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Mapping quantitative trait loci of morphological attributes using drought mapping population 5 (Opata × SH349) under drought stress

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Key words: Drought, doubled haploid mapping population, awn color, QTL.

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Abstract

Background, Synthetic hexaploid wheat has drought tolerant characteristics and Opata is high yielding wheat cultivar. In order to investigate the genetic basis of this tolerance, a segregating population composed of 84 double haploid mapping population 5 (DR.MP. 5) derived from the cross Opata × SH349, was evaluated under drought stress in wheat in field and tunnel environment. During the present research, the morphological study such as awn color, prostrate vs erect habit and pubescent vs glabrous habit was done for the two consecutive years. Results, The QTLs found during the recent study were all novel except the four QTLs located on 2D chromosome. The QTLs found by Multiple QTL Mapping and Interval Mapping were major QTLs. The LOD value varies from 11.63 to 21.8 by Multiple QTL Mapping and from 10.72 to 28.66 by interval mapping. Conclusions, No QTL was found for prostrate vs erect habit and pubescent vs glabrous habit. All QTLs were found for awn color. Novel QTLs for awn color were identified during the present studies, which are very important from practical point of view. These QTLs can be further utilized in Marker assisted selection or gene cloning.

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Introduction

Wheat (*Triticum aestivum* L.) is the first important cereal crop for the majority of world's populations (Tunio, 2006). It is the utmost vital staple food of Pakistan (Malik, 2006). Owing to land limitations, increase in wheat production must come from higher yield by increasing efforts in plant breeding along with biotechnological tools and expanded genetic diversity (Rajaram, 2001). According to Pakistan Bureau Statistics, 2012, wheat (*Triticum aestivum* L.) adds 2.6 % to GDP and 12.5 % to the value added in agriculture productivity. The size of wheat crop was 23.3, 25 and 23.5 million tons in 2010, 2011 and 2012 respectively (GOP, 2012). Synthetic wheats will be resilient enough to feed billions of people well in the next century. It has been manipulating accessions of the primary gene pools diploids (2n=2x=14, DD) wheat relative for the past few years. Synthetic wheat has increased levels of resistance/tolerance for many biotic and abiotic traits (Schmidt *et al.,*2005; Reynolds *et al.*, 2007). The genes introgressed from synthetic hexaploid wheat were found to improve quality and agronomic traits of spring wheat (del Blanco *et al.,* 2000, 2001).

SSR markers are abundant, high polymorphic, randomly and throughout distributed in most eukaryotic genomes, PCR-based and suitable for genome mapping application (Sun *et al.,* 1998; Dreisigacker *et al.,* 2004). The development of linkage maps (containing of DNA markers) and QTL analysis is done by correlating of genotypic data with phenotypic data (McCouch and Doerge, 1995). A mapping population can be divided into different groups by using markers based on specific marker function (Young, 1996). Molecular markers have the ability to characterize and discriminate different genotypes (Ijaz and Khan, 2009). Trait of interest can be measured by using SSR markers, which reduce the time and cost of quantitative trait loci (QTL) analysis.
It is the need of time to develop the varieties, which have drought tolerant potential to increase area under cultivation and yield of wheat crop. The present project is designed for QTL mapping in wheat mapping population through development of linkage map of the doubled haploid population and elucidation of phenological responses of wheat mapping population to drought stress.

Materials and methods

The present research was conducted in PMAS-AAUR (Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi) and NARC (National Agriculture Research Centre) Islamabad.

Plant Material

Drought mapping population 5 (DR. MP.5) was used in this research which was a double haploid mapping population. A double haploid mapping population (DR. MP.5) was constructed from the F1 derived from the cross between a drought susceptible bread wheat CIMMYT variety, Opata M-85 / SH (349), and a drought tolerant synthetic hexaploid line (SH349). Total eighty four lines and two parents were used for morphological, physiological and molecular evaluation.

Experimental Design

There were two treatments that is control (Fully irrigated) and stress (Under a rain-out plot shelter). For control the plant material was planted in field and there was a single row of each genotype in three replicates. For stress application, the plant material was planted in tunnel, where a plastic sheet was used to withhold water. Stress administration was done by withholding water at pre-anthesis stage until harvesting and stress was monitored by calculating soil moisture contents. There were 25% soil moisture contents at control and 13% under stress conditions. TDR soil moisture determination meter was used to determine soil moisture at different time interval to monitor the stress level.

Phenological Attributes

Eighty four DH lines and two parental lines of DR. MP. 5 were grown in the research field and tunnel of National Agriculture Research Centre, Islamabad over the regular wheat crop cycles during November 2010. Data was taken for three plants from each line and then arithmetic mean was taken. Phenological data was recorded for the following characters.

Awn Color

Data was recorded for awn color when plants fully turned golden brown at maturity from field and tunnel. The parameters to be used were white, light brown, dark brown or black. The awn color grading was done as white (1), light brown (2), dark brown or black (3) respectively. Opata belonged to first class i.e. white and SH349 belonged to second class i.e. dark brown and combination of these two colors resulted in variation in color which spread in the progeny which was shown in figure 1

Glabrous vs Pubescent Habit

Data for pubescent vs glabrous habit was taken using hand lens by observing hairs on the base of spikes i.e. the base of the peduncle and also on lemma and palea plus on awns when the plant were close to maturity. The pubescence grading was done as glabrous (0) or pubescent (1). Opata belonged to first class i.e. glabrous and SH349 belonged to second class i.e. pubescent and these habits spread in the progeny which was shown in figure 1.

Erect vs Prostrate Habit

Prostrate vs erect habit was recorded at earlier stage of vegetative growth from field and tunnel. The prostrate and erect habit grading was done as erect (0) or prostrate (1). Opata belonged to first class i.e. erect and SH349 belonged to second class i.e. prostrate and these habits spread in the progeny which was shown in figure 1.

Molecular Diagnostics

A linkage map of Opata × SH349 was used which has been published (Fatima *et al.*, 2014). The SSR linkage map data and morphological data recorded were subjected to analyze for QTLs in computer program MapQTL 5 (Van Ooijen, 2004). Corel draw 4 and Map chart were used to design the QTLs on chromosomes. Linkage (LOD) between markers was calculated using odds ratios (i.e. the ratio of linkage versus no linkage). LOD values of > 3 are typically used to construct linkage maps. QTL can be categorized in major and minor QTL. The QTLs with greater than 3 LOD score is considered as major QTL and the QTL which has LOD score less than 3 is categorized as minor QTL. Permutation test was performed to know the LOD value. First Interval Mapping (IM) was applied to detect QTLs and when major QTLs were obtained by IM then Multiple QTL Mapping (MQM) was used to get more refine results (Table 5 and Table 6).

Results

Frequency Distribution of Phenological Attributes

The distribution of phenological attributes was shown in Figure 1 and Table 1.

For awn color, there are three classes, white (1), light brown (2), dark brown or black (3). Two parents belong to different classes *i.e* Opata belongs to first class (light brown) while SH349 is the second parent and belongs to second class possessing a white color. As DR.MP.5 is a double haploid mapping population so these two parental awn colors segregate in the progeny and as a result of transgressive segregation, new combinations of dark brown or black (3) also observed in the population. The values on x-axis i.e 1, 2 and 3 were actual/meaningful values for traits under consideration. In case of awn color-control, the third category *i.e* dark brown or black most frequently distributed across the mapping population as compared to under stress conditions where first category i.e white most frequently distributed. In case of pubescent, there were only two categories, pubescent (1) versus glabrous (0) and two parents belong to different categories. The parents also behaved differentially as opata is high yielding cultivar but drought sensitive and showed glabrous habit. The second parent is SH349, which showed drought tolerant characteristics in most of cases and possessed a pubescent character. These characters also segregated in mapping population. In field conditions the glabrous character observed most frequently distributed as compared to stress condition where most of lines possessed pubescent character as this is the character of drought stress environment. Pubescent is the adaptive character of drought tolerant lines, which enables them to survive under harsh conditions. It reduces the rate of transpiration.

The last trait, for which observations were recorded, was prostrate versus erect habit, erect (0) or prostrate (1). There were only two classes and here parents belonged to different classes. Here parental lines also behaved differentially and belonged to different classes that Opata which is first parent showed erect habit while SH349 showed a prostrate habit which is an adaptation in drought tolerant lines. In case of erect vs prostrate habit, the prostrate habit observed most frequently distributed under stress conditions as compared to control, in field and tunnel experiment. Erect habit is the feature of Opata, drought sensitive parent, while prostrate habit is the characteristic of drought tolerant parent that is SH349. The drought tolerant characteristics in SH349 was contributed by durum which donated A and B genome while D genome was denoted by *Aegilops tauschii.* The parental position in population also clears from Table 2 in which their exact positions are mentioned and range also available.

Table 1. Analysis of frequencies of categorically traits.

Trait	Category	Frequency	% Frequency
Awn Color			
Control	White (1)	35	41.667
	Light brown(2)	4	4.762
	Dark brown (3)	45	53.571
Stress	White (1)	52	61.905
	Light brown(2)	14	16.667
	Dark brown (3)	18	21.429
Pubescence			
Control	Negative (0)	49	58.333
	Positive (1)	35	41.667
Stress	Negative (0)	38	45.238
	Positive (1)	46	54.762
Erect vs Prostrate Habit			
control	Erect (0)	38	45.238
	Prostrate (1)	46	54.762
Stress	Erect (0)	30	35.714
	Prostrate (1)	54	64.286

Table 2. Basic statistics for each yield component trait, (Field and tunnel experiment) from parents and DHs between individual for control and drought treatments.

Trait	Mean	Minimum	Maximum	Range	Variance	Std.Dev.	Coef.Var.	Skewness	Kurtosis	Opata	SH349
AC-C	2.105	1.000	3.000	2.000	0.942	0.970	46.112	-0.214	-1.934	1	2
AC-S	1.593	1.000	3.000	2.000	0.668	0.817	51.295	0.885	-0.914	1	2
Pub-C	0.419	0.000	1.000	1.000	0.246	0.496	118.542	0.336	-1.933	0	1
Pub-S	0.547	0.000	1.000	1.000	0.251	0.501	91.627	-0.190	-2.011	0	1
Pros-C	0.547	0.000	1.000	1.000	0.251	0.501	91.627	-0.190	-2.011	0	1
Pros-S	0.640	0.000	1.000	1.000	0.233	0.483	75.516	-0.592	-1.690	0	1

AC-C- Awn Color-Control,

AC-S- Awn Color-Stress,

Pub-C- Glabrous vs Pubescent -Control,

Pub-S- Glabrous vs Pubescent- Stress,

Pros-C- Erect vs Prostrate Habit -Control,

Pros-S- Erect vs Prostrate Habit- Stress.

Correlation among Phenological Attributes

The correlation was shown in Table 3.

Significant correlation was found between different traits under control and stress conditions. In field and tunnel experiment, significant and positive correlation was found in awn color under control and stress conditions, having value r = 0.6181. Significant and positive correlation was found between pubescent vs glabrous under control and stress conditions, having value r = 0.5362. Significant and positive correlation was found between prostrate vs erect habit under control and stress conditions, having value r = 0.3377.

Table 3. Correlation of phenological attributes under Control and Stress Conditions.

Trait	AC-C	AC-S	Pub-C	Pub-S	Pros-C	Pros-S
AC-C	1.0000					
	p= ---					
AC-S	.6181	1.0000				
	p=.000	p= ---				
Pub-C	.1278	-.0972	1.0000			
	p=.241	p=.373	p= ---			
Pub-S	.0504	-.0538	.5362	1.0000		
	p=.645	p=.623	p=.000	p= ---		
Pros-C	.0988	.1187	.2048	.1555	1.0000	
	p=.365	p=.276	p=.059	p=.153	p= ---	
Pros-S	-.0441	.0413	.0479	.1431	.3377	1.0000
	p=.687	p=.706	p=.661	p=.189	p=.001	p= ---

AC-C- Awn Color-Control,
AC-S- Awn Color-Stress,
Pub-C- Glabrous vs Pubescent -Control,
Pub-S- Glabrous vs Pubescent- Stress,
Pros-C- Erect vs Prostrate Habit -Control,
Pros-S- Erect vs Prostrate Habit- Stress.

Table 4. Density and Length of Genetic Map of OPATA x SH349.

Chromosome	SSR	cM	cM/Marker
1A	2	13.9	6.95
2A	4	35.6	8.90
4A	6	85.9	14.32
5A1	4	33.9	8.48
5A2	3	55.2	18.40
6A	5	96.8	19.36
7A1	4	30.8	7.70
7A2	2	15	7.50
Total Genome A	30	367.1	12.24
1B1	8	60.1	7.51
1B2	2	1.2	0.60
2B	2	15.1	7.55
3B	2	16.5	8.25
5B	4	53.8	13.45
7B	3	30.5	10.17
Total Genome B	21	177.2	8.44
2D	5	33.7	6.74
7D	5	87.3	17.46
Total Genome D	10	121	12.1
Total	61	665.3	10.91

Linkage Map

The map was taken from Fatima *et al.,* 2014. A linkage map of Opata × SH349 was constructed by using fluorescent labeled SSRs. 174 total SSRs were used to screen the parental lines to search the polymorphic loci. These SSRs were selected on basis of literature. Seventy nine polymorphic SSR genomic loci were utilized to construct the genetic map and to detect QTLs by using PCR and Capillary Electrophoresis. All loci were verified by chi-square test for goodness of fit to an estimated 1:1 segregation ratio. Sixteen linkage groups were obtained, covering 13 chromosomes. Linkage groups were allocated to chromosomes by relating the marker locations to formerly publish the wheat maps (Roder *et al.,* 1998 and Somers *et al.,* 2004). The primers were allocated on the chromosomes by taking information from GrainGenes 2.0. The length of linkage map was 665.3 cM. Linkage maps for the A genome chromosomes extended from 13.9 cM (1A) to 96.8 cM (6A) and concealed a total of 367.1 cM (55.17 percent of the total map length). The linkage groups in the B and D genomes extended from 1.2 cM (1B2) to 60.1 cM (1B1) and 33.7 cM (2D) to 87.3 cM (7D), respectively. The B genome spanned 177.2 cM (26.63 percent of the total map length) and the D genome spanned 121 cM (18.18 percent of the total map length). The most marker-dense chromosome regions were found on chromosomes 4A, 6A, 1B1, 5B and 7D (Figure 2 and Table 4).

Table 5. QTLs detected by Interval Mapping for Awn Color.

Sr #	Name of QTL	QTL Interval[a]	Peak marker	Chr[b]	Env	LOD	Adtv eft[c]	R^2 (%)[d]
1	*QAC.C.IM.wwc-7D.1*	wmc606d-wmc606b	wmc606d	7D	Field	27.51	0.65	35.4
2	*QAC.C.IM.wwc-7D.2*	wmc606b-wmc606c	wmc606b	7D	Field	27.06	0.75	65.3
3	*QAC.C.IM.wwc-6A.3*	gwm1017a-gwm1017b	gwm1017a	6A	Field	24.43	-0.67	45.2
4	*QAC.C.IM.wwc-6A.4*	gwm169-gwm1089b	gwm169	6A	Field	25.05	-0.65	55.3
5	*QAC.C.IM.wwc-6A.5*	gwm1089b-gwm1089a	gwm1089b	6A	Field	24.36	-0.89	15.3
6	*QAC.C.IM.wwc-2B.6*	wmc630f-wmc630h	wmc630f	2B	Field	26.07	-0.54	65.3
7	*QAC.C.IM.wwc-7A.7*	gwm698a-gwm698b	gwm698a	7A	Field	28.66	0.67	35.3
8	*QAC.C.IM.wwc-4A.8*	wmc718a-wmc718c	wmc718a	4A	Field	22.37	-0.63	55.3
9	*QAC.C.IM.wwc-2D.9*	wmc630d-wmc630e	wmc630d	2D	Field	26.8	-0.50	35.3
10	*QAC.C.IM.wwc-2A.10*	wmc453c-gwm122b	wmc453c	2A	Field	27.8	0.83	25.3
11	*QAC.S.IM.wwc-7A.1*	wmc826c-wmc826b	wmc826c	7A	Tunnel	12.52	-0.55	45.3
12	*QAC.S.IM.wwc-6A.2*	gwm1089b-gwm1089a	gwm1089b	6A	Tunnel	10.72	-0.78	32.1

Table 6. QTLs detected by Multiple QTL Mapping for Awn Color.

Sr #	Name of QTL	QTL Interval[a]	Peak marker	Chr[b]	Envi	LOD	Adtv eft[c]	R^2 (%)[d]
1	*QAC.C.MQ.wwc-1B*	gwm153e-wmc611c	gwm153e	1B	Field	21.8	-0.95	45.3
2	*QAC.S.MQ.wwc-2D*	wmc630d-wmc630e	wmc630d	2D	Tunnel	11.63	-0.78	34.2

a Marker interval where the QTL has been detected.

b Chr Chromosome

c Effects on the examined characters of the alleles from the 'Opata'

d R2 (%) is the quantity of phenotypic variation clarified by the QTL.

QTLs Detected by Interval Mapping (IM)

The QTLs detected by Interval Mapping was shown in Table 5 and figure 3.

Awn color

During field experiment, ten major QTLs for awn color were found on different chromosomes 7D, 7D, 6A, 6A, 6A, 2B, 7A, 4A, 2D and 2A respectively. Two major QTLs were found during the stress condition in tunnel experiment on 7A and 6A chromosome with different LOD values that is 12.52 and 10.72 respectively.

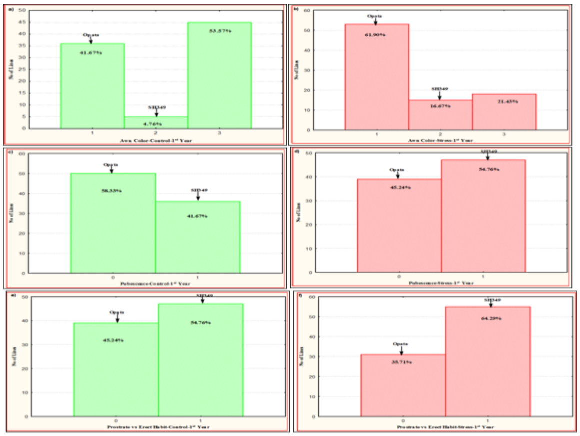


Fig. 1. Histogram of morphological attributes of Awn Color (cm) in field and tunnel (AC-C and AC-S), (c,d) Pubescence in field and tunnel (Pub-C and Pub-S), (e,f) Prostrate and Erect in field and tunnel (Pros-C and Pros-S).

No QTL was found for pubescent vs glabrous and prostrate vs erect habit.

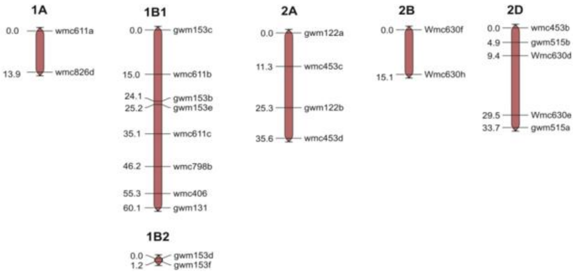


Fig. 2. Genetic Map of OPATA x SH349 for the 84 DHs population.

Continued....

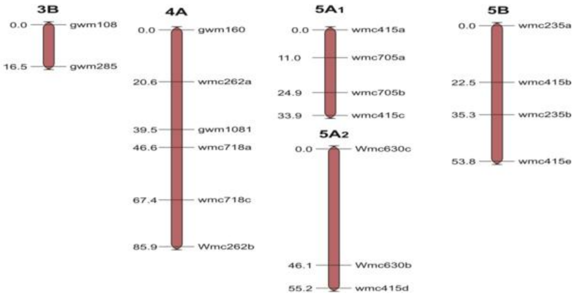


Fig. 2. Genetic Map of OPATA x SH349 for the 84 DHs population.

Continued....

QTLs Detected by Multiple QTL Mapping

The QTLs detected by Multiple QTL Mapping was shown in Table 6 and figure 4.

Continued....

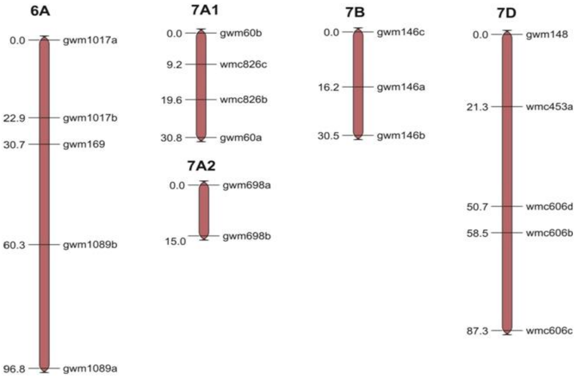
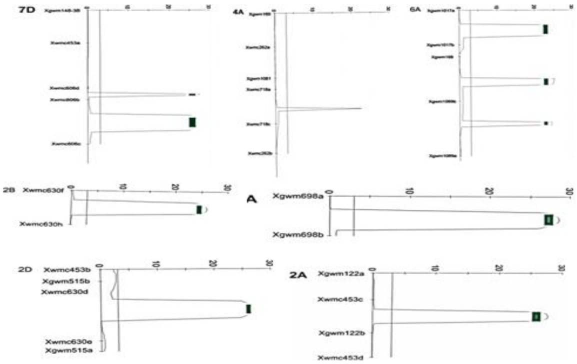


Fig. 2. Genetic Map of OPATA x SH349 for the 84 DHs population

Awn color

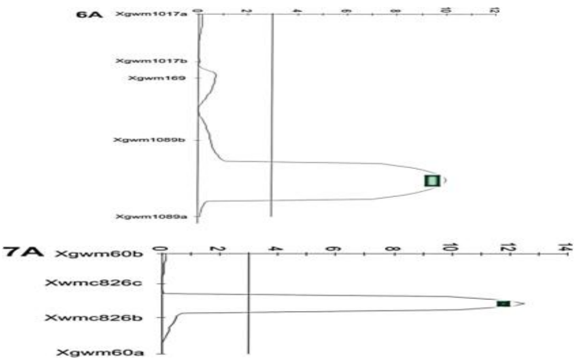
Two major QTLs were found for awn color in field and tunnel experiments. During the experiment, one major QTL was found in field experiment on 1B chromosome having a LOD value 21.80 and second major (11.63) QTL was detected in tunnel under stress condition on 2D chromosome.



Awn Color-Field

Fig. 3. Interval Mapping (IM) for the 84 DHs population.

Continued

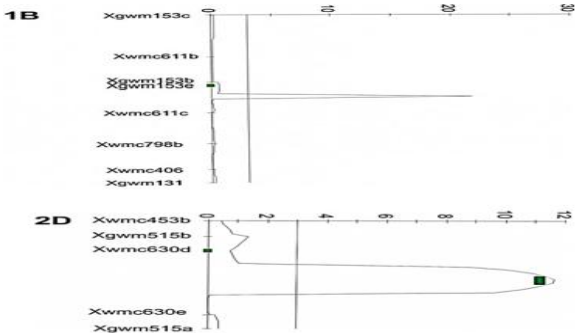


Awn Color-Tunnel

Fig. 3. Interval Mapping (IM) for the 84 DHs population.

Discussion

Drought tolerant characteristics are denoted by SH349, which may be due to D genome donnar, *Aegilops tauschii,* so these lines may contain alleles from SH349, which is drought tolerant parent (del Blanco *et al.,* 2000, 2001; Schmidt *et al.,* 2005). Some lines behaved much better under drought condition as compared to control e.g. some lines behaved much better than others as compared to control, this is because of transgressive segregation. During the study such genomic regions are identified that affect traits under observation. Most of the favorable SH349 alleles were at QTL on the A and B genomes. Form the results of the present research it is concluded that both parental lines i.e. Opata and SH349 have some good alleles for traits of interest, which can be transferred to selected/ susceptible genotype by MAS (Marker Assisted Selection). The results of QTLs were affected by many factors such as population under consideration, number of lines using during the analysis and density of markers. There is some indication the wild species *Ae. tauschii* donated some alleles for better yield. Overall, many QTL were detected across multiple traits, and the wild or synthetic line contributed both positively and negatively to these traits. There is some indication the wild species *Ae. tauschii* donated some alleles because QTL for some traits were associated with the D-genome and with the synthetic line.



Awn Color -Field and Tunnel

Fig. 4. Multiple QTL Mapping (MQM) for the 84 DHs population.

This work showed that the 'Opata' × 'SH349' segregating population was well adapted for QTL detection. Estimation of genetic diversity is a prerequisite for improvement of any species through genetic means. Drought is very complicated trait, which is controlled by many factors at a time such as soil, plant and environment. Asian countries are facing drought so it is difficult to maintain the agriculture in these areas (Huaqi *et al.,* 2002). During the present research, the morphological study such as awn color, prostrate erect habit and waxiness was done for the two consecutive years. No QTLs were found for prostrate vs erect habit and waxiness but major QTLs with very high LOD values and R^2 values were detected during the recent work. Many references are present to support the present work as Marone *et al.,* 2009 found QTLs with high LOD score and R^2 values. A QTL for relative disease severity was found with 77.7 LOD score and 96.7% R^2 values. Similarly QTLs with very high LOD score and R^2 values were reported by Do (2007) in unpublished PhD thesis where many QTLs were found for kernel number per plant, kernel weight per plant, single kernel weight per main spike and single kernel weight per plant with very high LOD values that is 323 and R^2 values that is 100.

According to previous record on grain gene and published data, very little work was done in the present scenario. Few references were found for the work under consideration. Twenty one QTLs were detected during the research by interval mapping under different environmental conditions that is irrigated and stressed. Ten QTLs found in the first year of sowing in irrigated conditions and out of ten, allele for six QTLs were denoted by SH349, synthetic hexaploid wheat which is a drought tolerant parent and the tolerance in synthetic wheat in contributed by durum and *Aegilops tauchii.* Only one supporting reference was found for the QTL, which was located on 2D chromosome by Sourdille *et al.,* 2003 who found the same results for awn color, and found QTLs for awn color on 2D, 4A but he has also found QTL on 6B. 6B chromosome is missing from the present study so no comment can be contributed in this regards. He has found QTLs for awn color and spike length at the same region of the chromosome. During the study awn color QTLs found on different chromosomes. It was difficult to compare the regions of chromosomes occupied by QTLs with previous work as the map was not of high resolution and large gaps were present but references of previous work were present on the same chromosomes for different agronomic traits.

This study provided evidence that the QTL strategy is useful and able to enhance performance of existing cultivars. Further QTL studies will assist in the contribution of positive allelic diversity in the coming time. The identification of numerous complementary promising alleles on chromosomes proposes that restoring wild alleles into domesticated wheat cultivars could result in improved drought resistance. The QTLs found during the recent study were all novel except the four QTLs located on 2D chromosome. As these are novel QTLs so these can be used in future for marker assisted selection and gene cloning.

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