

QTL Analysis for Drought Resistance in Wheat Using Doubled Haploid Lines

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ABSTRACT

A segregating population of 96 doubled haploid lines derived from a cross between Chines spring (CS) and SQ1 were evaluated in a drought stress (limited irrigation) and normal irrigated conditions in a green house to investigate QTLs of drought tolerance and its associated traits. Four agronomic traits *viz* peduncle length, grain yield per ear, number of kernel per spike and 1000 kernel weight were measured and five drought tolerance indicators including: stress susceptibility index (SSI), mean productivity index (MP), tolerance index (TOL) geometric mean productivity (GMP) and stress tolerance index (STI) were calculated. Using composite interval mapping (CIM) analysis 6, 1, 3 and 5 QTLs were detected for 1000 grain weight, grain yield, peduncle length, number of grain per ear, respectively. The QTL effects ranged from 13 to 34%. Marker alleles from SQ1 were associated with a negative effect for the majority of QTLs in studied traits. For SSI three QTLs on chromosomes 7A, 4B and 6B, for MP two QTLs on chromosomes 5A and 5B and for each STI, GMP and TOL only one QTL were detected. The QTL effects drought tolerance indices ranged from 13 to 36%. The identification of genomic regions associated with agronomic traits and drought tolerance indicators under drought stress will be useful for marker-based approaches to improve these characters and its stability for farmers in drought environments.

Key Words: QTL-analysis; Drought tolerance; Linkage map; Doubled haploid; Wheat

INTRODUCTION

Drought is the main environmental constraint, which occurs in many parts of the world every year, often having devastating effects on crop productivity. Hence, improved tolerance to drought has been a goal in crop improvement programs since the dawn of agriculture (Ludlow & Muchow, 1990). Drought tolerance is not a simple response, but is mostly conditioned by many of components responses, which interact and may different for crops, in relation to types, intensity and duration of water deficit. Moreover, most agronomical characters inherit differently in normal and stress conditions and are known to be affected by environmental factors. Therefore, selection based on the phenotype would be difficult for such traits (Hittalmani *et al.*, 2003).

Advent and development of molecular markers in quantitative genetics greatly facilitates the study of complex quantitatively inherited traits by the construction of high density genome linkage maps for crops such as wheat (Xiao *et al.*, 1996). This implies that the use of molecular markers and interval mapping is a powerful approach, which permits the identification and genetic mapping of loci controlling complex traits like grain yield and its contributing traits having great importance in plant breeding. The knowledge of the number and effects of quantitative trait loci (QTLs) can help breeders to understand the genetic control of these traits and to design more efficient selection strategies for improvement (Broman *et al.*, 1999).

The mapping of QTLs has been reported by several workers (Hyne *et al.*, 1994; Campbel *et al.*, 1999; Shah *et al.*, 1999; Borner *et al.*, 2002; Sourdille *et al.*, 2003; Marza *et al.*, 2006; Su *et al.*, 2006), but reports on the identification of QTL in greenhouse condition are scarce. Moreover, we could not find any report for QTLs controlling drought tolerance indices. The objective of this study was to investigate QTLs controlling agronomic traits and drought tolerance indices in a doubled haploid population derived from the cross between two cultivars of wheat, Chines spring' and 'SQ1'.

MATERIALS AND METHODS

Ninety-six doubled haploid (DH) line from the wheat 'Chines spring' (CS) and 'SQ1' cross population were used in this study. The inbred lines along with map of 338 markers (AFLP, RFLP, SSR & protein) were taken from Jon Inn's Institute, UK. The DH population and parental lines were raised under both normal and stress conditions in a greenhouse using completely randomized design (CRD). Five plants were grown each pots. Normal moisture (control) pots were irrigated two times, while limited irrigation pots were irrigated one time in a week.

The agronomic traits were evaluated based on following description: peduncle length from last node to the base of spike, grain yield per ear, number of kernel per spike and 1000 kernel weight. Drought tolerance indices including stress susceptibility index (SSI) (Fischer & Maurer, 1978),

mean productivity (MP), tolerance index (TOL) (Rosielle & Hamblin, 1981), geometric mean productivity (GMP), stress tolerance index (STI) (Fernandez, 1992) were calculated as following formulas:

$$SSI = \frac{[1 - (Y_S/Y_P)]}{[1 - (\bar{Y}_S/\bar{Y}_P)]}$$

$$MP = [Y_P + Y_S]/2$$

$$TOL = [Y_P - Y_S]$$

$$\sqrt{(Y_P * Y_S)}$$

$$STI = [Y_P * Y_S] / [\bar{Y}_P * \bar{Y}_S]$$

In the above relationships, Y_S , Y_P , \bar{Y}_S and \bar{Y}_P are yield amount in stress and non-stress conditions for each genotype, mean yield in stress and non-stress conditions for all of genotypes, respectively. Analysis of variance (ANOVA) was performed for data recorded under both condition, respectively. Pair-wise Pearson correlations were computed between traits and indices using SPSS software. A genetic map consisting of 338 markers was constructed using the computer package MAPMAKER/EXP 3.0 (Lander *et al.*, 1989). QTL mapping was carried out for each of the environment by composite interval mapping (CIM) at 2 cm intervals using Windows QTL Cartographer version 2.0 (Wang *et al.*, 2004). A logarithm of odds (LOD) threshold of 2.0 was used to declare the presence of putative QTL in a given genomic region. The percentages of phenotypic variation explained by a QTL (R^2) for each trait and the additive effect of an allelic substitution at each QTL were obtained.

RESULTS AND DISCUSSION

The result of ANOVA and narrow sense heritabilities (h^2) for 4 traits under both conditions were presented in Table I. The highest heritability was obtained for peduncle length in both the environments, indicating that this was controlled by additive effects. The lowest h^2 was obtained for grain yield in both environments. In general the obtained h^2 were greater under normal than stress environment, indicating differential response of genotypes in both the environments.

Comparison between means of parents different traits (Table II) showed that in normal conditions both parents CS and SQ1, were significantly different only for 1000 kernel weight and peduncle length, while in limited irrigation condition, peduncle length and number of kernel per spike different significantly between parents. Under both conditions however peduncle length was greater in CS than SQ1. In previous study, Quarrie *et al.* (1994) reported that

Table I. Analysis of variance and narrow sense heritability in normal and limited irrigation for different traits

Trait	Normal irrigation			Limited irrigation		
	MST	MSE	h^2	MST	MSE	h^2
Grain yield	**1.098	0.399	0.17	**0.064	0.036	0.13
Number grain per ear	***52.00	8.960	0.33	**24.49	10.200	0.18
1000kernel weight	***30.00	6.342	0.30	**35.63	13.800	0.20
Peduncle length	***62.00	7.631	0.42	**36.72	13.700	0.31

Significant at $\alpha = 0.01$ and * $\alpha = .001$ respectively

the amount of ABA in SQ1 was more than CS. And alleles inherited from SQ1 increased ABA. Therefore, SQ1 could be more resistant to drought condition, although in this study both parents did not show marked different for yield. It seemed that in both parents there were some alleles for drought resistance. Significant MSTs for all traits (Table I) showed a transgressive segregation for these traits in this DH population, which may explain existence of some QTLs for drought resistance in both parents.

Peduncle length in normal environment was positively correlated with peduncle length (0.47) under stress and with number of kernel per spike (0.45) in normal environment (Table III). Moreover the correlation between peduncle length and number of kernel per spike was positive (0.28*). Y_S was positively correlated with GMP ($r = 0.81^{**}$), STI ($r = 0.80^{**}$), Y_P ($r = 0.678^{**}$) and SSI ($r = 0.60^{**}$). GMP was rather more positively correlated with STI ($r = 0.98^{**}$). It is concluded that GMP, SSI and STI can be used as drought tolerance indices.

Knowledge regarding the number, genomic location and effect of quantitative trait loci (QTL) would facilitate marker-assisted selection and the development of cultivars with desirable characteristics (Marza *et al.*, 2006). The QTL for various traits along with marker interval of peak LOD, number of locations in which QTL appeared, additive effect and direction of the QTL, percentage variation explained and the peak LOD in (Table IV & V). In total, 20 QTLs were detected for 4 traits and 5 indices. The congruence of the QTL loci on the chromosome for various traits may be due to either linkage or pleiotropism. This signifies the plural selection efficiency by selecting markers closely associated with these traits (Hittalmani *et al.*, 2003).

In this research, 4 QTLs were identified for 1000 grain weight at stress condition. One of the QTL, which identified in 2A located in interval *M77P64 g - tell. 4* and comprises 13% of phenotypic variation. Zanetti *et al.* (2001) also found 8 QTLs for 1000 grain weight and 10 for 100 grain weight in 11 chromosomes of 226 RILs that included 2A chromosome. Two QTLs were also identified on 4A, which explained 12.5 and 17.9% of phenotypic variation under normal condition. The forth QTL was on 7A and explained 27% of variation. Campbel *et al.* (1999) also reported QTLs for 1000 grain weight on 7A. Of the four QTLs in normal condition, three had a negative influence. Two QTLs were

Table II. Comparison between means of parents in normal and limited irrigation for different traits

Trait	Normal irrigation				Limited irrigation			
	CS	SQ	CS-SQ	5 %LSD	CS	SQ	CS-SQ	5 %LSD
Grain yield	1.21	1.23	ns -0.02	1.01	0.31	0.17	0.14	0.34
Number grain per ear	11.28	14.13	ns 2.85	4.8	7.93	13.2	*-5.27	5.13
1000 kernel weight	15.7	11.2	*4.5	4.05	14.53	9.11	5.41	5.9
Peduncle length	27.5	20	*7.5	4.44	21	14.5	*6.5	5.96

*, ns, significant at ($\alpha=0.05$) and non significant**Table III. Correlation between traits and drought resistance indices**

	PDL-N	PDL-S	NG/SP-N	NG/SP-S	TKW-N	TKW-S	Y-S	GMP	STI	TOL	MP
PDL-S	**0.47										
NG/SP-N	**0.45	0.08									
NG/SP-S	0.22	*0.28	0.12								
TKW-N	0.10	0.00	-0.14	0.00							
TKW-S	-0.10	-0.07	-0.21	0.20	0.03						
Y-S	-0.03	0.18	0.17	*0.27	0.05	0.24					
GMP	0.16	0.21	**0.48	*0.40	0.07	0.10	**0.8				
STI	0.06	0.16	*0.37	*0.34	0.08	0.06	**0.8	*0.98			
TOL	0.17	0.15	0.12	**0.50	-0.08	-0.02	0.10	0.10	0.10		
MP	0.06	0.05	0.05	-0.07	0.00	-0.22	0.07	0.04	-0.02	**0.8	
SSI	0.13	0.29	0.05	-0.02	*0.32	-0.09	**0.6	0.15	0.07	0.00	0.04

*, ** significant at ($\alpha=0.05$) and ($\alpha=0.01$) respectively.

Peduncle length in normal irrigation (PDL-N), peduncle length in limited irrigation (PDL-S), number grain per spike in normal irrigation (NG/SP-N), number grain per spike, limited irrigation (NG/SP-S), 1000kernel weight in normal irrigation (TKW-N), 1000 kernel weight in limited irrigation (TKW-S), yield in limited irrigation (Y-S), geometrical mean productivity (GMP), stress tolerance index (STI), tolerance (TOL), mean productivity (MP) Stress susceptibility index (SSI)

Table IV. Position, additive effects and explained phenotypic variance of the QTLs

Trait	Irrigation	Chromosome	(Interval)	QTL's distance from the left marker	LOD	R ²	Additive effect ($\overline{BB} - \overline{AA}$)/2
1000kernel weight	normal	2A	M77P64g-tell.4(22.2)	22	2.16	0.130	-1.42
		4A	M60P64g-Psr490.2(4)	2	2.11	0.125	+1.45
		4A	Psr392.1-Psr392.3(25)	18	2.7	0.179	-2.4
		7A	P78m63f-P78m69a(14)	4	3.6	0.270	-2.6
	limited	7A	Psr558-Psr490.3(13)	8	3.6	0.210	-6.1
		5A	Wg832e-wg232c(33)	24	4.2	0.340	-9
Grain yield	normal	1B	Psr162-M17P65a(36.4)	0	3.5	0.220	-0.35
Peduncle length	normal	1B	M78p65e-mwg77(5.8)	2	4.2	0.230	3.15
		4B	Rht ₁ -Psp3030b(24.6)	6	2.3	0.188	-2.20
	limited	3B	M62P64g-60P64k(39.1)	10	2.68	0.220	-2.05
Number grain per ear	normal	4A	M17P65e-mwg58(4.6)	0	2.33	0.200	+1.86
		1B	M17P65e-67P77e(85)	50	2.6	0.320	-3.45
		5B	Teil 3-M51P65d(13)	11	2.3	0.140	+3.6
		5B	M78m49c-Psr725(21)	0	2.5	0.150	-3.5
	limited	7A	M71P77l-M62P64n(8)	0	3.1	0.230	-2.8

 \overline{BB} and \overline{AA} are mean of individuals similar to SQ and CS, respectively

also found on 7A and 5A under stress condition; the alleles from SQ1 have reduced 1000 grain weight in both.

For grain yield one QTL was identified on 1B under normal condition, which formed 22% of phenotypic variation. This QTL exactly located on *Psr162* and allele inherited from SQ1 parent decreased (-0.35) grain yield. As the parents showed no difference for this trait, so the identified QTL is from transgressive segregation of grain yield in double haploid population. No QTL was identified in stress condition.

Two QTLs on 1B in normal condition, which explained 23 and 18% of phenotypic variations were found for peduncle length. One of this QTL allele inherited from

SQ1 parent increased peduncle length (3.15), but another decreased (-0.2.2) it. Another QTL on 4B located near *Rht1* decreased plant height of SQ1. As peduncle length is a part of plant height, therefore probably this QTL is the effect of *Rht1* on this attribute (Sourdille *et al.*, 2003). One more QTL was identified on 3B in stress condition in interval *M62P64 g-M60P64 k*, which comprised 22% of phenotypic variation.

A total of 4 QTLs were found for number of kernel per spike in normal condition 2 of which were located on 4A and 1B, explained 20 and 32% of phenotypic variation, respectively. The alleles inherited from SQ1 parent on 4A increased (1.18), but on 1B decreased (-3.45) number of

Table V. Position, additive effects and explained phenotypic variance of the QTLs detected on different chromosomes

index	Chromosome	(interval)	QTL's distance from the left marker	LOD	R ²	Additive effect ($\overline{BB} - \overline{AA}$)/2
MP	5A	Psr967.3 – Psr575.2(9)	4	2.8	0.194	+1.13
SSI	7A	M83P65d – M21P76n(12)	10	2.9	0.210	-0.33
STI	1B	Glia-M85P65e(32)	7.3	3	0.170	+0.04
GMP	3B	M86P65d-Psr120.5 (26)	25	2.05	0.130	+0.09
SSI	4B	M62P64d-Rht ₁ (23)	10	2.34	0.150	-0.28
TOL	5B	Psr725-Psr3037(25)	0	3.2	0.180	-2.25
MP	5B	(25) Psr725-Psp3037	0	2.5	0.160	-1.4
SSI	5B	M51P65-Psr136(12)	0	3.1	0.360	+0.55

\overline{BB} and \overline{AA} are means of individuals similar to SQ and CS, respectively

kernel per spike. Two other QTLs located on 5B with 5 cm interval and explained 14 and 15% of phenotypic variation. The alleles inherited from SQ1 parent for one of these QTLs increased and another that exactly located on M78 m 49 c decreased number of kernel per spike.

For MP two QTLs on 5A and 5B were identified, which explained 19 and 16% of phenotypic variation. The last QTL located exactly on *Psr725* and allele inherited from SQ1 parent decreased (-1.4) MP. For SSI three QTLs on 7A, 4B and 6B were identified, which explained 21 - 36% of phenotypic variation, which the major one on 6B located exactly on *M51P65*.

Only one QTL was found for each of the other indices including STL, GMP and TOL on 1B, 3B and 5B, chromosomes, respectively. All these QTLs showed that the allele inherited from SQ1 parent decreased these indices. QTL analysis carried out by Quarrie *et al.* (1994) for ABA, using the same double haploid material, showed more ABA in SQ1 than CS under water stress. They found QTLs on *Psr426* and *Psr575.2* markers were located on 5A. While the QTL found in 5A of CS located on *Psr326* marker and increased ABA. In this research one QTL was identified on 5A located 5 cm from *Psr575.2*, which explained 19.4% of phenotypic variation and the allele inherited from SQ1 increased MP.

In conclusion the results of this research showed that there are some drought tolerant or resistant genes in both parents (SQ1 & CS), which can be transferred to susceptible genotypes using MAS. A variety of factors may affect the outcome of a QTL analysis; for instance the selection of the cross, population structure and size, number of measured replications and environments number and density of markers (Pillen *et al.*, 2003). Magnitude of QTL effect and accurate chromosome map location are also important for verifying identified QTLs.

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