# QTL mapping for physiological maturity in synthetic hexaploid wheat (*Triticum aestivum* L.) under drought stress

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**ABSTRACT**

Wheat is the main staple food and largest grain crop of Pakistan. An extended period of water deficits over months or years is the main cause of drought. Synthetic hexaploid wheat has drought tolerance characteristics and Opata is high yielding wheat cultivar. In order to investigate the genetic basis of the tolerance, a segregating mapping population (DR.MP. 5), composed of 84 double haploid lines derived from the cross Opata × SH349, was evaluated under field and drought stress conditions. The main objective was to identify early and late maturing wheat lines and to find out QTLs under drought stress. A field and tunnel experiment was performed for find out drought tolerant lines. Days to physiological maturity was selected as a crucial stage to find out best lines, as this stage is most vulnerable to drought stress. Ten early maturing lines were identified(84, 83, 7, 73, 43, 70, 22, 16, 6 and 15). Eight QTLs were identified during the present studies which were very important from practical point of view as these QTLs may be used in marker assisted selection (MAS) or gene cloning. Four QTLs were found under control and four under stress. These were all major QTLs. The source of tolerance in this germplasm is attributed to alleles on the A and B genomes of durum parents, or on the *Aegilopstauschii*'s D genome, or is a combination of genes that are pyramided as a result of A, B and D genome hybridizations.

**Keywords:** Drought stress, hexaploid wheat, Days to physiological maturity, QTL

## Introduction

Water stress is considered as the major stress that reduces the yield of crops including wheat in the areas with limitation of water [1]. Drought stress effects agriculture and in Asian countries this problem is very high as agriculture production mainly dependent on water [2]. During the evolution, wheat (*Triticumaestivum*L.), (2n = 6x = 42, AABBDD) is originated by three different monocot plants, having three sets of chromosomes that is A, B and D, each genome contained seven chromosomes. Wheat has very large genome of $16\times10^9$bp per chromosome [3]. Less than ten percent of the genome contains more than eighty five percent of the genes [4].

Wheat consumption is increasing day by day and there is need to enhance the food quantity, as during the next fifty years the demand for the food would be doubled [5]. Therefore, it is essential to improve its yield to meet this requirement rapidly. Mujeeb-Kazi synthesized hexaploid wheats [6]. *Ae.tauschii* cultivars are very important as these are D genome donars and enhance every type of stress tolerance in wheat[7]. 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 [8].

Microsatellite primers have been developed for *Triticum* along with other of *Triticeae* [9]. Molecular markers have the ability to characterize and discriminate different genotypes [10]. QTL mapping methods can be used to split the genetic design underlying difficult traits and to recognize QTL for marker assisted selection programs [11].Agronomic trait of interest can be measured by using SSR markers which lesson the time and cost of quantitative trait loci (QTL) analysis.

The present project is designed for QTL mapping in wheat mapping population throughdevelopment of linkage map of the doubled haploid population and elucidation of phenological responses of wheat mapping population to drought stress.

## Material and Methods

### Research area

The present research was conducted at PirMehr Ali Shah Arid Agriculture University, Rawalpindi and Wheat Wide Crosses and Cytogenetic (WWC), National Agriculture Research Center, (NARC) Islamabad.

### Plant material

Drought mapping population 5 (DR. MP.5) was used in this research which was a double haploid mapping population. Drought Mapping Population 5 is developed from the F1 between Opata M-85 / SH (349). Opata is a drought susceptible bread wheat CIMMYT variety. SH 349 is a synthetic produced by crossing durum wheat with *Aegilopstauschii*. This was done by Dr. Abdul MujeebKazi's program in CIMMYT Mexico in 1992 (Detail below in the pedigree). The pedigree is: Opata // Decoy (DOY) / *Ae. tauschii* (*Squarrosa*) {458} where 458 is the number of the *tauschii* accession. The SH 349 (D genome based drought tolerant synthetic

hexaploid wheat) has the cross number CIGM92.1727. This is the pollen parent and it is drought tolerant. F1 seed from the cross was crossed with Maize; haploids were produced and doubled with colchicine. The population obtained had 113 doubled haploids and numbered from 1 to113. Total eighty four lines and two parents were used for morphological 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. Stress administration was done by withholding water at pre-anthesis stage and stress was monitored by calculating soil moisture contents. There were 25% soil moisture contents at control and 13% under stress conditions.

**Phenological attributes**

DHs and parental lines were evaluated for days to physiological maturity (d).STATISTIX software was used for two factors factorial analysis of variance. Genotypes were highly different at $\alpha$ 0.05 and significant interaction was found between genotypes and treatments at $\alpha$ 0.05. Statistica (Statsoft Inc., Tulsa, OK, USA) was used for frequency distribution.

**Molecular diagnostics**

The map was taken from unpublished PhD thesis[12]. Total 174 fluorescent labeled 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.

**QTLs by using PCR and Capillary Electrophrosis**

Following marker were used: Gatersleben wheat microsatellites, 38+24gwm[13]; Wheat Microsatellite Consortium, 41+9 wmc[14]; Wheat Microsatellite Consortium); Beltsville Agricultural Research Centre, 12 BARC [15]; 1 CFD [16]and Clermont-Ferrand A genome 3 CFA[17], 56 mag[18], 15 swes[19], 17 F, 1 STS-PSR[18], 2 MGBE and 1TaPGAM [18].

The SSR linkage map data and physiological data recorded were subjected to analyze for QTLs in computer program MapQTL 5 [20]. Corel draw 4 and Map chart were used to design the QTLs on chromosomes. Permutation test was performed to know the LOD value.Interval Mapping (IM) was applied to detect QTLs (Fig. 2 and Table 4&5).

**Results And Discussion**

**Phenotypic analysis**

During the field and tunnel experiment, Drought mapping population exhibited wide segregation as compare to the Opata and SH 349 for most the traits studied. It showed that there were recombination events which produce such offspring which were better than their parents [21].

The DHs showed a normal distribution of most yield components under drought stress (Fig. 1). The phenotypic data for days to physiological maturity was collected. Opata, SH349 and the DHs showed wide phenotypic variation for these characters. Opata and SH349 behaved differently in all observations. During the statistical analysis of drought mapping population, two factors factorial analysis of variance was applied to see the interaction between the genotypes and genotypes and treatments. There was a significant interaction was found among all genotypes and treatments and genotypes (Table 2). This may be because of major genotype × environment interaction resulting from the noticeable changes between environments noticed during the research. The parental position also clears from Table 1 in which their exact positions are mentioned and range also available.

Ten early maturing lines were identified (84, 83, 7, 73, 43, 70, 22, 16, 6 and 15) on the basis of days to physiological maturityduring the study which performed best under drought conditions for days to physiological maturity and these lines performed best for other traits also such as grain per spike, chlorophyll contents and soluble sugar. Ten late maturing lines were also presented here for comparison.

These lines can be used as parents in breeding programs to select varieties with improved values of these traits. Drought tolerant characteristics are contributed by SH349, which may be due to its D genome donnar, *Aegilopstauschii*. Therefore, these lines may contain alleles from SH349, which is drought tolerant parent. Some lines behaved much better under drought condition as compared to control; i.e. because of trangressive segregation (Table 3).

**QTLs Detected by Interval Mapping (IM)**

QTLs detected by interval mapping are showed in Table 5 and figure 3.

***Days to physiological maturity***

Eight QTLs were detected by Interval mapping in field and tunnel experiment.Four QTLs were identified under stress condition. These were all major QTLs. Two QTLs which were located on 2D chromosome having LOD values 8.68 and

**Table 1:** Basic statistics for each yield component trait, (Field and tunnel experiment) from parents and DHs between individual for control and drought treatments
DPM (Days to Physiological Maturity, C (Control), S (Stress)

| Trait | Mean | Minimum | Maximum | Range | Variance | Std.Dev. | Coef.Var. | Opata | SH-349 |
|---|---|---|---|---|---|---|---|---|---|
| DPM-C | 146.60 | 135.00 | 160.00 | 25.00 | 45.91 | 6.78 | 4.62 | 144 | 160 |
| DPM-S | 152.11 | 113.00 | 175.00 | 62.00 | 113.95 | 10.67 | 7.02 | 163 | 175 |

**Table 2:** Two Factor Factorial Analysis of Variance for days to physiological maturity (DPM), (Field and tunnel experiment) from parents and DHs between individual for control and drought treatments

| Trait | Source | DF | SS | MS | P |
|---|---|---|---|---|---|
| DPM | LINE | 85 | 57022.40 | 670.85 | 0.000 |
| | TREAT*LINE | 85 | 51678.10 | 607.98 | 0.000 |
| | TREAT | 1 | 344686.00 | 344686.00 | 0.000 |

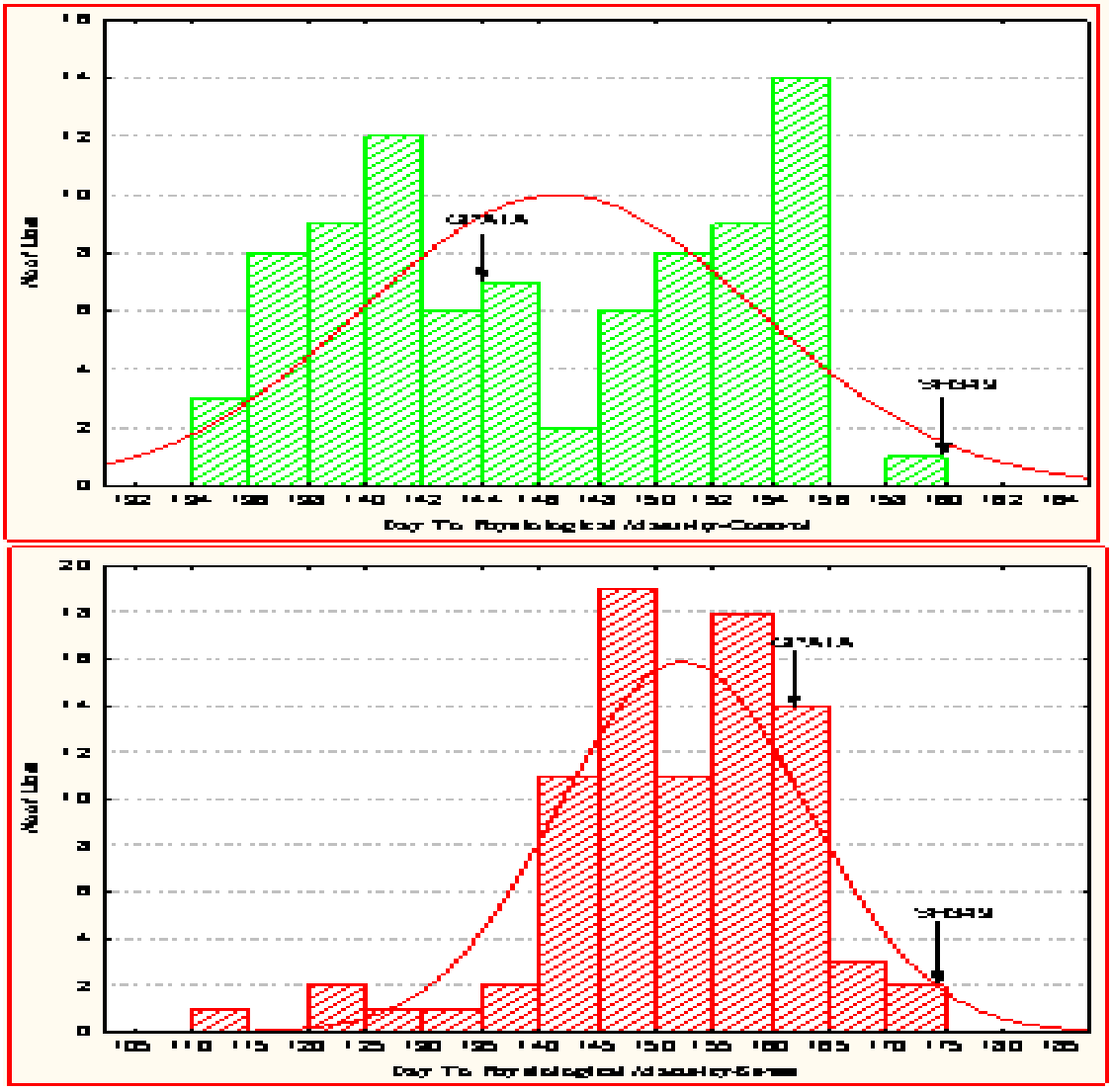

**Figure 1**: Histogram of phenological attributes of days to physiological maturity in field and tunnel (DPM-C and DPM-S)

**Table 3:** Early and Late Maturing Hexaploid Wheat

| Sr # | Line # | Days to Physiological Maturity (Early) | Line # | Days to Physiological Maturity (Late) |
|---|---|---|---|---|
| 1 | 84 | 113 | 17 | 163 |
| 2 | 83 | 121 | 33 | 163 |
| 3 | 7 | 122 | 23 | 164 |
| 4 | 73 | 130 | 32 | 164 |
| 5 | 43 | 132 | 37 | 165 |
| 6 | 70 | 138 | 40 | 165 |
| 7 | 22 | 140 | 38 | 166 |
| 8 | 16 | 141 | 61 | 167 |
| 9 | 6 | 144 | 81 | 170 |
| 10 | 15 | 144 | 77 | 173 |
| Parent 1 | Opata | 163 | | |
| Parent 2 | SH349 | 174 | | |

**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** |

**Table 5:** QTLs detected by Interval Mapping

| Sr # | Name of QTL | QTL Interval[a] | Peak marker | Chr[b] | Trait | Env | LOD | Adtveft[c] | $R^2$ (%)[d] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *QDPM.S.IM.wwc-2D.1* | wmc453b-gwm515b | wmc453b | 2D | DPM | Tunnel | 8.68 | 4.20 | 39.8 |
| 2 | *QDPM.S.IM.wwc-2D.2* | wmc630d-gwm515a | wmc630e | 2D | DPM | Tunnel | 6.86 | -4.15 | 34.6 |
| 3 | *QDPM.S.IM.wwc-7B.3* | wmc606d-wmc606b | wmc606d | 7B | DPM | Tunnel | 4.04 | 6.00 | 81.1 |
| 4 | *QDPM.S.IM.wwc-7A.4* | gwm698a-gwm698b | gwm698a | 7A | DPM | Tunnel | 5.09 | 5.91 | 78.7 |
| 5 | *QDPM.C.IM.wwc-7A.5* | wmc826c-wmc826b | wmc826c | 7A | DPM | Field | 4.81 | -5.9 | 77.9 |
| 6 | *QDPM.C.IM.wwc-2B.6* | wmc630f-wmc630h | wmc630f | 2B | DPM | Field | 4.02 | 5.95 | 79.9 |
| 7 | *QDPM.C.IM.wwc-6A.7* | gwm1017a-gwm1017b | gwm1017a | 6A | DPM | Field | 4.45 | 5.94 | 79.6 |
| 8 | *QDPM.C.IM.wwc-6A.8* | gwm1089b-gwm1089a | gwm1089b | 6A | DPM | Field | 3.85 | -5.87 | 76.6 |

a Marker interval where the QTL has been detected, bChr 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

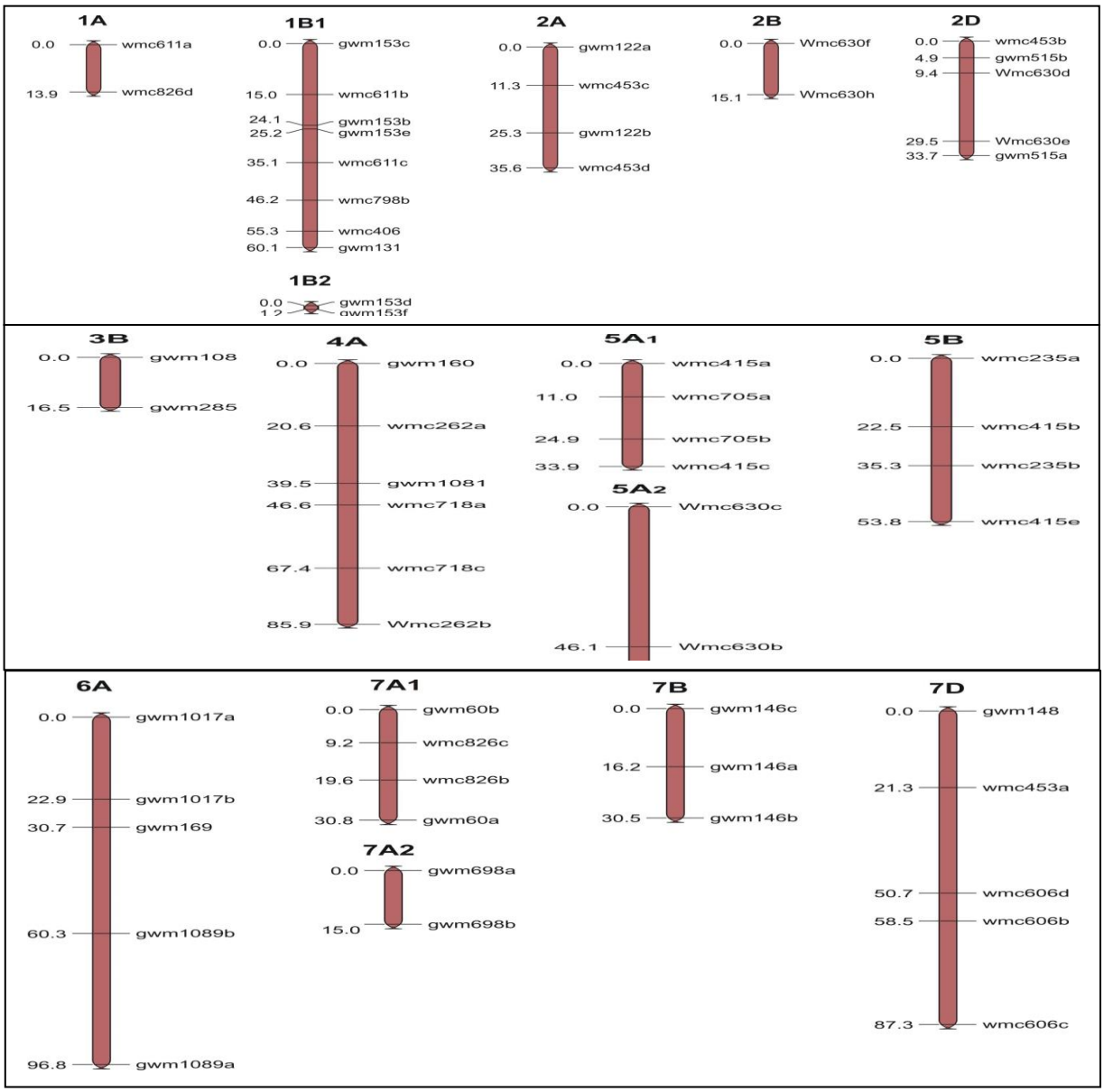


**Figure 2**: Genetic Map of OPATA x SH349 for the 84 DHs population

6.86. The alleles for these QTLs were denoted by Opata and SH349 respectively showing the 2D, 5D and 7D chromosomes **[22]**so two QTLs which are located on 2D were in agreement with this result. Third major QTL was found under stress condition on 7B chromosome having a LOD value 4.04. The allele for this QTL was contributed by SH349 with very high $R^2$ value i.e. 81.1%. QTL for days from heading to maturity at 7B chromosome were detected **[23]**. The fourth major QTL under stress condition was found on 7A chromosome with 5.09 LOD value and 78.7% phenotypic variation. Rests of four QTLs were found under control condition. The first QTL was found on 7A chromosome with 4.81 LOD value and 77.9% phenotypic variation. Allele for this QTL was contributed by SH349. QTL for days to physiological maturity at 7A chromosome were also detected [23]. The next QTL was found on 2B chromosome with LOD value 4.02 and large phenotypic variation 79.9%. QTLs for days from heading to maturity at 1B, 2B, 4A, 4B, 5A, 5B, 7A and 7B chromosomes were also detected **[23]**. Last two QTLs were found on 6A chromosome having LOD values 4.45 and 3.85. The alleles for these QTLs were denoted by Opata and SH349, showing phenotypic variation 79.6% for both. Three QTL for days to physiological maturity in the 2007 and 2008in wet environments were found on chromosome 6A **[24]** so all QTLs, which were found during the recent study, in agreement with previous research.

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. QTLs with high LOD scores and $R^2$ values were found in 2009 **[25]**. 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 **[26]** 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.

**Conclusions and Implications for Wheat Improvement**

As world population is increasing, food demand is also increasing. With the increase in world population, the man exploits natural resources in very bad manner, which is depleting the water availability and urbanization of farmlands. This is the big channel for world of agriculture that how to protect natural resources for increasing food demand. To meet the increasing food phenotypic variation 39.8 and 34.6% respectively. QTLs for DPM were identified on demand, it is necessary to increase the crop productivity. It is demand of time to produce such cultivars, which produce high yield under stress conditions as most part of the world is under stress conditions, which may be biotic or abiotic. As stress is polygenic trait, which is controlled by many genes, so it is necessary to enhance the stress tolerance in plants to compete the challenging environment. Drought is one of the most devastating stresses, which affect the plant productivity. Wheat is the staple food of more than 36 percent of world population so it is the need of time to produce drought tolerant wheat lines by advanced backcrosses of elite wheat cultivars. The identification of numerous complementary promising alleles on homoeologous chromosomes proposes that restoring wild alleles into domesticated wheat cultivars could result in improved drought resistance.

In spite of the fact that a lengthier growing period worth higher yields, early maturity genotypes are more required under our climatic conditions, as their smaller growing season offers the avoidance of drought during pollination and grain filling when the adverse consequences are the extreme. That's why the present project was designed to find out early maturing line to compete with drought and to locate loci controlling this trait under drought stress **[26]**.

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