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QTL analysis for diamondback moth resistance in canola (*Brassica napus* L.)

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Abstract

Diamondback moth (DBM), *Plutella xylostella* L. is the most injurious defoliage insect pest of canola in Ardabil province of Iran. It occurs annually and causes damage in canola fields. This study was performed to identify QTLs controlling resistance to diamondback moth using SSR and RAPD markers. An $F_{2:4}$ population of 180 families derived from crossing between cv. 'SLMO46' and cv. 'Quantum' were used. The number of DBM eggs (EPL) and larvae per leaf (LPL) were recorded in each F_4 families on 10 randomly selected plants at rosette stage. The intensity of damage (ID) was scored as 0 to 4 according to the relative size of leaf eaten area. QTL analysis was performed using the previously constructed linkage map of SSR and RAPD markers. QTL mapping based on composite interval mapping (CIM) method identified seven QTLs for the studied traits. The explained phenotypic variance by the QTLs ranged from 13 to 35%. The QTLs showed positive and negative additive effects and inherited from both parents to the progenies. Three QTLs on linkage group three were common for LPL, EPL and ID indicating pleiotropic gene effect or linked genes for these traits. Two QTLs on linkage group 14 were also common between studied traits.

Keywords: Diamondback moth**;** *Brassica napus*; Microsatellite; QTL mapping; RAPD; Resistance.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a destructive insect pest on cruciferous crops with a cosmopolitan distribution (Talekar and Shelton, 1993; Li et al., 2000). This pest occurs annually in Ardabil province of Iran and causes economic damage in canola fields. Its natural host range is limited to cultivate and wild *Brassicaceae* that are characterized by having glucosinolates and sulfur-containing secondary plant compounds (Sarfraz et al., 2006). Host cues accelerated egg maturation, increased the incidence of mating and shortened the time between adult emergence and the onset of oviposition (Hillyer and Thorsteinson, 1971; Pivnick et al., 1990).

The exceptional status of this pest is due to the diversity and abundance of host plants, the lack or disruption of its natural enemies, its high reproductive potential (over 20

generations per year in the tropics) and its genetic elasticity facilitating rapid development of resistance to insecticides (Mohan and Gujar, 2003; Vickers et al., 2004). Therefore, there has been an increasing interest to developing and using resistant cultivars to DBM in canola.

Resistance of canola cultivars to DBM depends on the oviposition and feeding preference for the certain canola cultivars. For many plant feeding insects the selection of an oviposition site is a critical stage in their choice of host. This is especially true when the newly hatched offspring are not capable of searching for additional hosts or at least not until they have fed on the plant chosen by their mother (Singer, 1986). Certain plant characteristics including biochemical or morphological factors, or a combination of both may promote resistance (antibiosis, antixenosis, or both antibiosis and antixenosis) to *P. xylostella* (Painter, 1951; Thorsteinson, 1953; Reed et al., 1989; Eigenbrode and Espelie, 1995; Li et al., 2000; Marazzi et al., 2004; Sarfraz, et al., 2006). Certain glucosinolates, cardenolides, plant volatiles, waxes, as well as host plant nutritional quality, leaf morphology and leaf color, or a combination of these factors, may trigger reproductive and feeding activities of DBM (Justus and Mitchell, 1996; Badenes-Perez et al., 2004; Bukovinszky et al., 2005).

The number of eggs and larvae of DBM and the relative size of leaf eaten area by its larvae can be used as DBM resistance indices in canola. Plant genes participating in the recognition of DBM oviposition and feeding in concert with plant genes involved in defense against herbivores, mediate plant resistance to DBM. Several genes involved in plant disease and nematode resistance have been characterized in detail, but their existence has only recently begun to be determined for arthropod resistance (Smith, 2005; Boyko et al., 2006). Kliebenstein *et al*. (2002) reported four QTLs for resistance to *P. xylostella* L. in *Arabidopsis thaliana*.

The objective of the present study was to identify QTLs involved in resistance to DBM. By confirming of these QTLs in future studies, those could be used more efficiently in marker assisted selection for resistant varieties to DBM.

Materials and Methods

A set of 180 $F_{2:4}$ families of canola (*Brassica napus* L.) derived from cross between cv. SLMO46 and cv. Quantum was used as genetic materials. The parental lines differed in resistance to DBM. SLMO46 and Quntum cultivars were resistant and susceptible to DBM respectively. F_4 families were sown based on randomized complete block design with 2 replications in May 10, 2007 in Ardabil province, northwest of Iran (elevation from sea surface 1332 m; longitude 48^{o} 16$^{'}$ E; latitude 34^{o} 15$^{'}$ N). Each family was sown in a single row with 4 m long per replication, considering 40 and 10 cm space between rows and plants in each row, respectively. In July 2, 2007, ten plants were randomly selected and one outer leaf per plant was sampled and placed in vials containing 70% ethyl alcohol solution. EPL and LPL of DBM in each sample were counted under stereomicroscope. The intensity of damage (ID) was scored as 0 to 4 according to the relative size of leaf eaten area as follows: 0, no damage; 1, small spots with 0.1 - 0.5 mm in diameter between the marginal intervene; 2, medium spots with 0.6 - 1 mm in diameter between the marginal intervene; 3, large spots with 1.1 - 2 mm in diameter between the primary sub-vascular and 4, very large spots with

2.1 - 4 mm in diameter that extending around the primary subvascular. Previously constructed linkage map of SSR and RAPD markers (Asghari et al., 2008) was used to rescanning the canola genome for identification of QTLs controlling resistance to DBM. The DNA extraction and linkage map construction methods were published by Asghari et al., 2008.

QTL analysis was performed using composite interval mapping (CIM) method of QTL Cartographer v. 2.5 software (Basten, et al., 2001). Putative QTLs were chosen based on LOD score of 3.0 or above.

Results

The DBM resistance indices of the susceptible parent (Quantum), the resistant parent (SLMO46) and the minimum as well as maximum values of the traits in F_4 families were shown in table 1. Significant differences were observed between parents and F_4 families with respect to the studied traits ($P \leq 0.01$). Presence of transgressive segregation for all the studied traits indicates the combination of parental genes in the progenies (Table 1). Based on CIM, one, three and three QTLs were identified for ID, EPL and LPL, respectively. The percentage of explained phenotypic variance ranged from 13 to 35 % (Table 2).

Table 1. The amounts of measured traits in parents Quantum (Q), SLMO46 (S) and F_4 families.

Trait	Q	S	F_4 families			Families lower than S (%)	Families' upper than Q (%)
			Max	Min	Mean		
LPL	1.67	1	7.75	0	1.69	33	40
EPL	3.2	2.4	10.5	0.25	4.17	6	70
ID	2.83	1.57	4	1	2.09	19	9

Q: Qunatum; S: SLMO46; LPL: Larva Per Leaf; EPL: Eggs Per Leaf and ID: Intensity of Damage

Table 2. The linkage group, additive effect, peak position and LOD score of the identified QTLs and phenotypic variance of traits explained by each QTL.

Trait	QTL	Linkage group	Peak position (cm)	Interval (cm)	Flank markers	LOD	Additive effect	Explained phenotypic variance (%)
ID	1	3	30	16-56	528b-593b	16.4	0.71	13
EPL	1	3	34.7	26-45	528c-682a	10.2	3.5	26
	2	3	66	60-68	593b-682b	3.4	0.4	35
	3	14	13	10-16	Ol10D03-BN12AF	5.9	5.5	23
LPL	1	3	34.9	25-45	528c-682a	4.6	3.01	24
	2	7	44.1	43-55	536-547	3.7	-0.1	31
	3	14	13.9	10-14.5	Ol10D03-BN12AF	181	5.5	22

LPL: Larva Per Leaf; EPL: Eggs Per Leaf and ID: Intensity of Damage.

The detected QTL for ID located on linkage groups 3 (figure 1) and explained 13 % of the phenotypic variance of this trait. This QTL had positive additive effect (Table 2), indicating that the allele conferring resistance inherited from resistant parent to progenies. In linkage group 3, two QTLs with positive additive effect explaining 26 and 35% of EPL phenotypic variance as well as a QTL with positive additive effect explaining 24% of LPL phenotypic variance were co-located with the putative QTL for ID (Figure 1 and Table 2).

For six QTLs, recessive alleles inducing resistance to DBM were transmitted from resistant parent (SLMO46) to F_2 plants and F_4 families.

In linkage group 14, two QTLs were identified in the same genomic region with positive additive effects explaining about 23 and 22 % of EPL and LPL phenotypic variance, respectively (Figure 1 and Table 2). The recessive alleles in these QTL loci that increased resistance to DBM passed from resistant parent (SLMO46) to the progenies.

One QTL for LPL, with negative additive was detected in linkage group 7 which explained 31% of phenotypic variance. The dominant allele of this locus that increased resistance to DBM transmitted from resistant parent (SLMO46) to the offspring.

Discussion

The alleles of identified QTLs which increased resistance to DBM were transmitted to the progenies from both parents. Negative and positive additive effects of the QTLs showed that the dominant and recessive alleles controlled DBM resistance in canola. Kliebenstein et al. (2002) reported that in *Arabidopsis thaliana*, QTLs with positive and negative additive effects control resistance to *P. xylostella* L. In our study the numbers of recessive alleles were more than dominants ones and only one QTL with negative additive effect was detected indicating transmission of its recessive allele from susceptible parent (Quantum).

Table 3. The name, sequence, type and linkage group of adjacent markers with identified QTLs in $F_{2:4}$ population of canola cross 'SLMO46'×'Quantum'.

Markers name		Sequence	Marker type	Linkage group
Ol10-D03	Forward	5′- GCCAAAGACCTCAAAGATGG -3′	SSR	14
	Reverse	5′- AAGCCACGTGAAGAAAGTCC -3′		
BN12Af	Forward	5′-GCC GTT CTA GGG TTT GTG GGA-3′	SSR	14
	Reverse	5′-GAG GAA GTG AGA GCG GGA AAT CA-3′		
528	-	5′-GGA TCT ATG C-3′	RAPD	3
682	-	5′-CTG CGA CGG T-3′	RAPD	3
593	-	5′-CGA GCT TTG A-3′	RAPD	3
536	-	5′-GCC CCT CGT C-3′	RAPD	7
463	-	5′-AGG CGG AAG C-3′	RAPD	7
547	-	5′-TAT GAC CTG G-3′	RAPD	7

The explained phenotypic variance by each QTL ranged from 13 to 35% indicating the contribution of minor and major effects QTLs in controlling resistance to DBM in canola. The three detected QTLs for EPL explained 84% of variations in this trait. Also, 77% of LPL variations were explained by three other QTLs.

In recent years very studies were done in order to identify and characterization of QTLs and genes controlling signaling pathway and resistance to environmental stresses (Asghari et al., 2008; Navabpour et al., 2007). By confirming of these QTLs in next generations after several meiotic cycles or in other mapping populations, they could be used more efficiently in marker-assisted selection for DBM resistance in breeding programs.

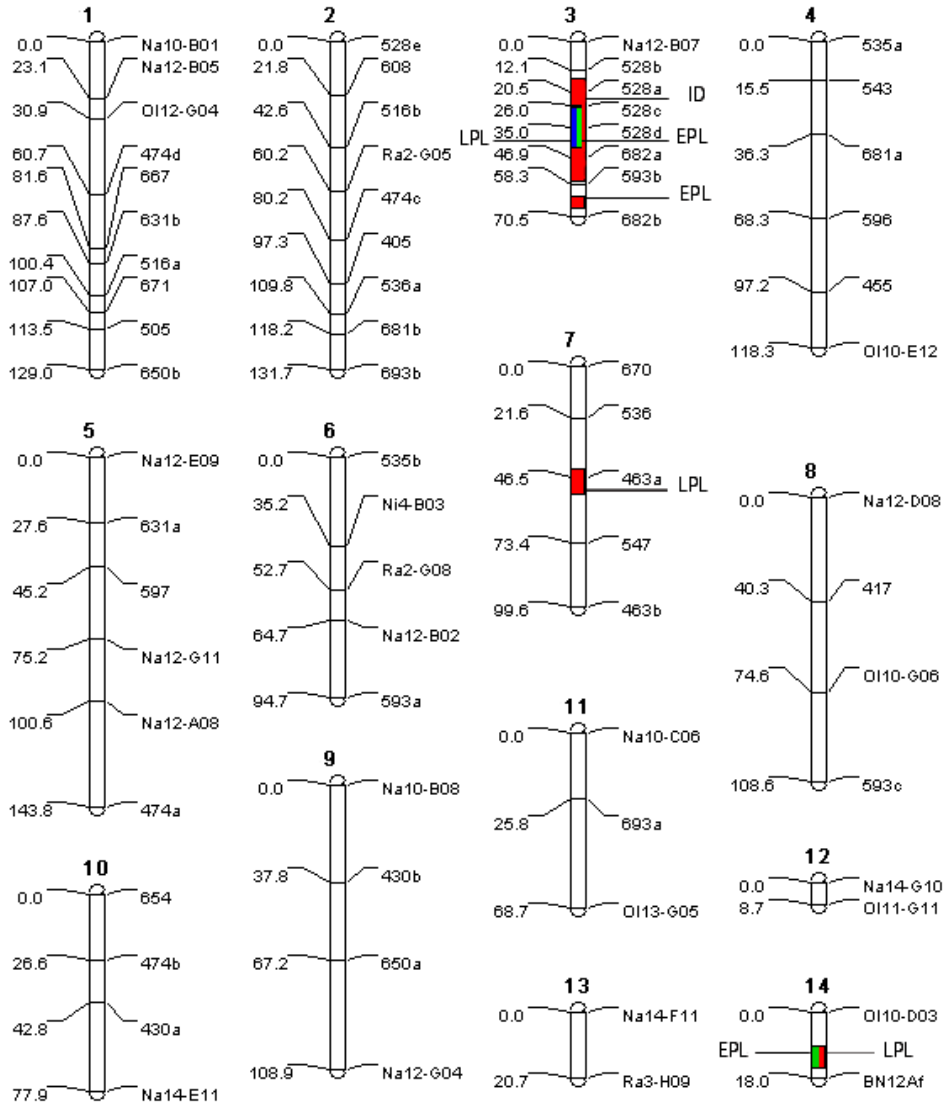

Figure 1. Linkage groups of SSR and RAPD markers (Asghari *et al*., 2008) and the position of QTLs controlling resistance indices (intensity of damage (ID); the number of larvae (LPL) and eggs per leaf (EPL) of diamondback moth in the $F_{2:4}$ populations of *Brassica napus*_cross 'SLMO46'×'quantum'. RAPD markers are shown with numbers and the name of SSR markers is the same as the name of these markers in Brassica data base. The sequences of flanking markers with identified QTLs are shown in Table 3. Map distances in cM were indicated on the left side of the linkage groups.

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References

Asghari, A., Mohammadi, S.A., Moghaddam, M., Toorchi, M., Mohammadinasab, A.D., 2008. Analysis of quantitative trait loci associated with freezing tolerance in canola (*Brassica napus* L.). Biotechnol. and Biotechnol. Eq. 22(1): 548-552.

Badenes-Perez, F.R., Shelton, A.M., Nault, B.A., 2004. Evaluating trap crops for diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). J. Econ. Entomol. 97: 1365–1372.

Basten, C.J., Weir, B.S., Zeng, Z.B., 2001. QTL Cartographer: a reference manual and tutorial for QTL mapping. North Carolina State University. USA.

[4] Boyko, E.V., Smith, C.M., Thara, V.K., Bruno, J.M., Deng, Y., 2006. The molecular basis of plant gene expression during aphid invasion: wheat Pto- and Pti-like sequences are involved in interactions between wheat and Russian wheat aphid (Homoptera: Aphididae). J. of Econ. Entomol. 99: 1430–1445.

Bukovinszky, T., Potting, R.P.J., Clough, Y., Van Lenteren, J.C., Vet, L.E.M., 2005. The role of pre- and post-alighting detection mechanisms in the responses to patch size by specialist herbivores. Oikos, 109: 435–446.

Eigenbrode, S.D., Espelie, K.E., 1995. Effects of plant epicuticular lipids on insect behaviors. Annu. Rev. Entomol. 40: 117–142.

Hillyer, R.J., Thorsteinson, A.J., 1971. Influence of the host plant or males on programming of oviposition in the diamondback moth (*Plutella maculipennis* (Curt.): Lepidoptera). Can. J. Zool. 49: 983–990.

Justus, K.A. Mitchell, B.K., 1996. Oviposition site selection by the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). J. Insect Behav. 9: 887–897.

Kliebenstein, D., Pedersen, D., Barker, B., Mitchell-Olds, T., 2002. Comparative analysis of quantitative trait loci controlling glucosinolates, myrosinase and insect resistance in *Arabidopsis thaliana*. Genetics, 161: 325–332.

Li, Q., Eigenbrode, S.D., Stringam, G.R., Thiagarajah, M.R., 2000. Feeding and growth of *Plutella xylostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. J. Chem. Ecol. 26: 2401–2419.

Marazzi, C., Patrian, B., Stadler, E., 2004. Secondary metabolites of the leaf surface affected by sulfur fertilization and perceived by the diamondback moth. Chemoecology, 14: 81–86.

Mohan, M., Gujar, G.T., 2003. Characterization and comparison of midget proteases of *Bacillus thuringiensis* susceptible and resistant diamondback moth (*Plutella xylostella*). J. Invertebr. Pathol. 83: 1–11.

Navabpour, S., Bagherieh-Najjar, M.B., Soltanloo, H. 2007. Identification of novel genes expressed in *Brassica napus* during leaf senescence and in response to oxidative stress. Journal of Plant Production, 1: 35-44

Painter, R.H., 1951. Insect resistance in crop plants. University of Kansas Press, Lawrence, KS, USA. PP. 234-238.

Pivnick, K.A., Jarvis, B.J., Gillott, C., Slater, G.P., Underhill, E.W., 1990. Daily patterns of reproductive activity and the influence of adult density and exposure to host plants on reproduction in the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Environ. Entomol. 19: 587–593.

Reed, D.W., Pivnick, K.A., Underhill, E.W., 1989. Identification of chemical oviposition stimulants for the diamondback moth, Plutella xylostella, present in three species of Brassicaceae. Entomol. Exp. Appl. 53: 277–286.

Sarfraz, M., Dosdall, L.M., Keddie, B.A., 2006. Diamondback moth-host plant interactions: Implications for pest management. Crop Protection, 25: 625-639.

Singer, M.C. 1986. The definition and measurement of oviposition preference in plant-feeding insects. In: T.A. Miller and J.A. Miller (Eds.), Insect-plant interactions, Springer-Verlag, New York. PP. 65-94.

Smith, C.M. 2005. Plant Resistance to Arthropods, Molecular and Conventional Approaches. Springer, Berlin, Germany.

Talekar, N.S., Shelton, A.M., 1993. Biology, ecology, and management of the diamondback moth. Annu. Rev. Entomol. 38: 275–301.

Thorsteinson, A.J., 1953. The chemotactic responses that determine host specificity in an oligophagous insect (*Plutella maculipennis* (Curt.) Lepidoptera). Can. J. Zool. 31: 52–72.

Vickers, R.A., Furlong, M.J., White, A., Pell, J.K., 2004. Initiation of fungal epizootics in diamondback moth populations within a large field cage: proof of concept of auto-dissemination. Entomol. Exp. Appl. 111: 7–17.