INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–5–820–823
http://www.ijab.org

QTL Analysis for Phenologic Traits in Doubled Haploid Population of Barley

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ABSTRACT

Drought is one of the main constraints for barley production, especially in dryland conditions. Drought adversely affect the yield by reduction in other yield related traits. Identification of genetic factors of these traits might be helpful in evaluating drought tolerance cultivars. 158 Doubled haploid lines with their drought resistant parents (Wi 2291×Tadmor) evaluated in two environments with 100 mm difference in rainfall. They were planted in alpha-lattice design with 2 replications. In order to easiness in marker assisted selection, number of QTLs ,their genomic location, additive effects and the nearest markers were identified for phenologic traits. Linkage map was constructed using 50 SSR and 93 AFLP markers and included 8 groups for all seven chromosoms. QTL analysis performed using composite interval mapping.Transgressive segregation observed for all three traits, so, positive and negative alleles for most of the traits were found in both parents. For phenologic characteristics, 27 QTLs were determined. Sixteen and 11 QTLs identified in relatively favorable and unfavorable environments, respectively while 4 QTLs were observed in both environments. Total phenologic variance accounted for days to heading (DHE) was about 51% at each region and was 42% at Tel Hadya and 43% at Breda for days to maturity. Maximum explained percentage of individual variation for DHE, Grain filling period (GFP) and DMA was 27, 11 and 11%, respectively. Although the detected QTLs located on all seven chromosomes, most of them were identified on chromosomes 2 and 7. In addition to individual QTLs, some of the QTLs were found which affect all three traits or have close genomic location for some genes cluster that control these traits.

Key Words: QTL; Barley; Stress; Drought; Phenology

INTRODUCTION

Drought is an important abiotic factor affecting yield and yield stability of food cereals in the Mediterranean basin. The water stress acts simultaneously on many traits, leading to a decrease in yield. It causes various physiological and biochemical effects on plants (Bohnert & Jensen, 1996; Tabaeizadeh, 1998). Drought tolerance could, therefore, be studied by identifying the traits which have a significant impact on yield and the genetic factors controlling them. For this purpose, barley could serve as a simple genetic model as it is known to be well adapted to several abiotic stresses, especially to water deficit (Ceccarelli *et al.*, 1998).

The trait having the most dominant effect on fitting a plant to its environment for maximum productivity is the appropriate phenological development (Muchow *et al*., 1994; Passiora, 1994; Richards, 1996). In the Mediterranean type of climate, it is important for the life-span of small grain crops to be completed while water is still available and plants are not dehydrated completly. Ceccarelli *et al.* (1998) showed a significant negative correlation between the grain yield and flowering date under drought stress, but no significant correlation obtained between the two traits under non-stress conditions.

A short grain filling period followed by earlier maturity in the adapted Mediterranean germplasm group under terminal drought conditions genetically associated with high yield. Earliness allow the plants to escape extreme moisture stress, which is often accompanied by heat stress. However there is the risk of frost at flowering in some parts of the Mediterranean regions.

Most of drought traits in crops are quantitative in nature. In barley, the polygenic nature of flowering time has been pointed out in numerous studies (Noli *et al.*, 2000). They are controlled by polygenes, displaying interaction among genes and with drought treatment as well as, heat treatments. These make their genetic inheritance complicated and difficult to be understood. The procedure for finding and locating the quantitative trait loci (QTL) and analyzing their magnitude of genetic effects are called QTL analysis. This bridges the gap between continuous between phenotypic variation and the inherited mechanisms by dissecting genetic variation into individual loci. QTL finding might open up new possibilities for marker based on selection in plant breeding (Zhao, 2002).

The objectives of this work were to identify QTLs, their effects and locations on chromosome for heading date, grain filling period and maturity time in the doubled haploid barley populations WI 2291*Tadmor.

MATERIALS AND METHODS

158 doubled haploid (DH) lines derived from spring barley cross between Wi 2291 and Tadmor were used in this study. They were obtained through anther culture of F1 plants at ICARDA. DH lines and Parents were evaluated at Tel Hadya and Breda in Syria in 2003-04. The experimental design was alpha-lattice with 2 replications.

Three phonologic traits, such as days to heading (DHE), takes as the number of days from emergence to awns appearance in 50% of plants per plot, grain filling period (GFP), takes as number of days from awns appearance to physiological maturity, days to maturity (DMA) takes as number of days from emergence to yellowing of peduncle length in 50% of plants per plot. Transgressive segregation was determined by significant difference between the best doubled haploid and the best parent for a given trait.

Doubled haploid lines were used to construct genetic map. Total genomic DNA was extracted following the procedure described by Saghai and Maroof *et al.* (1984) with minor modifications. The DNA was RNAs treated and quantified using a Spectrophotometer. The quality of extracted DNA was visually checked on 1% agarose gel.

Genetic mapping was carried out using 93 AFLP and 50 SSR markers in 8 groups for all seven chromosomes (Fig. 1) using Mapmaker (lander *et al*., 1987). They were amplified on a PE-9700 system using the published protocols for the respective markers (Zabeau & Vos, 1993; Ramsay *et al.*, 2000). Products which obtained electrophoretically were done electrophoretically with florescence dyes were used to establish linkage between these data set. The QTL analysis was performed using Composite interval mapping while threshold LOD was 2.5 with 2 centimorgan in each step through QTL-Cartographer V. 1.15 (Christopher *et al*., 2001).

RESULTS AND DISCUSSION

Mean and range of days to heading (DHE), grain filling period (GFP), days to maturity (DMA) showed that these traits in parents and DH progeny have enough variation. All three traits showed transgressive segregation at least in one direction (data have not shown).

Days to heading for Wi 2291 was shorter in average 2.2 days in comparison to Tadmor in Tel Hadya and Breda, but GFP for Tadmor was 6.3 and 3.6 shorter in abovementioned regions respectively.Wi 2291 reached to physiological maturityin 4 and 1.4 days later at Tel Hadya and Breda. Therefore, in spit of earliness of flowering in Wi 2291, GFP cause lateness for Wi 2291 that could due to reduction of grain filling rate, cause lateness for Wi 2291.

In this research, 8 QTLs that are located on chromosomes 1, 2, 4, 6 and 7, were identified for DHE at Tel Hadya and 8 QTLs were determined on chromosomes 1, 2, 3, 5 and 7, at Breda. Explained percentage of individual variation was 4 to 14 at Breda and 5 to 27 at Tel Hadya. Four QTLs were common in both environments. Reducing alleles of the strongest common QTL that have close correlation with *BMag813* was on the third chromosome and reduce 0.8 and 1 day of flowering time at Tel Hadya and Breda, respectively (Table I). It is originated from Wi 2291. The most probable QTL that had the closest linkage with *BMS64* on chromosome 7, come from Tadmor. The third probable QTL that originate from Wi 2291 have marked by *p104m95b*. Total phenotypic variance accounted for DHE was about 51% at each region.

Table I. Position, location, effects, significant level (LOD) and explained phenotypic variance (R^2) of detected QTLs

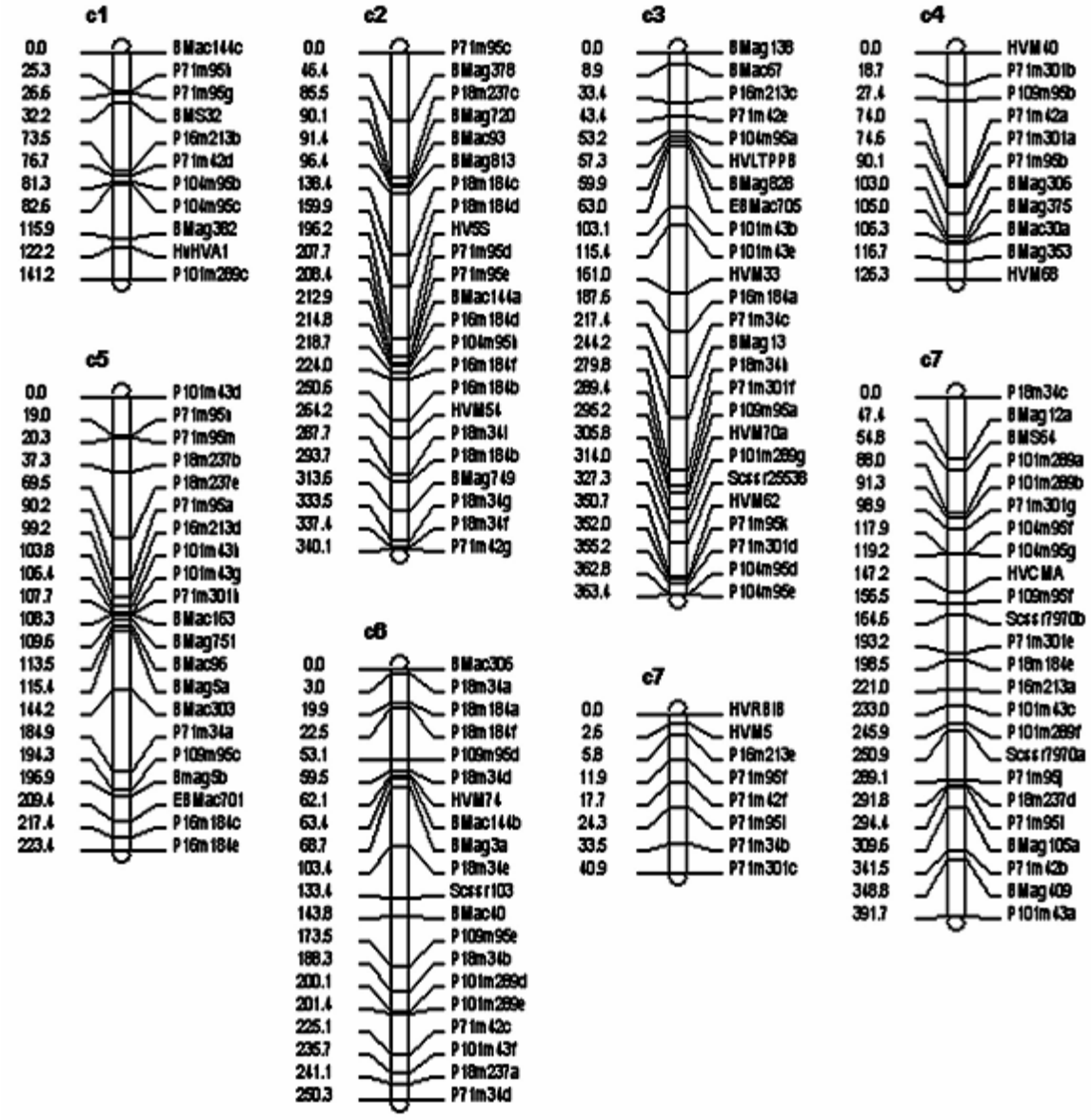
Trait	Environment	Chromosom	LOD	R^2	Effect	Nearest marker
DHE	Tel Hadya	1	5.1	0.13	0.68	*P104m95b*
DHE	Tel Hadya	4	3.3	0.07	-0.55	*P71m42a*
DHE	Tel Hadya	6	2.8	0.07	-0.53	*BMag3*
DHE	Tel Hadya	2	5.6	0.14	0.80	*BMag813*
DHE	Tel Hadya	2	2.9	0.04	-0.54	*P16m184f*
DHE	Tel Hadya	7	5.1	0.14	-0.74	*BMS64*
DHE	Tel Hadya	7	3.5	0.11	-0.75	*P101m289a*
DHE	Tel Hadya	7	5.3	0.14	-0.69	*Scssr7970a*
DHE	Breda	1	5.5	0.05	0.41	*P104m95b*
DHE	Breda	2	2.6	0.10	0.61	*P18m237c*
DHE	Breda	2	3.8	0.13	0.66	*BMag72*
DHE	Breda	2	6	0.27	1.01	*BMag813*
DHE	Breda	7	8.7	0.16	-0.76	*BMS64*
DHE	Breda	5	4.4	0.08	-0.53	*Bmac163*
DHE	Breda	3	2.7	0.04	0.44	*P104m95a*
GFP	Tel Hadya	1	2.5	0.06	0.59	*P104m95c*
GFP	Tel Hadya	7	4.1	0.06	0.58	*P104m95g*
GFP	Tel Hadya	7	6.9	0.11	0.77	*Scssr7970a*
GFP	Tel Hadya	5	4.2	0.06	0.58	*Bmac96*
GFP	Tel Hadya	3	5.3	0.08	0.66	*P101m289g*
GFP	Breda	7	2.7	0.06	0.54	*BMS64*
DMA	Tel Hadya	2	2.7	0.06	0.64	*P18m237c*
DMA	Tel Hadya	7	3.2	0.06	0.64	*BMag12*
DMA	Tel Hadya	5	2.5	0.05	0.58	*P18m237b*
DMA	Breda	6	3.3	0.11	0.43	*P18m184f*
DMA	Breda	7	7.5	2.7	2.1	*BMag12*

Laurie *et al.* (1995), Noli *et al.* (2000) and Teulat *et al.* (2001), Baum *et al.* (2003), reported some QTLs on chromosomes 2, 3, 5 and 7. Thomas *et al.* (1995), Backes *et al.* (1995) and marquez *et al.* (2001) identified some QTLs on chromosomes 2, 3, 4, 6 and 7. The QTL on chromosome 1 that was determined in both environments in this study, only reported by Yin *et al.* (2001).

For GFP, one QTL found on chromosome 7 in Breda and 5 QTLs on chromosomes 1, 3, 5 and 7 in Tel Hadya. The strongest QTL in Tel Hadya comes from Wi 2291 in average reduce 0.8 day of this period (Table I).

BMS64 was the nearest marker with a QTL that control DHE and GFP at Breda and DHE at Tel Hadya. One of the QTL which control DHE was the same for GFP at Tel Hadya. *Scssr7970a* has close linkage with this QTL. *P18m237c* has a linkage with the QTL that control DHE and DMA at Tel Hadya. These QTLs might have peliotropic

Fig. 1. Linkage map of the cross Wi 2291 * Tadmor



effects on the traits or might have close genomic location for some genes cluster that control these traits.

Four QTLs on chromosomes 2, 5 and 7 were identified for DMA at Tel Hadya and two QTLs were determined on chromosomes 6 and 7 at Breda.The QTL that was located on chromosome 7 has close linkage with *Bmag12*. This QTL was common in both regions. Reducing allele of this QTL originated from Tadmor. Total percentage variation justified phenotypic variation of DMA was about 42 and 43% at Tel Hadya and Breda, respectively (Table I).

Some inconsistency of detected QTLs for given traits across environments may be due to chance, i.e. QTLs of relatively small effects, which are constant over environments, may simply go unnoticed in one experiment but show up in another. However this inconsistency is more likely to be caused by real QTL × environment interaction, as it can be explained physiologically. For example, the expression of QTLs grain filling period varied between years. The physiological basis for such interaction is that grain-filling period varies depends on plant N status as a result of the translocation from vegetative organs to meet N requirement for grain growth (Backes *et al*., 1995).

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(Received 31 May 2005; Accepted 30 August 2005)