marker and phenotypic data can each be analyzed with a marker. If any, linkage to a locus QTL markers are studied, and the results of tests carried out for each of the markers is independent of the marker. Single marker linkage detection methods are simple and do not require a linkage map of markers. QTL through its contribution to the phenotypic variance are studied and evaluated. The

share of R^2 value is calculated based on the fraction of 1 to 100 percent. In this way, large and small effect of the QTL effect is clear. A major QTL of relatively large amounts of more than 10 percent and a minor QTL usually less than 10% of the observed phenotypic variance in the population is allocated. From another

perspective, a major QTL effect on the stability refers to the different experimental conditions, while a small QTL effect may be characteristic of the environment. The QTL effects can be classified as significant and very significant (Lander *et al.*, 1989).

Table 1. Single marker analysis of leaf traits.

Trait	Marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
Ln	WMS 111	7D	0/498	5%	5/716	5/9
Ln	WMS 131	1B	-0/446	5%	4/819	4/9
Ln	WMS 1043	5B	3/405	1%	13/190	16/6
Ln	WMS 1235	4D	-0/445	5%	4/916	4/8
Ls	WMS 95	2A	-0/558	1%	7/702	7/4
Ls	WMS 157	2D	-0/431	5%	5/089	4/9
Ls	WMS 715	5D	-0/684	5%	4/189	4/1
Ls	WMS 721	2D	0/714	1%	7/087	7/3
Ls	WMS 1130	5D	0/637	1%	9/894	9/2

Table 2. Single marker analysis of root length.

Trait	Marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
RLn	WMS 99	1A	-1/747	1%	7/931	7/7
RLn	WMS 102	2D	1/531	5%	6/203	6/1
RLn	WMS 122	2A	-1/868	1%	7/227	6/9
RLn	WMS 762	1B	3/128	5%	12/117	14/1
RLn	WMS 1200	5B	-1/586	5%	5/397	5/3
RLn	WMS 1241	2D	1/746	5%	6/754	6/6
RLs	WMS 18	1B	2/618	5%	6/505	6/2
RLs	WMS155	3A	3/605	1%	12/437	11/9
RLs	WMS 778	1A	-2/090	5%	6/221	6

Table 3. Single marker analysis of seedling length.

Trait	Marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
SLn	WMS 122	2A	-2/264	%5	5/441	5/3
SLn	WMS 1093	4A	4/601	%1	15/617	17/5
SLs	WMS 88	6B	2/173	%5	4/649	4/7
SLs	WMS 715	5D	-2/726	%5	5/176	5
SLs	WMS 1223	6A	3/048	%1	13/247	14/2

Table 4. Single marker analysis of root wet weight.

Trait	Marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
RWWn	WMS 111	7D	0/068	%5	5/210	5/4
RWWn	WMS 1070	2B	4/045	%1	15/456	17/2
RWWn	WMS 1093	4A	-0/077	%5	6/675	6/6
RWWs	WMS 149	4B	0/025	%5	4/068	4/1
RWWs	WMS 1241	2D	0/030	%5	6/165	6/1

Single marker analysis for number of leaves traits indicate that a major QTL on chromosome 5B was

found in non-stress conditions (Table 1). We see this trait A and D genomes in a stressful situation to have

more QTL. Two major QTL affecting root length traits were determined in 1B and 3A (Table 2). Stress conditions A and B genomes share the QTL further. Sanguineti *et al* (1999) both the 2A and 5A QTL associated with this trait were reported. For seedlings length two major QTL were found on chromosomes 4A and 6A. A major QTL for this trait kato *et al* (2000) have reported on the 4B. Two major QTL for length of the seedling on chromosome 4A and 6A, respectively, in terms of stress and salinity was found (Table 3). The characteristic length of the seedlings, the contribution of each QTL in wheat genome is the same stress conditions. A major effect QTL on 2B was determined for root wet weight (Table 4), while Zhou *et al* (2007) for the trait under stress have reported a QTL on 4D. Distribution of this trait QTL in stress conditions B and D genome and genomes share this

trait QTL is approximately equal. Two major QTL effect on root dry weight was determined on the 7D and 4A stress conditions, As Table 5 shows, a greater proportion of the D-genome QTL associated with salinity is accounted for. The wet weight of seedlings under salt stress conditions A and D genomes of most QTL into account (Table 6). In terms of salinity genomes A and D, to have more QTL. Seedling dry weight for a major QTL on chromosome 4B was found in the control condition (Table 7). Known QTL for this trait is evenly distributed over the entire genome. Zhou *et al* (2007) have reported eight QTL on chromosomes 1A, 2A, 3A, 2D, 3D, 6A under salinity stress. No major effect QTL for seedling root growth relative trait found only a small effect QTL was found on chromosome 6A (Table 8).

Table 5. Single marker analysis of root dry weight.

trait	marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
RDWn	WMS 18	1B	0/032	%5	4/584	4/5
RDWn	WMS 610	4A	3/032	%1	11/506	14/4
RDWn	WMS 732	1A	0/022	%5	4/336	4/3
RDWn	WMS 735	7A	0/022	%5	6/196	6/5
RDWn	WMS 1016	6B	0/040	%5	4/624	7/3
RDWn	WMS 1043	5B	-0/043	%5	7/810	7/7
RDWn	WMS 1093	4A	3/034	%1	13/009	15
RDWs	WMS 111	7D	3/89	%1	14/927	16/1
RDWs	WMS 721	2D	0/08	%5	4/857	5/1
RDWs	WMS 1241	2D	0/08	%5	5/293	5/3

Table 6. Single marker analysis of wet weight of seedling.

trait	marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
SWWn	WMS 111	7D	0/069	%5	4/866	5/1
SWWn	WMS 897	3D	0/057	%1	10/047	10/1
SWWn	WMS 1093	4A	-0/065	%5	4/515	4/6
SWWn	WMS 1243	1B	-0/067	%5	4/567	4/7
SWWs	WMS 155	3A	3/059	%1	12/962	14/1
SWWs	WMS 1235	4D	0/060	%5	4/441	4/3

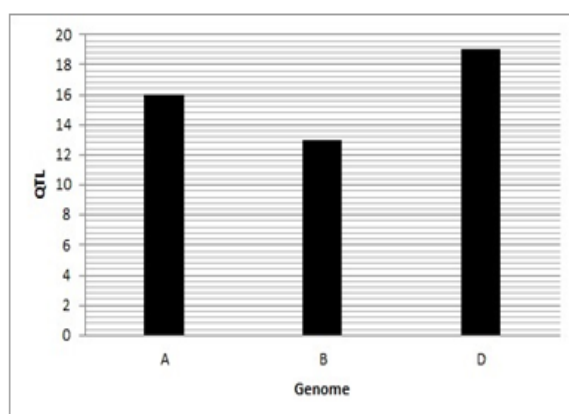
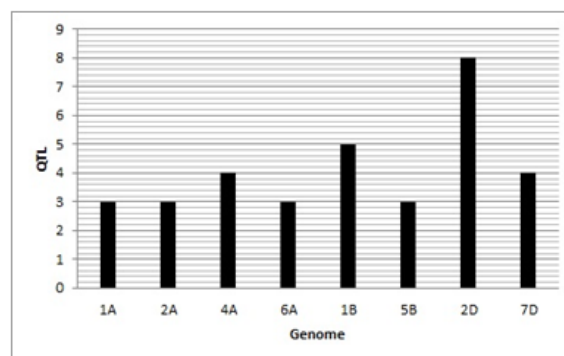
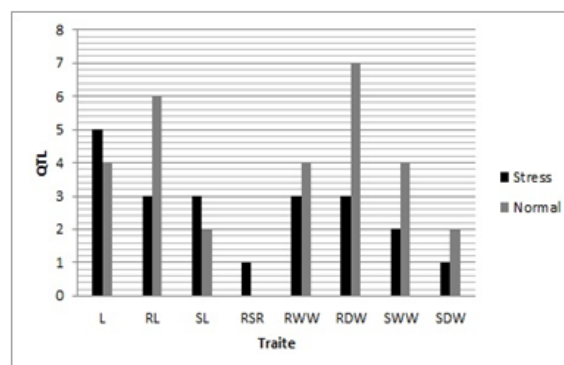
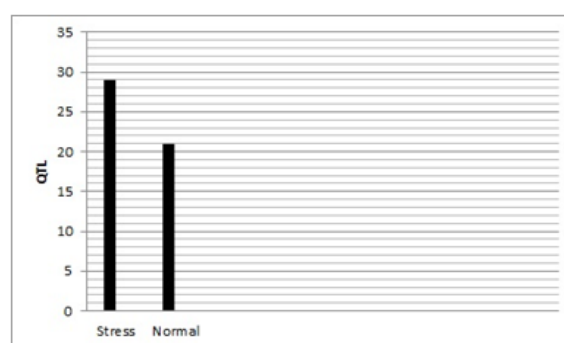
Table 7. Single marker analysis of dry weight of seedling.

trait	marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
SDWn	WMS 610	4A	-0/043	%1	7/495	7/1
SDWn	WMS 1084	4B	3/034	%1	12/558	14/5
SDWs	WMS 721	2D	0/032	%1	7/519	7/7

Table 8. Single marker analysis of root/shoot length ratio.

trait	marker	Chromosomal position	The regression coefficient	Significant level	F	R ² (%)
RSR	WMS 1223	6A	0/162	%5	4/368	4/4

Through genome chromosomes of wheat in this study, the genome of D, A and B, respectively 19, 16 and 13 QTL were in on. In this study, the characteristics of the seedling stage compared genomes, D genome was richer (Fig. 1). Most of the QTL on chromosome 2D is allocated (Fig. 2). Among the traits most QTL were detected for root dry weight, whereas trait QTL accounted for less than seedling root growth data (Fig. 3). Looks distributed groups of gene expression QTL, QTL particularly influenced by stress occurring at different stages of plant growth. While the study of Zhou *et al* (2007) where the salinity was applied at grain filling stage, A genome larger share of the QTL is involved in plant response to stress. One of the interesting points in this review is that the fifty QTL identified 29 QTL under stress and non-stress conditions was only 21 QTL (Fig. 4). This is consistent with the fact that the plant is essentially faced with stress in different growth stages, certain genes are expressed in groups. Although common QTL identified in terms of chromosomal location and determine where they are in terms of both developmental stage of the plant can suggest some groups for genetic activation of plant response to stress may be at different stages. In addition to the developmental stage, type and origin of the genetic factors in plant responses to stress gene activation group said.

**Fig. 1.** QTL categories based on gene groups.**Fig. 2.** Frequency distribution of QTL in the genome, chromosome segregation population.**Fig. 3.** QTL categories based on the characteristics of both normal and stress levels.**Fig. 4.** QTL categories in both stress and normal.

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