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Short Communication

QTL Analysis for Some Agronomic Traits in Barley (*Hordeum vulgare* L.)

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ABSTRACT

In this study, quantitative trait loci (QTL) were mapped in an F3 population (90 F3 families) derived from a cross between two barley genotypes. A molecular marker linkage map of this cross had been constructed based on 71 random amplified polymorphic DNA (RAPD) markers. Seven agronomic traits: 1000 grain weight, spike length, plant height, number of spike-lets per spike, awn length, number of grains per spike and number of spikes were measured in F3 population. Two environments (Karaj & Kerman) along with mean of environments were used to map quantitative trait loci (QTLs). QTLs controlling the 7 traits were detected by interval mapping analysis using MAPMAKER/QTL. A total of 28 QTLs were identified for 7 traits. Phenotypic variance explained by these QTLs varied from 12.5 to 48.9%. Mean environments could detect 11 QTLs, which 5 of them were new. The results showed relatively high R^2 values and low confidence limits for QTLs, so that the relevant linkage between markers and QTLs can be used in breeding program.

Key Words: *Hordeum vulgare*; Quantitative trait loci; F3 families; Agronomic traits

INTRODUCTION

Most agronomically significant characters inherited quantitatively and are known to be affected by environmental factors. The polygenic nature of many morphological characters has been described in many studies (Mohammadi *et al.,* 2005; Peighambari *et al.,* 2005), which makes genetic inheritance complicated and difficult to use the molecular markers. Interval mapping is a powerful approach that permits the identification and genetic mapping of loci controlling complex traits like grain yield and its components, having great importance in plant breeding. Knowledge of numbers and effects of quantitative trait loci (QTLs) can help breeders to understand the genetic control of these traits and design more efficient selection strategies (Broman *et al.,* 1999).

Several reports on the application of the QTL strategy are available for barley (Backes *et al.,* 1995; Marquez-Cedillo *et al.,* 2001; Pillen *et al.,* 2003; Mohammadi *et al.,* 2005; Peighambari *et al.,* 2005) in which favorable exotic QTL alleles for important agronomic traits have been identified. For example, Peighambari *et al.* (2005) by using 72 doubled haploid (DH) barley lines identified twenty-three QTLs controlling different traits. Phenotypic variance explained by these QTLs varied from 11.9 to 61.1%.

Favorable QTL-alleles are useful as a breeding resource after they have been fixed in nearly isogenic lines. However, these favorable QTLs often lose their effects after they are purified into elite lines (Pillen *et al.,* 2003). The objectives of this work was to identify QTLs, their effects and locations on linkage groups for 1000 grain weight, spike length, plant height, number of spike-lets per spike, awn length, number of grains per spike and number of spikes in 90 F3 barley families using RAPD markers.

MATERIALS AND METHODS

Plant materials and field experiments. A cross between two barley lines, Afzal and Cwb, was carried out. A single F1 plant was selected and selfed for the production of a segregating F2 population. Individual F2 plants were selfed to produce 90 F3 families, which were grown at Karaj and Kerman locations in (2003-04). The F3 populations were sown in a field plot. The entire F3 population was sown in rows of 1 m as randomized complete block design with three replications to measure 1000 grain weight, spike length, plant height, number of spikelets per spike, awn length, number of grains per spike and number of spikes. Seven traits were measured on 5 plants in the center of the row and average values were used for analysis. Seven seeds from each F3 family were harvested and DNA extraction was done based on mini prep protocol (Dellaporta *et al.,* 1983). Bulked DNA of each family was subjected to RAPD analysis.

Construction of RAPD map and QTL mapping. A genetic map consisting of 71 RAPD markers was

constructed using MAPMAKER/EXP 3.0 computer package (Lander *et al.,* 1989). QTL mapping was carried out for each environment using interval mapping approach in the computer package MAPMAKER/QTL1.1 (Lander *et al.,* 1989). A LOD threshold of 2.0 was used to declare the presence of putative QTL in a given genomic region. The percentages of variation explained by the QTL for the trait, the additive and dominance effects and the degree of dominance were also estimated by MAPMAKER/QTL analysis.

RESULTS AND DISCUSSION

Four linkage groups were obtained using 71 RAPD polymorphic bands, covering 1600 cM of the barley genome; the mean RAPD density was equal to 22 cM. This cover of map was lower than barley genome mapping prepared by Gerhard (2002) and higher than genome mapping from Pillen *et al.* (2003) Out of seven group chromosome only four linkage groups were obtained, resulting in some gap regions, which were not covered by any polymorphic RAPD. The occurrence of marker gaps was also observed in other QTL analyses. Backes *et al.* (1995), for instance, could map only a single marker to barley chromosome 1H using a set of 50 informative restriction fragment length polymorphisms (RFLPs). 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 we identified 22 markers, which associated with 7 traits (Table I).

QTLs for 1000 grain weight (TGW). In this research, 3 QTLs located on linkage groups one (2 QTLs) and four (1 QTL) were identified for TGW at Kerman and one on linkage group at mean of the environments. However no QTL was identified in Karaj. This explained that the percentage of variation (R^2) was 17.1 to 25.3% at Kerman and it was 15.8% at the mean of environments. One of the QTL, identified in linkage groups one at Kerman, was close (6 cM) to J-1826 marker and this QTL was also found in the mean of environments near 8 cM from J-1826. Peighambari *et al.* (2005) found 3 QTLs for 1000 grain weight on chromosomes 1, 5 and 7 H that explained variation ranging from 14 to 20% of the total phenotypic variation.

QTLs for spike length (SPL). One QTL was identified on linkage group four in Karaj and 2 QTLs found in the same linkage group in the mean environments for SPL. However, the nearest markers to these QTLs were different. As in Karaj, the F-1752 marker was a part of QTL (0 cM), but in the mean environments UB64 and F-1386 were the nearest markers (0 & 9.2 cM, respectively). These QTLs explained variation ranging from 24 to 46.2% of the total phenotypic variation.

QTLs for plant height (PHT). For PHT, in both environments and also in mean environments 7 QTLs were identified. In Karaj two QTLs on linkage group one with a distance of 4 and 14 cM from H-805 and H-441, respectively and one QTL on linkage group four with a distance of 16 cM from F-1384 marker were found. But in Kerman one QTL on linkage group one with a distance of 8 cM from nearest marker (J-1826) was obtained. Moreover, in the mean environments three QTLs were identified for PHT, and one of them (from J-1826) was like QTL that was found in Kerman with the same distance (8 cM). This common QTL explained 21.8 and 20.6% of variation (R^2) at Kerman and mean environments, respectively. J-1826 was also the nearest marker with a QTL that control TGW and PHT at Kerman and mean environments. Peighambari *et al*. (2005) also found 3 QTLs for plant height in barley that explained 16 to 30% of phenotypic variation. Kandemir *et al.* (1999) found a PHT QTL on chromosome 3H.

QTLs for number of spikelets per spike (NOS). In each environment (Karaj & Kerman), different QTLs were identified for NOS. These QTLs were all on linkage group one with distance of 0 to 14 cM from nearest markers. These QTLs explained variation ranging from 15.7 to 48.9% of the total phenotypic variation. Gerhard (2002) also found two QTLs for spikelets per spike in barley; each of them explained 16.5% of total phenotypic variation.

QTLs for awn length (ALH). For ALH in all environments, 6 QTLs were identified. In Karaj two QTLs on linkage groups (1 & 4) with a distance of 4 and 13 cM from B-1021 and UB64-736 markers, respectively were found. In Kerman one QTL on linkage group one with a distance of 2 cM from nearest marker (J-1152) was obtained. Moreover, in the mean environments three QTLs were identified for ALH, which one of them (from J-1152) was like QTL that was found in Kerman with the same distance (2 cM). This common QTL explained 27.1 and 17.3% of variation (R^2) at Kerman and mean environments, respectively.

QTLs for number of grains per spike (NOG). In each environment (Karaj & Kerman) and also in the mean environments different QTL was identified for NOG. Three QTLs were located on linkage groups 3 (in Kerman), 4 (in Karaj) and 2 (in mean environments), which explained phenotypic variation ranging from 21.5 to 31% of total.

QTLs for number of spikes (NSP). One QTL located on linkage group one were identified for NSP at Kerman and one on linkage group at Karaj. However no QTL was identified in mean of the environments. Explained percentage of variation (R^2) was 29.3% at Kerman and it was 17.8% at Karaj. The trial sites varied significantly with respect to geographical locations and provided different environmental conditions in Karaj and Kerman such as experimental conditions, planting dates and seasons. Furthermore, a third environment, 'mean environments', was derived by averaging of two locations. This environment reduced the variance due to error and increased the precision of QTL environment (Knapp & Bridges, 1990). In this study, mean environments could detect 11 QTLs; 5

Table I. Number of location, group Linkage number, nearest marker (distance), significant level (LOD) and explained phenotypic variance (R^2) of detected QTLs in Karaj, Kerman and mean of the environments

Environment	Trait	Number of Location	Group Linkage No.	Nearest marker (distance)	R^2	LOD	Additive effect	Dominanteffect
Karaj	**TGW**	-	-	-	-	-		
	SPL	1	4	F-1752 (0.0)	24.3	2.43	0.74	-0.67
		3	1	H-441(14.0)	23.3	2.07	-0.54	7.31
	PHT		1	H-805(4.0)	31.6	2.24	3.75	10.74
			4	F-1384 (16.0)	53.6	3.13	-5.76	13.65
	NOS	1	1	UB53(14)	48.9	2.89	1.31	4.85
Kerman								
		3	1	B-1012(10)	25.3	2.36	5.38	3.53
	TGW		1	J-1826(6.0)	17.1	2.45	-5.01	-1.67
			4	F-1384 (0.0)	24.9	2.30	-4.79	7.12
	SPL	-	-	-	-	-		
	PHT	1	1	(8.0)-1826J	21.8	3.52	-4.89	4
	NOS	1		H-926(0.0)	15.7	2.24	8.58	-12.27
Mean								
	TGW	1	1	J-1826(6.0)	15.8	2.25	-3.15	-0.003
	SPL	2	4	F-1386 (9.2)	46.2	2.87	0.55	-1.34
			4	UB64 (0.0)	33.9	2.25	0.58	-0.78
		3	1	H-805(2.0)	32.8	2.28	5.85	10.89
	PHT		1	J-1826(8.0)	20.6	3.23	-4.33	3.23
			4	UB64 (17.0)	41.3	2.98	-6.04	10.78
	NOS	1	1	A-372(12.0)	43.24	3.29	0.29	3.68

of them were new.

A variety of factors may affect the outcome of a QTL analysis; for example, the selection of the cross, population structure and size, number of measured replications and environments and type, number and density of markers (Pillen *et al.*, 2003). Magnitude of QTL effect and accurate chromosome map location are also important for verifying the QTLs (Romagosa *et al.*, 1999). In view of the fact that to determine exact localization of the QTLs on the linkage map and better estimation of explained variation, the QTL mapping is very sensitive to errors in marker placement and between marker distances. Hence, we recommend use of codominant markers to optimize the genetic linkage map.

In conclusion, this study revealed a total of 16 QTLs for four traits. A maximum of 7 QTLs were detected for spike length and no common QTL was detected from Karaj and Kerman. Most of the suitable QTLs were located on chromosomes linkage groups one and four. No favorable QTLs were detected on linkage groups two and only one QTL (for NOS) was identified in linkage groups three. This is because of low number of RAPD polymorphic markers used in this study.

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