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ARTICLE

The Effect of Pesticide Application on QTLs Controlling Traits in Barley

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ABSTRACT Among cereals, barley (Hordeum vulgare L.) ranks fourth in consumption worldwide. Among barley breeding goals, one can refer to gene mapping, studying their inheritance, and saturated genetic linkage maps. Problems with pesticide applications include reduced genetic diversity, reduced nitrogen fixation, and destruction of the habitat of especially endangered species. The effect of pesticide application on the emergence of QTLs expressing traits in experimental barley was investigated using 104 barley F2:4 families from Badia × Kavir cross. A total of 25 QTLs were mapped for all traits. In non-using pesticides, 12 QTLs were identified for peduncle length, stem diameter, flag leaf length, and awn length. It was found that qFL-4 has major effects on flag leaf length. For using the pesticide, 13 QTLs were detected that QTLs related to stem diameter, grain weight, flag leaf length explained a high percentage of phenotypic variation. The results of this study showed that pesticide application affects the expression of some genes in barley. Besides, major-effect trait-controller QTLs and their associated markers can be used in marker-assisted selection (MAS) programs.

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Introduction

Barley (*Hordeum vulgare* L.,) is a diploid crop with 2n = 2x = 14 chromosomes (Germán et al. 2000). Among cereals, barley ranks fourth after wheat, rice, and corn. However, barley has the first planting in terms of the extent of expansion because it can be cultivated under different climatic conditions (Feug et al. 2006). Over $136{\times}10^6$ tons of barley are produced worldwide each year, mainly used for livestock nutrition, and for industrial application (Zong-Yun et al. 2006). Due to the economic importance of barley and the widespread cultivation of this cereal, its breeding is on the agenda of breeders. To accomplish barley breeding goals, gene mapping, a study of their inheritance, and saturated genetic linkage maps were necessary. One of the major challenges of barley breeding programs is determining the inheritance and locus of genes controlling these traits (Li et al. 2005; Hassan et al. 2010).

All cereals, including barley and wheat, are exposed to various pests and diseases. A pesticide is a substance or mixture of substances used to prevent, control or reduce pest damage. It can be a chemical compound (synthetic or natural), or a biological agent (biopesticide, e.g., a bacterium) eliminating various pests (e.g., insects, pathogenic microorganisms, weeds, nematodes) of the cultivated plant (Shibamoto and Bjeldanes 2009). Alongside the benefits of the pesticide application, there are problems such as reduced genetic diversity, reduced nitrogen fixation, and destruction of living habitats, especially endangered birds and species. In addition, humans receive pesticides through various means, including food consumption; exposure to food (especially fruits and vegetables) is five times more than other ways, such as air and drinking water (Shokrzadeh and Saravi 2011; Bonnechère et al. 2012). When flour and bread prepared, the amount of pesticides is slightly reduced by the procedure of grinding and baking. The highest residual concentration was observed in bran because most residues of pesticides accumulate in the grain exosporium (Kaushik et al. 2009).

Molecular markers made it possible to generate high-density genomic (genetic) linkage maps for many plants, including barley. These high-density genetic linkage maps based on molecular markers and QTLs (Quantitative Trait Loci) allow using Marker-Assisted Selection (MAS), and in this way selection is possible in the early generations of a breeding program which highly improves efficiency (Ayoub et al. 2003; Han et al. 1994).

In the study of a barley population derived from Steptoe × Morex cross, 3 QTLs were identified for grain yield using 15 Restriction Fragment Length Polymorphism (RFLP),

4 Random Amplification of Polymorphic DNA (RAPD), one microsatellite, and 77 Amplified Fragment Length Polymorphism (AFLP) markers. In the same study, it was found that for plant height, the number of spikelet's per spike and grain weight, there was one QTL on chromosome 3 (Kandemir et al. 2000). By conducting research on germination traits in 85 wheat double haploids lines and their parents, Landjeva et al. (2010) identified 20 QTLs, most of which were clustered on the 1DS chromosome. In a study, the QTLs of some major crop traits in barley, a double haploid barley generation derived from a cross between two six-row barley cultivars (Botania × Rolfi) were studied and RAPD markers were used to prepare the linkage map. Finally, 654 cM of the genome was covered in this study; 1-7 QTLs were determined for each of the attributes of plant height, spike yield, the weight of one thousand grains. It is worth noting that many of these QTLs overlap with previously identified QTLs (Manninen 2000).

The objectives of this study were to identify genetic loci controlling the traits related to yield and its components, to determine the contribution and mode of QTLs identified to the phenotypic variation in the attributes under pesticide application and non-application in the Badia × Kavir cross barley population.

Materials and Methods

Plant materials and phenotypic evaluation

In this study, 104 $F_{2:4}$ families from the Badia × Kavir cross as well as two parents were planted at the research farm of Gonbad Kavous University. The examined families were cultured using augmented design in two separate experiments (pesticide application and pesticide-free). In the pesticide-using experiment Deltamethrin, Dimethoate, Diazinon and Trichlorofon were applied for controlling pests. Barley families were planted in 0.3 m^2 (2 meters long rows). The distance between the plants was 25 cm in the rows. Peduncle length, stem diameter, flag leaf

Table 1. Chromosomal location and primer sequences of SSR markers used for linkage map.

Marker	Chromosome	Forward / Reverse sequence
HVM20	1	CTCCACGAATCTCTGCACAA / CACCGCCTCCTCTTTCAC
Bmag0782	1	ATGTACCATTACGCATCCA / GAAATGTAGAGATGGCACTTG
Bmac0032	1	CCATCAAAGTCCGGCTAG / GTCGGGCCTCATACTGAC
Bmag0718	1	ATCGTGACATCTCAAGAACA / CCTGATACTGCCTAGCATTAG
HVM36	2	TCCAGCCGAACAATTTCTTG / AGTACTCCCACACCACGTCC
GBM1462	2	CTGTGGCTAAAGAAGGCACC / AAGATTGCTGCAGGATAGGC
GBMS160	2	ATCCAGTGGCCTTTGTATGG / TCAGCTCCTCTCTCTTCATGTG
GBMS247	2	ACACCACATTCATCTTCCTTCA / CATTGCTCTGCTTCCTGTCA
HVM27	3	GGTCGGTTCCCGGTAGTG / TCCTGATCCAGAGCCACC
Bmag0603	3	ATACCATGATACATCACATCG / GGGGGTATGTACGACTAACTA
Bmag0225	3	AACACACCAAAAATATTACATCA / CGAGTAGTTCCCATGTGAC
Bmag0013	3	AAGGGGAATCAAAATGGGAG / TCGAATAGGTCTCCGAAGAAA
HVM67	4	GTCGGGCTCCATTGCTCT / CCGGTACCCAGTGACGAC
Cit7	4	GCAGCCAAGACCTTGAGAAAGC / GCCTGAACTAGCCCGAGAAATG
EBmac0906	4	CAAATCAATCAAGAGGCC / TTTGAAGTGAGACATTTCCA
HVM40	4	CGATTCCCCTTTTCCCAC / ATTCTCCGCCGTCCACTC
HVM30	5	AGTGGGGAATGAGAGAATGG / TGCTTGTGGGGCATCACAC
GMS001	5	CTGACCCTTTGCTTAACATGC / TCAGCGTGACAAACAATAAAGG
EBmac0684	5	TTCCGTTGAGCTTTCATACAC / ATTGAATCCCAACAGACACAA
Bmag0113	5	GGAATCTTCTGGAACGTC / TTAAGAAGATCATTGTATTGAAGA
HVM65	6	AGACATCCAAAAAATGAACCA / TGGTAACTTGTCCCCCAAAG
Bmac310	6	CTACCTCTGAGATATCATGCC / ATCTAGTGTGTGTTGCTTCCT
Bmac0040	6	AGCCCGATCAGATTTACG / TTCTCCCTTTGGTCCTTG
GBMS0083	6	ACACTATACACATATAT / GAATCCCAACAGACACA
HVM4	7	AGAGCAACTACCAGTCCAATGGCA / GTCGAAGGAGAAGCGGCCCTGGTA
GBMS0111	7	ATATTTATGAAACGGTGTTCG / GGGTTTATCCTCTGCAGG
Bmag0135	7	ACGAAAGAGTTACAACGGATA / GTTTACCACAGATCTACAGGTG

Table 2. Sequences of ISSR, IRAP and iPBS markers used for linkage map.

Marker	Sequence
iPBS	
2231	ACTTGGATGCTGATACCA
2074	GCTCTGATACCA
2076	GCTCCGATGCCA
2415	CATCGTAGGTGGGCGCCA
2221	ACCTAGCTCACGATGCCA
IRAP	
IRAP56	TGAGTTGCAGGTCCAGGCATCA
IRAP54	ACCCCTTGAGCTAACTTTTGGGGTAAG
IRAP50	CACTTCAAATTTTGGCAGCAGCGGATC
ISSR	
ISSR16	CTCTCTCTCTCTCTCTG
ISSR20	CTCTCTCTCTCTCTCT
ISSR22	CTCTCTCTCTCTCTCTT
ISSR29	TCTCTCTCTCTCTCTCA
ISSR30	GAGGAGAGAGAGAGAG

length, flag leaf width, flag leaf weight, number of grains, grain weight, awn length, spike weight, and grain weight were measured for 20 plants per families and parents in both experiments.

DNA and PCR extraction

Twenty young leaves from each family were randomly selected and genomic DNA extraction was carried out using the CTAB method (Saghi Maroof et al. 1994). Horizontal gel electrophoresis (8% agarose gel) was used to check quality of DNA. Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), Inter Primer Binding Site (iPBS) and Inter Retrotransposon Amplified Polymorphism (IRAP) markers were used as for as providing of genetic linkage map (Table 1-2). Materials and PCR thermal program for different markers are listed in Tables 3-6.

Altogether, 28 SSR, 19 ISSR, 3 IRAP and 5 iPBS markers (93 alleles) were used to determine the genotype on the studied population. After the PCR reaction, amplified DNA was electrophoresed on 6% polyacrylamide gel. Fast silver nitrate method (An et al. 2009; Byum et al. 2009) was used for visualization of the gel.

Table 3. Materials used in polymerase chain reaction for ISSR, iPBS and IRAP markers.

Components	Concentration	Amount (µl)
Buffer PCR10X	1X	1
$MgCl_2$	50 mM	0.48
dNTP	10 µl	0.6
Taq DNA Polymerase Enzyme		0.12
Primer	60 ng	1.5
DNA diluted	0.75-0.5 ng	2.5
H_2O		3.8
Final volume		10

Table 4. Thermal program for amplification of ISSR, iPBS and IRAP markers.

Step	Temperature (°C)	Time	Number of cycles
Primary denaturing	95	5'	1
Denaturing	95	45"	
Annealing	-	45"	10
Synthesis	72	45"	
Denaturing	95	45"	
Annealing	-	45"	25
Synthesis	72	45"	
Final amplification	72	5'	1

Statistical analysis

Map Manager QTX17 software (Manley and Olson 1999) was used for genetic mapping and MapChart software (Voorrips 2002) for drawing of map. The point that had the highest LOD value was identified as the region most likely to have QTL. The critical limit for detecting QTLs was obtained by the permutation test (LOD = 2). The exact position of the QTL relative to both markers was determined in cM. QTLs were named according to the method of McCouch et al. (1997). First, the letter *q* and then the trait abbreviation was capitalized and separated using a dash from the chromosome number on which the QTL was identified. It is worth noting that the mean of each family was used for each trait in the QTL analysis. QTL was analyzed using QGene software (Nelson 1997).

Results

The phenotypic distributions of barley studied traits width in the $F_{2:4}$ populations grown in the using and no using pesticides environments are shown in Fig. 1. Means of traits among parents was significant for all traits in both of condition except shoot thickness. The genotypes in the $F_{2:4}$ population differed significantly ($P < 0.05$) for all traits (Table 7), indicating the presence of sufficient genetic variation. In all traits, $F_{2:4}$ progeny was observed that fell beyond the high or low mean of the two parents (Fig. 1-2). For all characters, the number of observed extreme individuals significantly ($P < 0.01$) exceeded the expected, suggesting transgressive segregation.

A genetic linkage map was generated using 28 SSR markers, 19 ISSR markers, 3 IRAP markers, and 5 iPBS

Table 5. Materials used in polymerase chain reaction for SSR markers.

Components	Concentration	Amount (µl)
Buffer PCR10X	1X	1
$MgCl_2$	50 mM	0.48
dNTP	10 mM	0.6
Taq DNA Polymerase Enzyme		0.12
Forward primer	60 ng	0.75
Reverse primer	60 ng	0.75
Diluted DNA	0.75-0.5 ng	2.5
H_2O		3.8
Final volume		10

Table 6. Thermal program for amplification of SSR markers.

Step	Temperature (°C)	Time	Number of cycles
Primary denaturing	94	5'	1
Denaturing	94	1	
Annealing	64	30"	18
Synthesis	72	1'	
Denaturing	72	1'	
Annealing	94	1'	30
Synthesis	55	1'	
Final amplification	72	5'	1

markers (93 alleles) as well as 104 F2 individuals. The markers used were divided into 7 linkage groups corresponding to 7 barley chromosomes. Based on the Kosambi's mapping function (Kosambi 1994), the map length was estimated to be 617.5 cM and the average marker interval was 5.41 cM (Fig. 3).

A total of 25 QTLs were mapped for traits. The minimum and maximum LODs were detected in the range of 2.038-4.537 for awn length and flag leaf length. The explained variance for traits in both conditions varied from 8.7 to 16.1 (Table 8).

In pesticide-free condition, 12 QTL were identified for peduncle length, stem diameter, flag leaf length, awn length and Peduncle length. qPL-3, in ISSR13-3-ISSR47-8 marker interval at position of 48 cM accounted for 9.2% of the total variation of peduncle length. Five QTLs controlled stem diameter (qSD-1, qSD-4a, arise-4b, qSD-6, qSD-7) at positions of 70, 0, 72.0, and 0 cM in ISSR47-1, ISSR31-6, ISSR20-1, ISSR38-6, ISSR48-1 flanked markers, that increased stem diameter with an additive effect from Badia. Three QTLs were identified at position 10, 68, 0 near Bmag0013, ISSR31-5, and ISSR31-6 markers for flag leaf length accounting for 11.5, 11.8, and 18.4% of the phenotypic variation, respectively (Fig. 4). For awn length, three QTLs (qRL-2a, qRL-3a, qRL-3b) on chromosomes 2 and 3 could account for about 29.3% of the total variation of this trait at positions 76, 2, and 68.

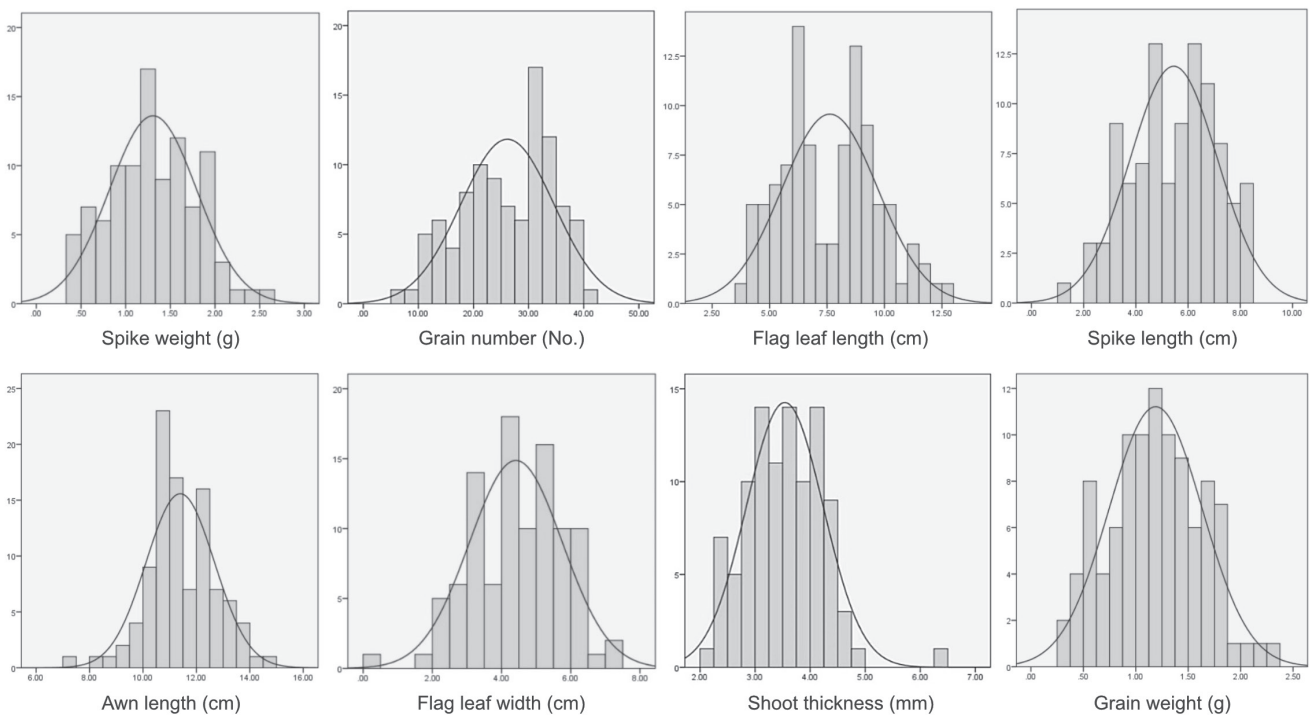

Figure 1. The histogram of studied traits in population where pesticides were not used.

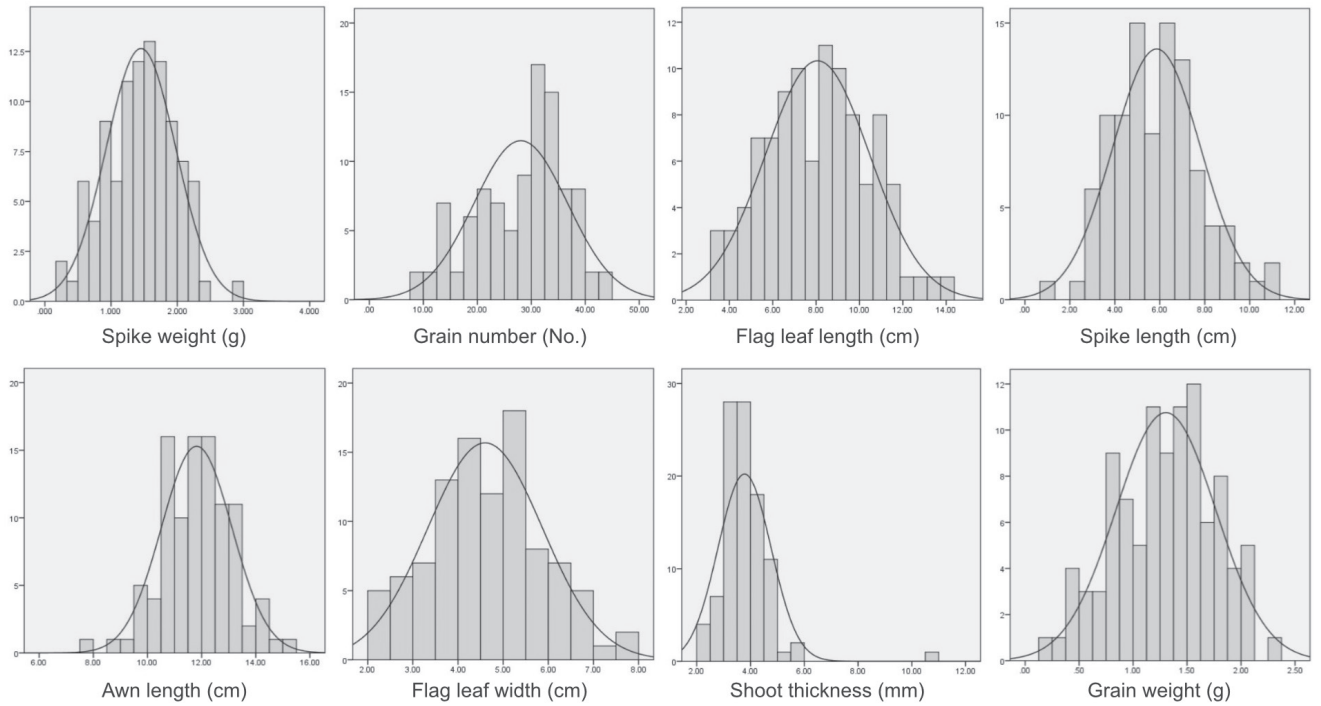

Figure 2. The histogram of studied traits in pesticide-treated population.

From these QTLs, qPL-3 was found to have major effects (R^2 = 11.8).

In pesticide-treated population, 13 QTLs were detected. For stem diameter, three QTLs (qSD-3, qSD-6a, qSD-6b) were detected on chromosomes 3, 6 (two QTLs) at positions 48, 2, and 18 cM in ISSR13-3 - ISSR47-8, ISSR38-6-Bmac310, and IRAP50-3-ISSR48-3 marker intervals. The stem diameter was found to have major effects, accounting for 10.9 and 16.5% of the phenotypic variation, respectively. For flag leaf length, 3 QTLs (qFL-3, qFL-7a, and qFL-7b) were identified on chromosomes 3, 7 (two QTLs) at positions 48, 102, and 106. For flag leaf width, qWI-6 at position 52 cM flanked by HVM65-Bmac0040 markers and was located with an LOD of 2.16. This QTL allele was transferred from Kavir with a negative additive effect.

For flag leaf weight, one QTL (qFWE-4) was identified at 2 cM from the beginning of the chromosome at

Table 7. Means comparison of parents for agronomically traits in no using pesticides and using pesticides.

Traits	Without pesticides			Treated with pesticides		
	Kavir	**Badia**	**T-test**	**Kavir**	**Badia**	**T-test**
Biomass (g)	126.378	149.077	**	95.632	116.321	**
Spike weight per plant (g)	34.421	51.746	**	27.632	46.325	**
Spike number per plant	34.000	45.000	**	26.000	33.000	**
Spike length (cm)	99.000	110.000	**	86.215	98.784	**
Grain yield per plant (g)	43.297	63.526	**	32.125	54.369	**
Peduncle length (cm)	3.000	6.420	**	2.690	5.632	**
Shoot thickness (mm)	4.100	4.480	ns	2.652	2.832	ns
Flag leaf length (cm)	11.300	9.900	**	8.321	7.954	**
Flag leaf weight (g)	0.140	0.020	**	0.122	0.018	**
Grain number	34.000	36.000	*	26.000	31.000	*
Grain weight (g)	1.340	1.820	**	1.126	1.325	*
Awn length (cm)	12.360	10.400	*	11.635	9.847	**

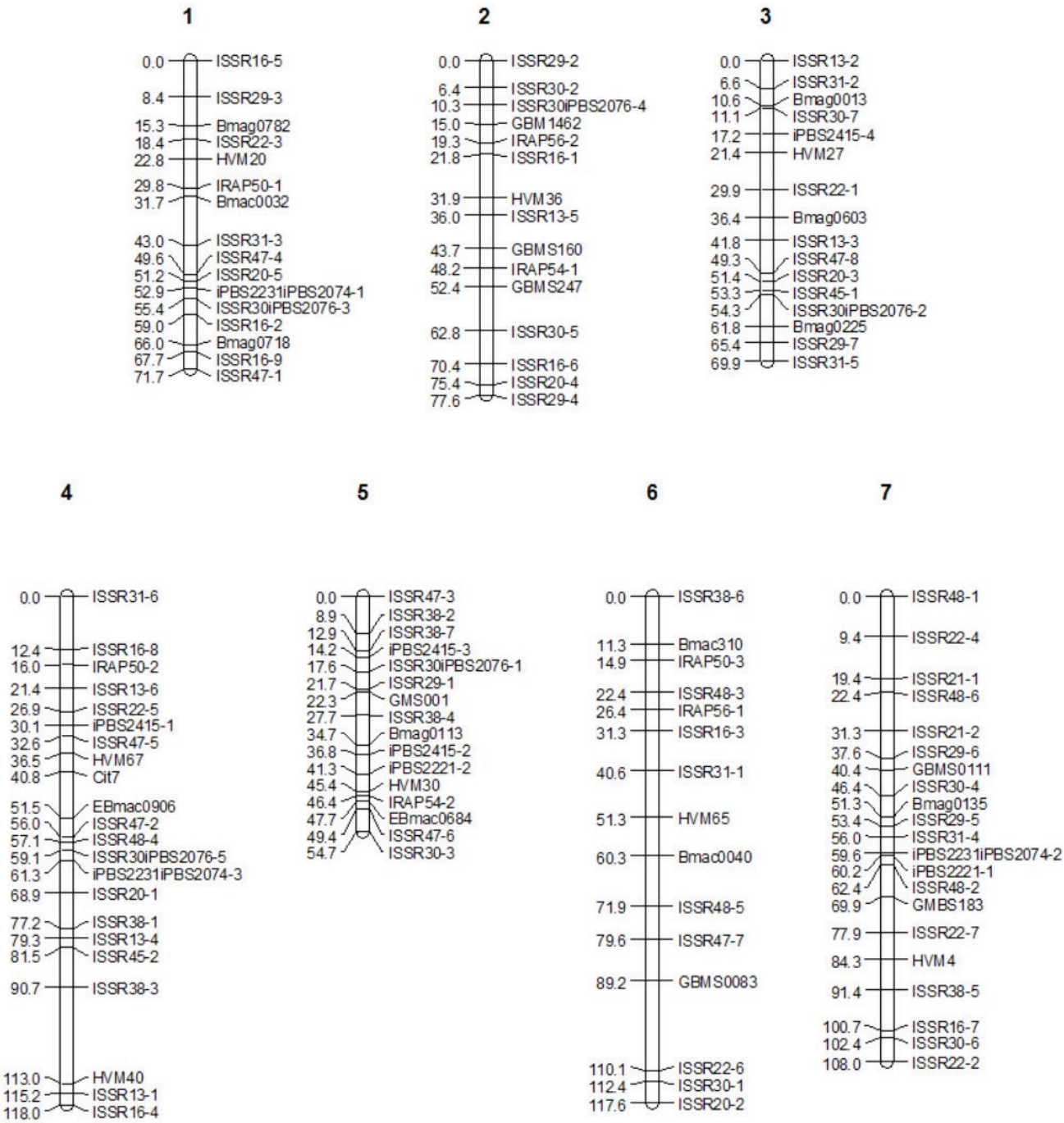

Figure 3. Linkage map caused Badia × Kavir cross based on 28, 9, 3 and 5 SSR, ISSR, IRAP and iPBS markers in F2:4 mapping population.

ISSR31-6-ISSR16-8 marker interval and LOD of 3.935 and phenotypic variance of 16.1 with a negative additive effect of Kavir. qSN-3 at 48 cM flanked by ISSR13-3-ISSR47-8 markers with an LOD of 2.213 accounted for 9.4% of the variation in grain number. For spike weight, one QTL was detected on chromosome 3 at 48 cM above the chromosome (Fig. 5). This QTL was within the ISSR13-3-ISSR47-8 marker interval and accounted for 13.2% of the variation with an LOD of 3.163. For grain weight, 3 QTLs (qSW-1a, qSW-1b, qSW-3) were identified that could account for 12.8, 12.8, and 15.7%, respectively, and had a positive additive effect for this trait at chromosomes 64,

Table 8. QTL detected for agronomical traits in F2:3 caused Badia × Kavir populations.

Trait	QTL	Chromosome	Position	Flanking marker	LOD	Additive	R^2	Allele direction
Without pesticides								
	qPLNUF-3	3	48	ISSR13-3- ISSR47-8	2.158	-0.813	9.2	Kavir
	qSTNUF-1	1	70	ISSR16-9-ISSR47-1	2.911	-0.713	12.2	Kavir
	qSTNUF-4a	4	0	ISSR31-6- ISSR16-8	2.289	0.581	9.7	Badia
	qSTNUF-4b	4	72	ISSR20-1-ISSR38-1	2.240	-0.583	9.5	Kavir
	qSTNUF-6	6	0	ISSR38-6-Bmac310	2.478	0.580	10.5	Badia
	qSTNUF-7	7	0	ISSR48-1-ISSR22-4	2.041	-0.437	8.7	Kavir
	qFLLNUF-3a	3	10	ISSR31-2- Bmag0013	2.745	-0.605	11.5	Badia
	qFLLNUF-3b	3	68	ISSR29-7-ISSR31-5	2.806	0.949	11.8	Kavir
	qFLLNUF-4	4	0	ISSR31-6- ISSR16-8	4.537	1.235	18.4	Kavir
	qALNUF-2	2	76	ISSR20-4 - ISSR29-4	2.038	-1.432	8.7	Badia
	qALNUF-3a	3	2	ISSR13-2 - ISSR31-2	2.072	-1.688	8.8	Badia
	qALNUF-3b	3	68	ISSR29-7 - ISSR31-5	2.802	-0.707	11.8	Badia
Treated with pesticides								
	qSTUF-3a	3	48	ISSR13-3 - ISSR47-8	2.57	-2.961	10.9	Badia
	qSTUF-6a	6	2	ISSR38-6 - Bmac310	4.025	-1.71	16.5	Badia
	qSTUF-6b	6	18	IRAP50-3 - ISSR48-3	2.303	-1.949	9.8	Badia
	qFLLUF-3	3	48	ISSR13-3 - ISSR47-8	2.2	-4.632	9.4	Kavir
	qFLLUF-7a	7	102	ISSR16-7- ISSR30-6	2.544	1.551	10.8	Badia
	qFLLUF-7b	7	106	ISSR30-6-ISSR22-2	2.413	2.525	10.2	Badia
	qFLWIUF-6	6	52	HVM65- Bmac0040	2.16	-0.672	9.2	Kavir
	qFLWEUF-4	4	2	ISSR31-6- ISSR16-8	3.935	0.194	16.1	Badia
	qGNUF-3	3	48	ISSR13-3-ISSR47-8	2.213	-11.546	9.4	Kavir
	qGWUF-1a	1	64	ISSR16-2- Bmag0718	3.06	0.272	12.8	Badia
	qGWUF-1b	1	66	Bmag0718-ISSR16-9	3.057	0.218	12.8	Badia
	qGWUF-3	3	48	ISSR13-3- ISSR47-8	3.809	-0.944	15.7	Kavir
	qSWUF-3	3	48	ISSR13-3- ISSR47-8	3.163	-0.945	13.2	Kavir

66, 48 cM in ISSR16-2-Bmag0718, Bmag0718-ISSR16-9, and ISSR13-3-ISSR47-8 marker intervals (Fig. 6).

To identify and map the dwarfism gene in 92 double haploid barley lines and its correlation with agronomical traits, Wang et al. (2010) identified two QTLs for spike emergence, two QTLs for spike length, one QTL for grain number, and one QTL for awn length on chromosome H3, which accounted for 70-81% of the relevant phenotypic variance of the trait. The results of Wang et al.'s (2010) work on chromosome #3 were consistent for awn length and grain number traits. In addition, Teulat et al. (2001a,b) identified two QTLs on chromosomes 3 and 4. The results of this study on chromosome #3 are consistent with the results of Teulat et al. (2001 a,b).

Discussion

The widespread use of various pesticides, including herbicides, insecticides, fungicides, and rodenticides, has long been a cause for concern for environmental pollution and endangering human health. Adverse effects of these compounds on living and non-living environment include accumulation and concentration of pesticides in the living body and entry into the food chain, and long-

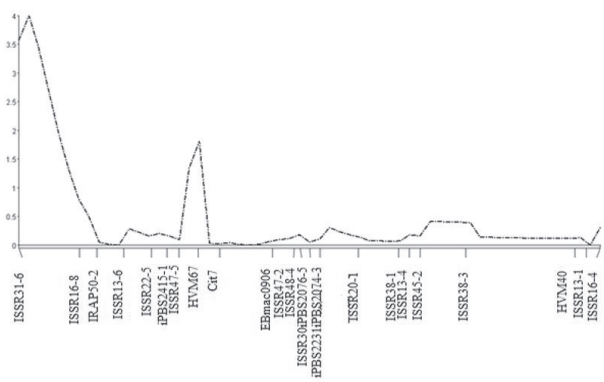

Figure 4. QTL mapping result for flag leaf length (on chromosome 3) in population where pesticides were not used.

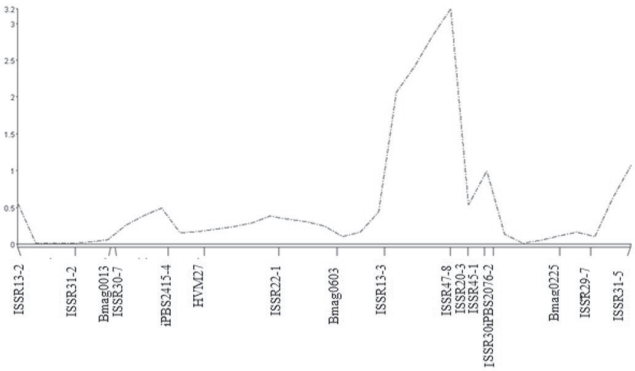

Figure 5. QTL mapping result for spike weight (on chromosome 3) in pesticide-treated population.

term contamination of water and soil resources with pesticides and their residues (Shaw et al. 1992; Newman 2008). The development of pesticide resistance in pest populations could increase with the elevated concentration of these compounds as well as with repeated applications (Hemingway and Ranson 2000; Brown and Pal 1971; Hemingway et al. 1992). The combination of different control methods, including environmental, biological, genetic, and chemical approaches, has been able to reduce environmental pollution and at the same time properly controls population of harmful pests.

This study sought to investigate the effect of pesticide application on the emergence and absence of QTLs in attribute controllers in barley. To do this, first a genetic map with molecular markers was developed. The obtained linkage map showed that the distribution of markers on linkage groups was not uniform and the highest number of markers belonged to linkage group #3 and the lowest number of markers to linkage group #5 (Fig. 3). Since the resulting map length was 617.5 cM and the interval between the two flanking markers was 5.41 cM, the resulting map was found to be suitable for gene mapping (Fig. 1).

The study identified 25 QTLs. Comparison of QTLs detected under both conditions, namely pesticide application and non-application showed that some chromosome regions play an effective role in controlling attributes under both conditions. For example, in ISSR13-3-ISSR47-8 and ISSR38-6-Bmac310 marker intervals, QTLs related to stem diameter, peduncle length, flag leaf length, and seed weight, were detected under both conditions; however, some regions played a role only in the absence of pesticide use, IRAP50-3-ISSR48-3, HVM65-Bmac0040. Therefore, it can be concluded that pesticide application is effective in the presence or absence of some genes in barley. The major-effect trait-controller QTLs concerned, and their associated markers can be used in MAS programs. The results showed that when plant populations are treated with pesticides, the expression of genes controlling traits is affected.

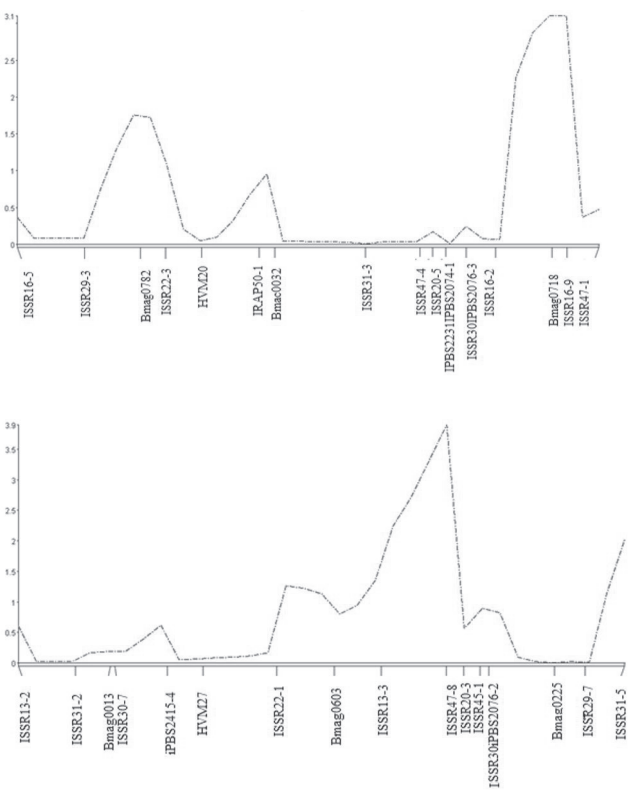

Figure 6. QTL mapping result for grain weight (on chromosome 1 and 3) in pesticide-treated population.

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