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Major quantitative trait loci for flowering time in lentil

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Abstract: Quantitative trait loci (QTLs) for flowering time (FT) in lentil were located using a recombinant inbreed line population derived from an intraspecific cross of Precoz × WA8649041. Experiments for FT were conducted in 2 locations (Haymana, Turkey and Pullman, WA, USA) for 5 years (from 1998 to 2002 in both Pullman and Haymana). A linkage map was constructed using 149 markers (RAPD, AFLP, ISSR, SSR, and 2 morphological markers) located on 11 linkage groups (LGs). Analysis of variance of FT was found to be significant for all locations and years. One major QTL for FT was identified on LG6 across all environments, indicating the stability of the QTL. The LOD scores for FT varied between 3.25 and 13.64 among the environments. The markers UBC318_2, SSR212_1, UBC220, M09a, and M09 on the QTL region were statistically significant in all environments. The SSR212_1 marker itself explained 57% of total genotypic variation according to the average of all environments, indicating the potential use of these markers in marker-assisted selection studies.

Key words: Early flowering time, genetic markers, *Lens culinaris*, linkage map, quantitative trait loci

1. Introduction

Lentil is a cool-season grain legume generally grown in temperate semiarid regions. Lentil is usually grown in rotation with cereals and also contributes to enhanced soil nitrogen levels by fixation from air. Lentil seeds are valued as a food source of both high-quality plant proteins and fiber; in addition, the remaining plant residues can be used as animal feed and fodder. Lentil (*Lens culinaris* Medik.) is a self-pollinated diploid (2n = 14) legume. Its DNA content is about 4063 Mbp (Arumuganathan and Earle, 1991).

Flowering time (FT) is a key stage in plant development that initiates grain production and is vulnerable to stress. In plants, this corresponds to the decision to flower, which, as well as being influenced by intrinsic factors, is induced by 2 environmental stimuli: day length (photoperiod) and extended exposure to cold temperatures (vernalization) (Welch et al., 2005). Plants at different latitudes and with different climate regions, seasons, sowing dates, and altitudes (Summerfield and Roberts, 1988) respond differently to flower-inducing environmental stimuli, and several quantitative trait loci (QTLs) for natural variation in FT have been identified (Chiang et al., 2009). FT is also a very significant factor in crop yield. As an example, in chickpea, as a legume crop, number of days to flowering is an important trait for adaptation and productivity under environments with late-season drought and higher temperatures (Kumar and Van Rheenen, 2000) as well as in short-season environments (Anbessa et al., 2006). Early flowering can increase yearly crop production by allowing different sets of crops, such as chickpea, per growing season.

Genetic studies on FT in lentil have been reported using various types of populations. According to Tahir et al. (1994), 4 QTLs for days to flowering were detected on linkage groups (LGs) LG1, LG2, LG4, and LG7 in lentil. Sarker et al. (1999) found that FT was determined by a single recessive gene in 4 different crosses of lentil. Fratini et al. (2007) detected 3 QTL regions for FT in lentil grown in greenhouse conditions. Additionally, Tullu et al. (2008) reported 2 major QTLs for earliness located on LG4 and LG12 in lentil in Saskatoon and Floral in Canada. They also identified 3 additional QTL regions in LG1, LG5, and LG9 at the Floral location.

The use of molecular markers to map genomic regions controlling FT and related traits would improve our understanding of the genetic control of these traits. One of the main goals in lentil breeding in Turkey is the development of early maturing lines combined with high and stable yield to escape terminal drought stress and also to grow 2 crops per year in southeast Anatolia, where

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99% of red lentil is produced. This study aimed to locate QTLs related to FT using recombinant inbred lines (RILs) derived from a cross between Precoz and WA8949041.

2. Materials and methods

2.1. Plant material

The RILs were developed from a Precoz (nonhardy and early flowering) × WA8649041 (winter hardy and late flowering) cross with single seed descent until the F_6 generation at Washington State University, Pullman, WA, USA. One hundred and one seeds of RILs were grown in Haymana, Turkey, in the winter season in the years of 1999, 2000, 2001, and 2003, and RILs were also grown in Pullman in the winter season in the years of 1998 and 2000. Thirty to 40 seeds of each of the 101 RILs were sown in the field in Haymana and Pullman. Seeds were sown in single plots, 2 m long, spaced 0.3 m apart and 0.5 m between plots in 3 replications in a randomized complete block design. Parental lines were included in each replication. FT was recorded as number of days from planting date until 50% of the plants within the plot had at least 1 open flower (Erskine et al., 1994). Analysis of variance was applied to calculate variations in FT of the RILs as affected by environment and interaction (SAS Institute, 1997).

2.2. DNA markers and genetic linkage map

A genetic linkage map of lentil using a RIL population developed from the cross of Precoz × WA8649041 was constructed previously (Tanyolac et al., 2010). To this existent linkage map, 13 SSR markers were integrated (Varlı, 2009). The new linkage map was used to locate QTLs that control FT in lentil. The details of the genetic map were presented by Tanyolac et al. (2010) and Varlı (2009). Briefly, the genetic map was constructed with 116 RAPD, 23 ISSR, 13 SSR, and 180 AFLP polymorphic markers consisting of 11 LGs covering 1396.3 cM.

2.3. QTL mapping

QTL analysis was performed using Qgene 3.0 (Nelson, 1997) and MapManager QT 2.8 (Manly, 2001). Qgene was used for simple interval mapping and multiple regression, and to determine epistatic interactions. MapManager QT was used to check data quality and to confirm the results generated by other programs. QTL positions were determined by the peak LOD score. Multiple peaks within 30 cM were considered as a single QTL (Kearsey and Pooni, 1996). The percentage of the phenotypic variation (R^2) explained by the detected QTL was determined by multiple regression analysis using those markers explaining the peak response of individual QTLs.

3. Results

3.1. Field results for flowering time

FT was evaluated in Haymana and Pullman in 1998, 1999, 2000, 2001, and 2003. Analysis of variance for FT in all environments is presented in Table 1. Effects of genotypes, environments (location and year), and genotype × environment were highly significant ($P \leq 0.01$). According to environments, FT of parents ranged between 195 (Precoz) and 252 (WA8649041) days (Table 2). FT values of RILs varied between 197 (2001, Haymana) and 261 (2003, Haymana) days (Table 2). Average value of FT in parents ranged between 206 and 236 in the overall experiment (Table 2). Precoz and WA8649041 differed in FT by 30 days (Table 2). Positive and significant correlations between environments were detected for FT values (Table 3). The highest correlation among the environments was between the Haymana-2000 and Pullman-1998 experiments (r = 0.96).

3.2. QTL analysis

QTL analysis results for flowering time are presented in Tables 4–11 and in Figures 1a–1g. One major QTL for FT was detected on LG6 (Figure 1) over LOD score 3 in all environments, indicating stability of the QTL across environments. The markers at the peak of the QTL region were UBC318_2 and SSR212_1 (Table 4). The strongest QTL effect was obtained in Haymana-2003 (LOD = 13, Table

Table 1. Analysis of variance for FT in all environments.

		1998-Pullman	1999-Haymana	2000-Pullman	2000-Haymana	2001-Haymana	2003-Haymana
Source	DF	MS	MS	MS	MS	MS	MS
Rep	2	3.48 ns	3.35 ns	1.72 ns	1.84 ns	2.78 ns	1.79 ns
Genotype	100	342.06**	415.39**	266.95**	375.26**	512.97**	421.47**
Error	200	1.53	3.81	3.98	2.54	4.84	6.32

*: Significant at $P \leq 0.05$.
**: Significant at $P \leq 0.001$.
ns: Not significant.

Table 2. Average days to FT for parents and RILs in Pullman-1998, Haymana-1999, Pullman-2000, Haymana-2001, and Haymana-2003 and across environments.

Locations	Parents		RILs	
	Precoz	WA8649041	Average	Range
Pullman-1998	199	229	212	198–231
Haymana-1999	215	244	208	193–235
Pullman-2000	201	239	230	214–246
Haymana-2000	195	229	214	195–235
Haymana-2001	206	222	215	197–244
Haymana-2003	221	252	235	217–261
Average	206	236	219	202–242

Table 3. Correlation coefficients for flowering time among environments.

Years and locations	Pullman-1998	Haymana-1999	Pullman-2000	Haymana-2000	Haymana-2001	Haymana-2003
Haymana-1999	0.88					
Pullman-2000	0.88	0.90				
Haymana-2000	0.96	0.89	0.90			
Haymana-2001	0.86	0.84	0.84	0.84		
Haymana-2003	0.86	0.86	0.83	0.86	0.87	
Combined	0.96	0.95	0.94	0.96	0.93	0.93

Table 4. QTLs for FT in lentil at Pullman, WA, USA in 1998 and 2000 and at Haymana, Turkey in 1999, 2000, 2001, and 2003.

Location	Linkage Group	QTL peak (cM)	Marker at QTL peak	LOD	R^2 (%)	P
Pullman-1998	LG6	95.50	UBC318_2-SSR212_1	10.60	60	0.000
Haymana-1999	LG6	95.50	UBC318_2-SSR212_1	10.15	42	0.000
Pullman-2000	LG6	95.50	UBC318_2-SSR212_1	10.58	44	0.000
Haymana-2000	LG6	95.50	UBC318_2-SSR212_1	10.70	44	0.000
Haymana-2001	LG6	95.50	UBC318_2-SSR212_1	11.29	54	0.000
Haymana-2003	LG6	95.50	UBC318_2-SSR212_1	13.24	57	0.000
Combined	LG6	95.50	UBC318_2-SSR212_1	13.03	57	0.000

Table 5. QTL analysis results for Pullman-1998.

Marker	Chrom	Dist	Source	F	RSq	LOD	P
UBC318_2	LG6	95.50	A	64.39	0.44	10.60	0.00
SSR212_1	LG6	91.70	A	70.10	0.60	9.65	0.00
UBC220	LG6	104.50	A	26.40	0.24	5.11	0.00
M09a	LG6	80.20	A	23.11	0.20	4.57	0.00
M09	LG6	74.10	A	22.40	0.20	4.44	0.00
sdcoat	Unlinked	-	A	14.82	0.15	3.04	0.00
B11	LG6	119.80	A	11.39	0.12	2.37	0.00

Table 6. QTL analysis results for Haymana-1999.

Marker	Chrom	Dist	Source	F	RSq	LOD	P
UBC318_2	LG6	95.50	A	60.84	0.42	10.15	0.00
SSR212_1	LG6	91.70	A	30.27	0.40	5.27	0.00
UBC220	LG6	104.50	A	23.43	0.22	4.60	0.00
M09a	LG6	80.20	A	21.31	0.19	4.25	0.00
sdcoat	Unlinked	-	A	18.79	0.18	3.78	0.00
M09	LG6	74.10	A	16.96	0.16	3.45	0.00
UBC79-2	LG2	20.10	A	9.13	0.14	1.90	0.00

Table 7. QTL analysis results for Pullman-2000.

Marker	Chrom	Dist	Source	F	RSq	LOD	P
UBC318_2	LG6	95.50	A	64.23	0.44	10.58	0.00
SSR212_1	LG6	91.70	A	29.90	0.39	5.22	0.00
UBC220	LG6	104.50	A	24.59	0.22	4.80	0.00
M09a	LG6	80.20	A	19.82	0.18	3.98	0.00
sdcoat	Unlinked	-	A	17.43	0.17	3.53	0.00
M09	LG6	74.10	A	13.80	0.13	2.85	0.00
AB08	LG2	82.90	A	12.30	0.12	2.56	0.00
U502-2	LG2	23.20	A	10.39	0.12	2.17	0.00
UBC79_2	LG2	20.10	A	10.23	0.16	2.11	0.00
B11	LG6	119.80	A	9.71	0.11	2.04	0.00

Table 8. QTL analysis results for Haymana-2000.

Marker	Chrom	Dist	Source	F	RSq	LOD	P
UBC318_2	LG6	95.50	A	65.19	0.44	10.70	0.00
SSR212_1	LG6	91.70	A	58.64	0.56	8.57	0.00
UBC220	LG6	104.50	A	31.48	0.27	5.95	0.00
M09	LG6	74.10	A	22.41	0.20	4.44	0.00
M09a	LG6	80.20	A	19.30	0.18	3.88	0.00
sdcoat	Unlinked	-	A	15.85	0.15	3.23	0.00
B11	LG6	119.80	A	11.78	0.13	2.45	0.00

Table 9. QTL analysis results for Haymana-2001.

Marker	Chrom	Dist	Source	F	RSq	LOD	P
UBC318_2	LG6	95.50	A	70.02	0.46	11.29	0.00
SSR212_1	LG6	91.70	A	53.66	0.54	8.06	0.00
UBC220	LG6	104.50	A	28.58	0.25	5.48	0.00
M09a	LG6	80.20	A	15.19	0.14	3.12	0.00
sdcoat	Unlinked	-	A	10.59	0.11	2.22	0.00
M09	LG6	74.10	A	9.60	0.10	2.02	0.00

Table 10. QTL analysis results for Haymana-2003.

Marker	Chrom	Dist	Source	F	RSq	LOD	P
UBC318_2	LG6	95.50	A	87.06	0.51	13.24	0.00
SSR212_1	LG6	91.70	A	59.78	0.57	8.68	0.00
M09a	LG6	80.20	A	31.97	0.26	6.08	0.00
UBC220	LG6	104.50	A	25.58	0.23	4.97	0.00
M09	LG6	74.10	A	20.45	0.19	4.09	0.00
sdcoat	Unlinked	-	A	9.29	0.10	1.96	0.00

Table 11. Combined QTL analysis results for all environments.

Marker	Chrom	Dist	Source	F	RSq	LOD	P
UBC318_2	LG6	95.5	A	85.13	0.50	13.03	0
SSR212_1	LG6	91.7	A	61.21	0.57	8.82	0
UBC220	LG6	104.5	A	31.03	0.26	5.88	0
M09a	LG6	80.2	A	24.36	0.21	4.79	0
M09	LG6	74.1	A	19.35	0.17	3.89	0
sdcoat	Unlinked	-	A	15.93	0.15	3.25	0.0001

4). Total phenotypic variation (r^2) for FT explained by this major QTL ranged from 44% to 60% across environments. Seed coat as a morphological marker was associated with a QTL for FT, but the morphological marker was unlinked (data not shown). The morphological marker with a LOD score above 3.0 was associated with QTL for FT in the Haymana-1998, -1999, and -2000 and Pullman-2000 environments, but the LOD score of the morphological marker was lower than LOD 3 in Haymana-2001 (LOD = 2.22) and Haymana-2003 (LOD = 1.96).

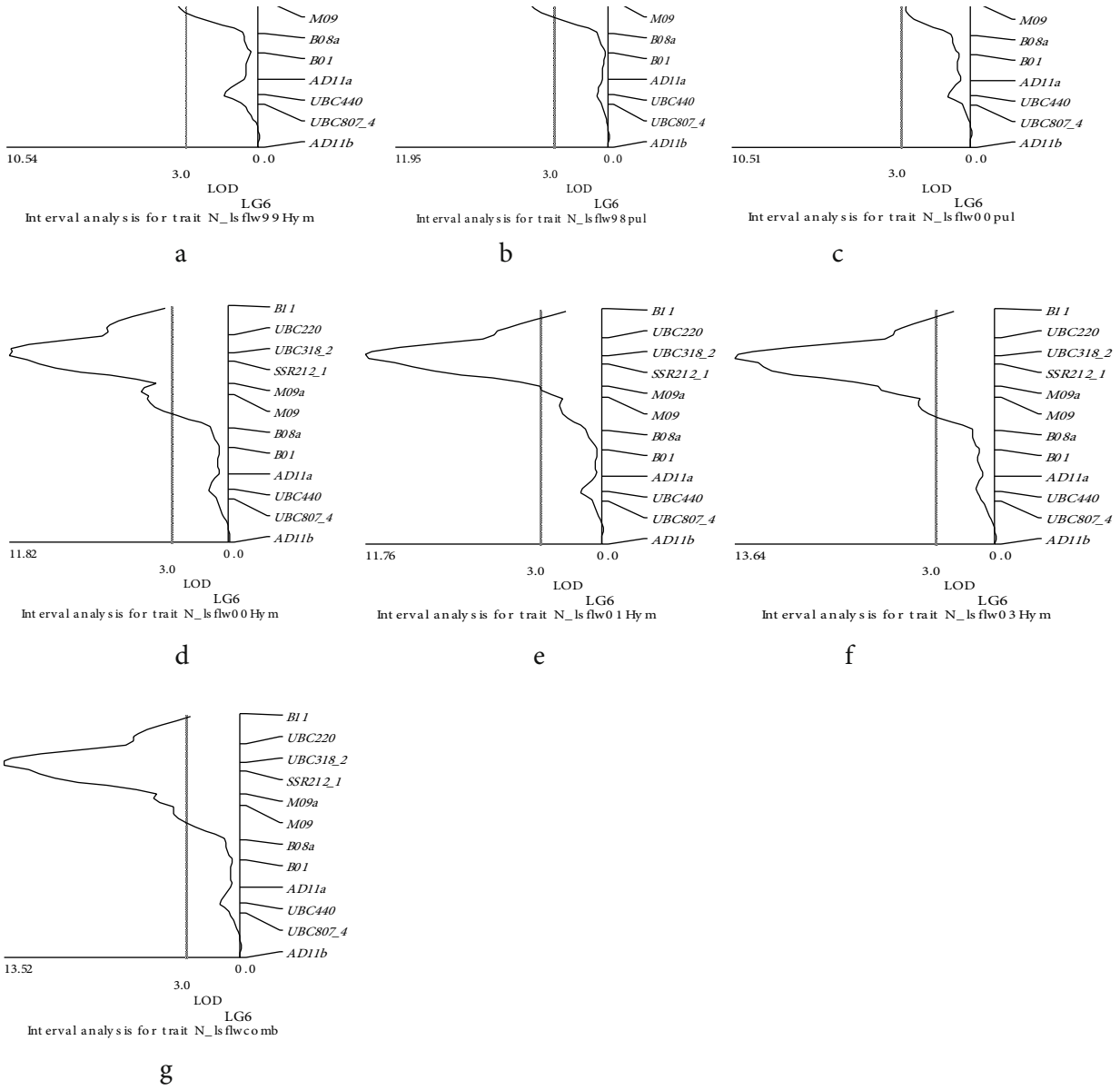

Figure 1. Interval mapping of markers controlling FT loci in Pullman-1998 (a), Haymana-1999 (b), Pullman-2000 (c), Haymana-2000 (d), Haymana-2001 (e), and Haymana-2003 (f) and combined QTL analysis of all environments (g).

4. Discussion

Significant genetic variation was detected among the RILs for flowering time at the 2 locations and in the 5 years (Table 1). This is in agreement with results previously reported by Erskine et al. (1990), Hamdi et al. (1991), and Tullu et al. (2008). FT is among the traits that can influence lentil yield in long day-length environments with a short growing period. Identifying of genomic regions of time to flowering harboring the genes responsible for this trait will be important in backcross breeding programs targeted at introgressing desirable traits into cultivars.

Environmental effect on the phenotypic and genotypic correlations of FT was minimal among diverse genotypes of lentil grown at 2 locations for 5 years. This indicates that the genotypic effects were consistent and were more important than the interaction effect. Tullu et al. (2008) carried out an experiment on FT in lentil at 2 diverse locations for 2 years and obtained minimal environmental effect on FT. Results of the highly significant correlations obtained from our experiments also support minimal effect of year and environment on FT. FT is one of the key factors for lentil yield and, according to Erskine et al.

(1990) and Sarker et al. (1999), FT in lentil is controlled by genetic factors.

One major QTL was detected from the 6 experimental sites. The QTL found between the marker interval of UBC318_2 and SSR212_1 on LG6 has a major effect and explains the largest proportion of the response variance. Among the markers on the QTL region, these markers had the stably highest QTL effect in all environments. According to Tahir et al. (1994), 4 QTLs for days to flower were detected on LG1, LG2, LG4, and LG7 using a small number of isozyme markers in lentil. Fratini et al. (2007) found 2 QTLs for FT in LG1 and LG10. Tullu et al. (2008) identified 2 QTLs for FT in 2 experimental locations in lentil. Since no common markers exist among the reported linkage maps, it was not possible to compare the results obtained to different works in relation to the chromosomal location of the QTL analyzed.

Sarker et al. (1999) found that the crosses between early and late parents indicated monogenic segregation for days to flower in Syria, and days to flowering was controlled by a single recessive gene (*Sn*) as well as by a polygenic system. They also found that the *Sn* gene is linked to the morphological marker seed coat (*Scp)*. In our research, the morphological marker seed coat was unlinked in the linkage map of lentil. The seed coat was associated with FT QTL in all locations except Pullman-2000 and Haymana-2001 and had minimal QTL effect on FT. Anbessa et al. (2006) proposed that time to flowering in chickpea was controlled by 2 major genes along with other polygenes, and late flowering was dominant over early flowering for both major genes with digenic interaction between them, mainly an additive × additive type.

As compared to other crops, flowering time is a very complex trait in terms of genetic background. Jonathan et al. (1995) found 5 QTLs affecting FT in induced mutation experiments in *Arabidopsis* and the existence of about 80 loci that affect FT was revealed (Levy and Dean, 1998), indicating that there are multiple genes controlling FT in plants. Mei et al. (2009) detected 6 QTLs for FT in *Brassica*. Our results indicated 1 major QTL region controlling FT and it seems that FT in lentil could be an exception among the plants discussed above, rather than multiple genes controlling FT.

A highly significant QTL effect was obtained from locations and years. The highest QTL effect was observed in Haymana-2003 (LOD = 13.64) and the lowest in Haymana-1999 (LOD = 10.15). Tullu et al. (2008) obtained the maximum LOD of 5.8 at Floral and LOD of 5.3 at Saskatoon. QTLs that show consistency in expression of earliness across environments could likely be QTLs with large effects, as also reported in pea by Pilet-Nayel et al. (2002) and in common bean by Tar'an et al. (2002). The QTL detected in all environments in this study could be used as a desirable marker in marker-assisted selection programs (Kahraman et al., 2004). Since the markers located on the QTL region (except marker SSR212_1) are RAPD markers, these dominant markers should be converted to codominant markers in order to be used efficiently in lentil breeding.

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