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MAPPING QTL(s) FOR RESISTANCE TO THE BEET CYST NEMATODE (*Heterodera schachtii* Schm.) IN RADISH (*Raphanus sativus* L.)

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ABSTRACT :

This is the first report of attempt to identify QTL(s) for the Beet Cyst Nematode (*Heterodera schachtii* Schm) resistant segregating in an F_2 population of the oil radish (*Raphanus sativus* L.) cross 'Pegletta x Silettanova'. A total of 290 F_2 individuals were derived from self-pollinating single F_1 plant of the cross 'Pegletta x Silettanova'. The F_2 individuals distributed in two discreet genotype classes, complying with the assumption of monogenic dominant inheritance of the BCN resistance in oil radish. Bulked segregant analysis (BSA) revealed 16 RAPD and AFLP markers differentiating the resistance and the susceptible bulks based on band intensity. The RAPD OPD-04-568 and the AFLP E39M48-268(D) markers were the most differentiated candidates be the resistant and the susceptible bulks. Quantitative trait analysis was applied using MAPQTL 4.0 computer software. The Kruskal-Wallis and IM results the identified one major QTL dispread on one arm of linkage group 6. Multiple QTL Model (MQM) analyses indicated that the BCN resistance is controlled by one major QTL on LG 6. Moreover, the RAPD marker OPD-04-568 and the AFLP marker E39M48-268(D) were candidate to flanking the QTL of BCN resistance. These markers explained 62% and 48% of the total phenotypic variability, respectively. The detected QTL explanted 83% of the total phenotypic variations. The implications of these findings for breeders are discussed.

INTRODUCTION:

The Beet cyst nematode (BCN), *Heterodera schachtii* Schm., is a serious threat in beet cultivating areas of the world. The BCN is a widespread parasite and the host-range includes many arable plant species among them horticultural crops belonging to several families (*Cruciferae*, e.g *Brassica*, *Raphanus* and *Sinapis* species; *Leguminosae* and C*henopodiaceae*, e.g. *Beta* species) (Baukolh, 1976; Lange *et al*., 1990; Raski, 1950). In Egypt, one of the major obstacles facing the agricultural production is the damage caused by the pests including nematode (El- Sherif, 1992). The genus *Heterodera* is becoming an important pest in Egyptian agriculture. It has been detected in most vegetable and field crops (e.g. legume crops and maize). The virtual damage and the yield losses are not assessed. However, in some individual fields and pot experiments it was observed that the yield losses reached up to 25% (El Sherif 1992).

Oil-radish (*Raphanus sativus* L.) and white mustard (*Sinapis alba*) are considered to be useful source of the resistance to BCN (*Heterodera schachtii* Schm) (Baukolh, 1976).

Therefore, many breeding programs have used oil radish cultivars to transfer the resistance to the commercial cultivars of the related *Brassica* species, e.g. oil-seed rape (Lelivelt *et al*., 1991; Levlivelt and Krens, 1992; Voss *et al*., 1999). Nevertheless, there are few number of investigations focused on the genetic behaviour of this pathogen in oil radish and white mustard. Baukolh (1976) studied the inheritance of the beet cyst nematode resistance in oil-seed radish. He reported that a major dominant gene controls the resistance trait. The resistance inheritance in white mustard (*Sinapis alba*) has not been described.

Recently, many PCR-based marker techniques were developed. These technologies encouraged and supported the geneticists and plant breeders to construct high-density genetic maps of different crop species. Moreover, there are numerous established studies for tagging and locating gene(s) controlling important traits of most economically important crops. Otherwise, DNA polymorphisms are now used extensively to elucidate the genetic nature of agriculturally important traits in a wide range of crops. For example, RAPDs, AFLPs were used efficiently to clarify the genetic behaviour of the fruit quality in tomato (Causse *et al*., 2002)

Bulked segregant analysis (BSA) is a powerful novel technique being used for identifying marker candidates linked to genes of agronomic treats (Michelmore *et al*., 1991). The method involves comparing two DNA pools of specific individuals from a segregating population. The number of individuals in each bulk depends on the type of marker techniques and the type of population used to construct the bulks. BSA is used successfully for identifying genes of qualitatively and quantitatively inherited traits (Ajisaka *et al*., 1999; Bryan *et al*., 2002). Our objective in the current study was to use this previously studied population to identify QTL(s) associated with the BCN resistance trait. Through the BSA we were able to identify RAPD and AFLP markers associated with the BCN resistance gene(s).

MATERIALS AND METHODS:

Plant material:

The F_2 segregating population was produced by self-pollinating single F_1 plant originating from the cross between two oil radish (*Raphanus sativus* L.) cultivars 'Pegletta' (P_1, resistant cultivar) and 'SilettaNova' (P_2, susceptible cultivar) (Kandeel *et al*., 2004).

BCN resistance testing:

The resistance test was done as described by Toxopeus and Lubberts (1979). Seeds from the P_1 (resistant parent), P_2 (susceptible parent), F_1 hybrid, F_2 and the *Brassica napus* cultivar 'Madora' populations were germinated in petri dishes on moist filter papers. Three days later, a total of 290, 30, 80, 80 and 240 from the total 720 seedlings of the F_2, F_1 hybrid, P_1, P_2 and the *Brassica napus* control, respectively, were transplanted individually into polyethylene folding boxes (40x20x120 mm) filled with 100 cm^3 of an autoclaved soil and sand mixed medium (1:1 by volume). Soil medium was mixed by ratio of 7:1 with clay growing stone (Leni, Gebr. Lenz GmbH, 51691 Bergneustadt, Germany). Because of the restricted seeds of the F_1 hybrid, 30 seeds were sown only in the first three containers. The boxes were subsequently arranged in 8 containers and placed into two growth chambers. The growth chamber programmed to provide 16 h lighting (5000 Lux) and 8 h darkness. The lighting (day) and the darkness (night) temperatures were 22°C and 18°C, respectively.

One week after transplanting, each plant was inoculated twice with a suspension of pre-hatched Juvenile II (J_2) larvae from *Heterodera schachtii* Schm. The first inoculation was performed one week after transplanting and each plant was inoculated with 1000 J_2 larvae (1 ml of the larvae suspension) on the right side of the plant place (approximately 2 cm from the plant base) at depth of 5 cm from the soil surface. Each plant was inoculated again at the next day with 1000 J_2 larvae on the left side of the plant and at the same depth. The infection was performed by 10 ml pipette.

Quantitative measurement of the visually resistance:

About six weeks after the inoculation, the plants were transferred with soil from the boxes into dishes filled with water and the plant development was observed. Poor developed plants were excluded from further analysis. Thereafter, the soil was carefully discarded from the roots. Then the plants were transferred separately into other dishes filled with water. The number of cysts on the periphery and the main roots was counted. Otherwise, the cysts number in the water was also counted and added to the cyst number on the roots to calculate the total number of cysts per plant. A binocular microscope was used to estimate number of cysts on the roots and in the water.

Generation means, standard error and variances were determined from individual plants within each experimental unit and pooled over replications.

Molecular marker analysis:

1- Bulked Segregant Analysis (BSA):

Bulk segregant analysis (Michelmore *et al*., 1991) was used to identify RAPD and AFLP markers linked to the nematode resistance gene(s). The 24 F_2 individuals showing the lowest and highest number of cysts was selected to compose the resistant and susceptible bulks, respectively. The bulks were constructed by mixing equal DNA amount from each selected F_2 individual. The pair of bulks was screened with 60 RAPD primers and 72 AFLP *Eco*RI+3/*Mse*I+3 primer combinations (PCs) to identify the candidate markers revealing clear qualitative polymorphisms (presence or absence of bands) and/or revealing quantitative polymorphisms (band intensity differences) between the bulks.

2- Map construction:

The existed RAPD and AFLP integrated map for the same mapping population (Mousa, 2004) was used. The map was reconstructed after excluding RAPD and AFLP markers that were generated using a sub-set of the mapping population (64 F2 individuals) and/or has shown more missing genotypes. Therefore, this map consisted of 391 markers (66 RAPDs and 325 AFLPs) distributed over 9 LGs. The map covered a total of 1070 cM of the radish genome with a mean distance 2.73 cM between adjacent markers.

3- QTL analysis:

MAPQTL 4.0 software (Van Ooijen and Maliepaard, 2001) was used to perform the quantitative trait loci analysis of the Beet cyst nematode resistance. The program uses the trait means and marker data in the non-parametric Kruskal-Wallis test (Lehmann, 1975) in order to search each locus separately for segregant QTL(s). Marker-QTL(s) associations are detected based on the differences level in average rank between the marker genotype classes. Stepwise, MAPQTL 4.0 applied Single-QTL Analysis Model using interval mapping

(IM) function to the trait means and marker data. This method calculates the QTL likelihood (LOD score) for each position on the genome for the presence of a segregate QTL (Van Ooijen, 1992). The position on the target linkage group that revealed largest LOD score value is the estimated position of the QTL(s). Finally, Multiple-QTL analysis Model (MQM) (Jansen, 1994; Jansen and Stam, 1994) is applied for precisely detection putative QTL(s). MQM consists of two steps: 1) called *'restricted MQM mapping*' and comprises the selection of markers to be used as cofactors and 2) called *'MQM mapping*' and consists of the estimation of putative QTL(s) effects on the quantitative trait throughout the genome with correlation to the cofactor markers effects (Voorrips *et al.*, 1997). The cofactor markers are selected based primarily on the results from Kruskal-Wallis test and the Interval mapping. It is advisable to select the markers that are located nearby the most closely linked markers to the putative QTL(s) as cofactors (Van Ooijen and Maliepaard, 2001). As a last step, we selected two markers left and right of the putative QTL position (with at least 2.0 LOD less than the highest LOD value) as support intervals. This has been done to obtain a 0.95 confidence interval around the putative QTL location.

RESULTS:

Resistance test:

Tables (1, 2) show the reaction of the four oil radish (*Raphanus sativus* L.) populations P_1, P_2, F_1 and F_2 and one B.n.cv. 'Madora' (susceptible control), to the BCN parasitism. The mean number of cysts on P_1, F_1, P_2, F_2 and 'Madora' root systems was 4.12±0.534, 15.73±1.68, 135.53±7.22, 24.57±2.02 and 157.3±3.23, respectively. The number of cysts ranged from 0 to29, 56 to 349, 3 to 34, 0 to 217 and 39 to 298 for the P_1, P_2, F_1, F_2 and the *B. n.*cv. 'Madora', respectively.

Frequency distributions for the number of cysts are presented in Fig. (1A, B and C). The majority of the resistant parent (Pegletta) plants (93%, 68 plants from total 73) belonged to the cysts class 0-10, 4 plants (5.5%) belonged to the cysts class 11-20 per plant and only 1 plant (1.4%) contained 29 cysts (Fig. 1A). On the contrary, 71.8 percent of the susceptible parent plants (46 plants of total 64 plants) showed more than 100 cysts/plants and 28.2 percent had from 50-100 cysts/plant. All F_1 plants were in the resistant parent classes. With respect to the distribution of the F_2 population, a bimodal distribution was observed (Fig. 1C). The results showed that 183 F_2 plants (74.7% of the total 245 individuals) had from 0 to 27 cysts/plant, 25 plants (10.2%) exhibited from 28 to 50 cysts/plant and 37 plants (15.1%) exhibited from 51 to 250 cysts/plant. Obviously, small portion of the F_2 individuals contained a cysts number comparable to the susceptible parent.

Bulked segregant analysis:

DNA samples from the resistant parent, the susceptible parent and both the resistant and susceptible F_2 bulks were screened with 60 and 40 pre-selected RAPD and AFLP primers and PCs, respectively. The results showed that 15 RAPD and 6 AFLP markers generated polymorphic patterns differentiating the two bulks on the base of bands intensity (Fig. 2). We did not observe either RAPD or AFLP candidates that qualitatively distinguished the resistance and susceptible bulks (present in one bulk only). However, the RAPD candidate OPD-04-568 and the AFLP candidate E39M48-268 revealed the clearest band intensities differences between the two bulks, suggesting that both candidates were the nearest markers from the BCN resistance locus.

Table (1): The number of the used radish (*Raphanus sativus* L.) plants for different populations (P_1, P_2, F_1, F_2 and cultivar 'Madora') in determining the resistance to the Beet Cyst Nematode (BCN).

Replications	Populations[a]	Total plants No.	Excluded plants	Excluded plants[b] %	Used plants
1	P1	10	0	0.00	10
	P2	10	3	30.00	7
	F1	10	3	30.00	7
	F2	30	5	16.67	25
	Madora	30	3	10.00	27
2	P1	10	1	10.00	9
	P2	10	2	20.00	8
	F1	10	1	10.00	9
	F2	30	6	20.00	24
	Madora	30	4	13.33	26
3	P1	10	1	10.00	9
	P2	10	2	20.00	8
	F1	10	0	0.00	10
	F2	30	6	20.00	24
	Madora	30	5	16.67	25
4	P1	10	0	0.00	10
	P2	10	3	30.00	7
	F2	40	5	12.50	35
	Madora	30	5	16.67	25
5	P1	10	1	10.00	9
	P2	10	2	20.00	8
	F2	40	7	17.50	33
	Madora	30	1	3.33	29
6	P1	10	1	10.00	9
	P2	10	0	0.00	10
	F2	40	6	15.00	34
	Madora	30	3	10.00	27
7	P1	10	1	10.00	9
	P2	10	2	20.00	8
	F2	40	5	12.50	35
	Madora	30	4	13.33	26
8	P1	10	2	20.00	8
	P2	10	2	20.00	8
	F2	40	6	15.00	34
	Madora	30	2	6.67	28

[a] P_1= 'Pegletta', P_2 = 'Siletta nova', F_1= 'Pegletta x Siletta nova', F_2 = the segregating population (obtained from selfing F_1), and 'Madora' = *Brassica napus* susceptible control.
[b] omitted due to poor development of root system.

Table (2): Range of cysts, standard error, average number of cysts and variance of generations P_1, P_2, F_1, F_2 and susceptible control *Brassica napus* cv. 'Madora'.

Population[a]	No. of plant	Range of cysts No.		Mean Cyst No.	Variance
		Min	Max		
P1	073	00	029	04.12±0.53[b]	0020.8
P2	064	56	349	135.5±7.20	3333.3
F1	026	03	034	015.7±1.70	0073.4
F2	245	00	217	024.6±2.02	0999.7
Madora control	212	59	298	157.3±3.23	2212.2

[a] P_1= 'Pegletta', P_2 = 'Siletta nova', F_1= 'Pegletta x Siletta nova', F_2 = the segregating population (obtained from selfing F_1), and 'Madora' = *Brassica napus* susceptible control.
[b] standard error

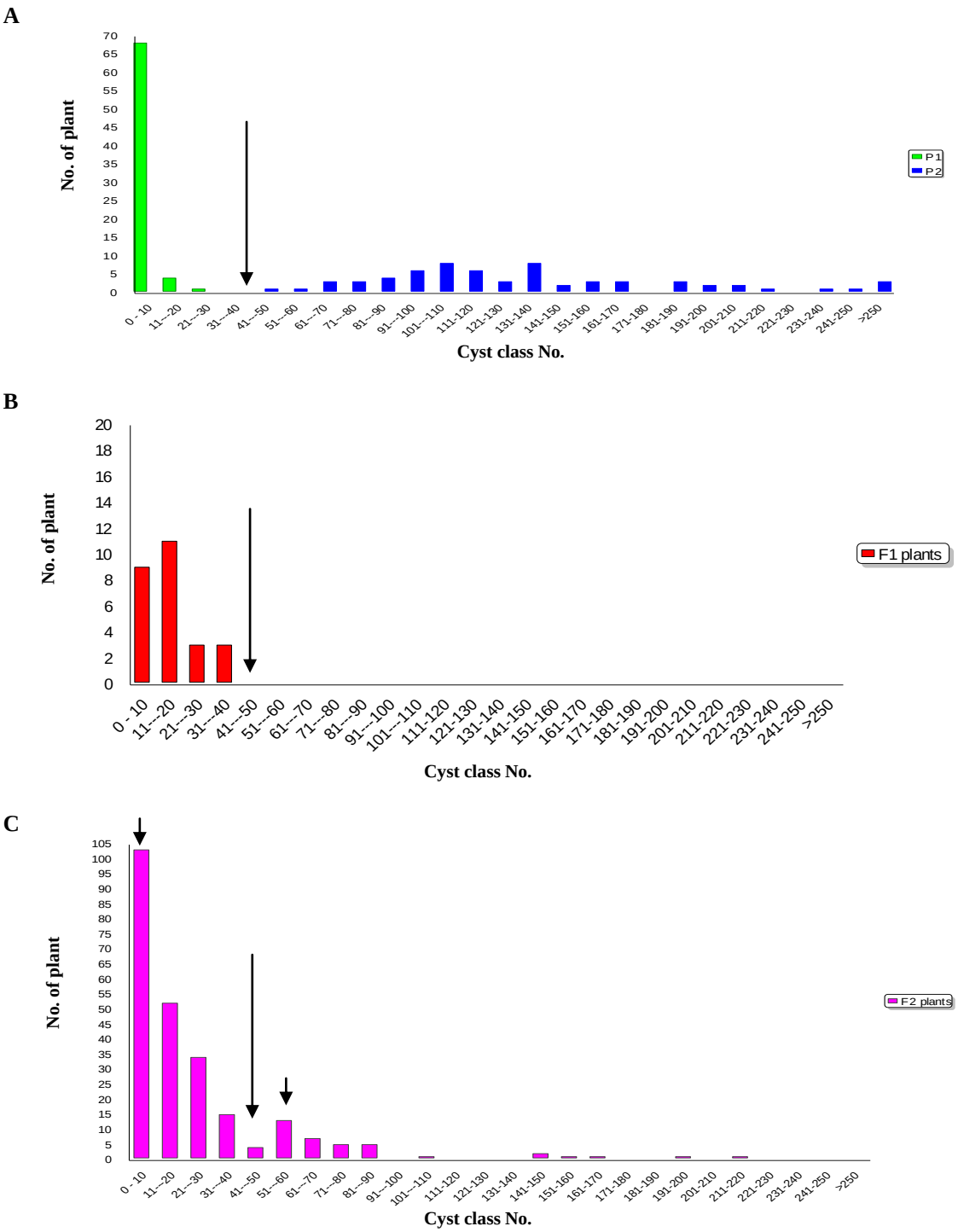

Fig. (1): The distribution of the reaction of *Raphanus* populations to the BCN parasitism: A) the parental 'Pegletta' and Siletta nova' cultivars, B) the F_1 hybrid from the intra-specific cross 'Pegletta x 'Silleta nove' and C) the segregating F_2 population. Short arrows refer to the distribution peaks while the long arrow points to the position of the threshold class.

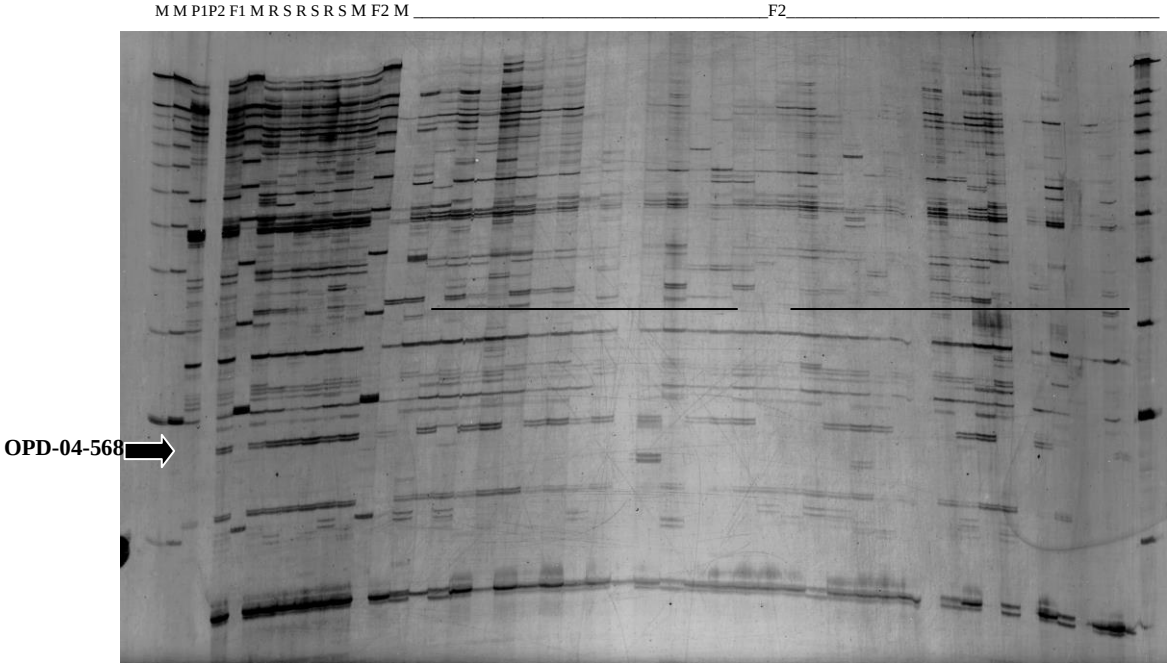

Fig. (2): DNA amplification patterns obtained with RAPD primer OPD-04 on DNA samples of the resistant and susceptible parents (P_1 and P_2), F_1 hybrid (F_1), resistant and susceptible bulks (R and S) and a sub set of F_2 population. The locus OPD-04-568 revealed a quantitative differences (band intensity differences) between both bulks and segregated in the F2 population. The molecular weight is evaluated with the 100-bp DNA ladder from Gibco-BRL (Bethesda, MD, USA).

QTL analysis:

Mapping for target linkage group:

For QTL analysis, only RAPD and AFLP markers that generated by the whole mapping population were used. This was done to either prevent or reduce false marker-QTL associations. The results obtained from the non-parametric Kurskal-Wallis test suggested the existence of QTLs for the resistance to *Heteodera schachtii* in LG6 (Table 3). The range of probability values suggested the existence of three QTLs distributed over a great number of markers on LG6. The first one is located near the AFLP marker E42M49-250, the second is located nearby the AFLP marker E41M60-484 at the centromeric region and the third appeared to be spread over a large number of markers that located at the telomeric region of LG6 starting from the AFLP marker E40M50-410 to the RAPD marker OPD-04-568. The 3 QTLs spanned more than 74% of the length of LG 6. Otherwise, 13 unmapped markers showed significant differences in the number of cysts between each marker genotypes ($P<0.005$). From these markers, the AFLP marker E39M50-142 and the RAPD marker OPI-17-644 showed the greatest Kruskal-Wallis values (14.41 and 19.62, respectively). These results suggested that both markers perhaps associated with the BCN resistant QTL.

Table (3): Testing the differences between marker phenotypic classes for linkage group 6 using the non-parametric Kurskal-Wallis test as a first indication for markers-QTL(s) accessions.

Map distance	Markers	Phenotypic classes		K*
		Pegletta[a]	Sillta nova[b]	
0.0	*OP-17-660*	20.75	25.85	0.595
12.4	*E38M59-850*	18.56	25.96	0.170
22.4	*E42M59-396*	22.29	32.26	3.375*
24.7	*E35M59-345*	22.63	29.87	0.541
25.4	*OPH-15-250*	23.98	24.54	0.000
26.5	*E42M60-750*	21.80	30.67	2.744
27.4	*E40M59-395*	22..41	27.48	0.238
29.4	*E33M50-147*	21.77	34.26	2.913*
33.5	*E42M60-190*	21.05	33.48	4.464**
37.6	*E42M49-250*	20.22	40.52	11.083*****
40.0	*E41M60-618(D)*	20.49	41.08	10.542****
42.3	*E39M50-148*	21.57	34.07	2.071
45.3	*E41M60-484*	19.76	44.04	14.965******
48.0	*OPE-19-462*	21.25	35.51	3.292*
59.0	*E40M50-410*	19.45	33.81	14.170******
69.2	*E33M59-265*	19.93	41.02	13.045******
78.1	*E33M62-141*	12.78	27.85	16.328*******
85.3	*OPH-03-780*	21.79	43.53	9.961****
93.0	*E41M61-479*	16.50	28.60	13.682******
99.9	*E32M48-430*	16.87	27.26	7.859***
106.5	*E39M48-268(D)*	16.06	27.28	10.798****
115.9	*OPD-04-568*	12.34	27.97	13.760******

[a] = Mean number of cysts for the resistance genotypes
[b] = Mean number of cysts for susceptible genotypes; *, **, *, ****, *****, ******, ******* significant at P<0.05, P<0.01, P<0.001, P<0.0001, P<0.00001, P<0.000001 and P<0.0000001, respectively.**

Mapping the nematode resistance gene (HsI^{Raph}):

In order to detect more accurate and rigorous QTL(s) for the BCN resistance trait, a Single-QTL Model analysis was applied for each single locus using the Interval Mapping (IM) option of the MAPQTL 4.0 software. The interval mapping analysis is searching for markers-QTL associations based on the algorithm of odd (LOD score) values of each single locus. The results presented in Fig. (3) show that the highest LOD score values were observed only for the markers assigned to LG6. The LOD values and the explained variance of the markers on LG6 suggested the existence of single QTL for the BCN resistance. This QTL spread over great number of markers that located at one side of the chromosome. The AFLP marker E33M60-141 showed the largest LOD value (LOD=7.14) and explained 14.9% of the total phenotypic variations. On the other hand, all unmapped markers (that were suggested to associate with the BCN resistance QTL in Kurskal-Wallis test), revealed no

association phenomena with the QTL of the resistance trait. Only one exception was observed for the unmapped RAPD marker OPI-17-644 which shown a LOD score value of 5.18 and explained 10.9% of the total phenotypic variance suggesting the reliable association between this marker and the BCN resistance gene.

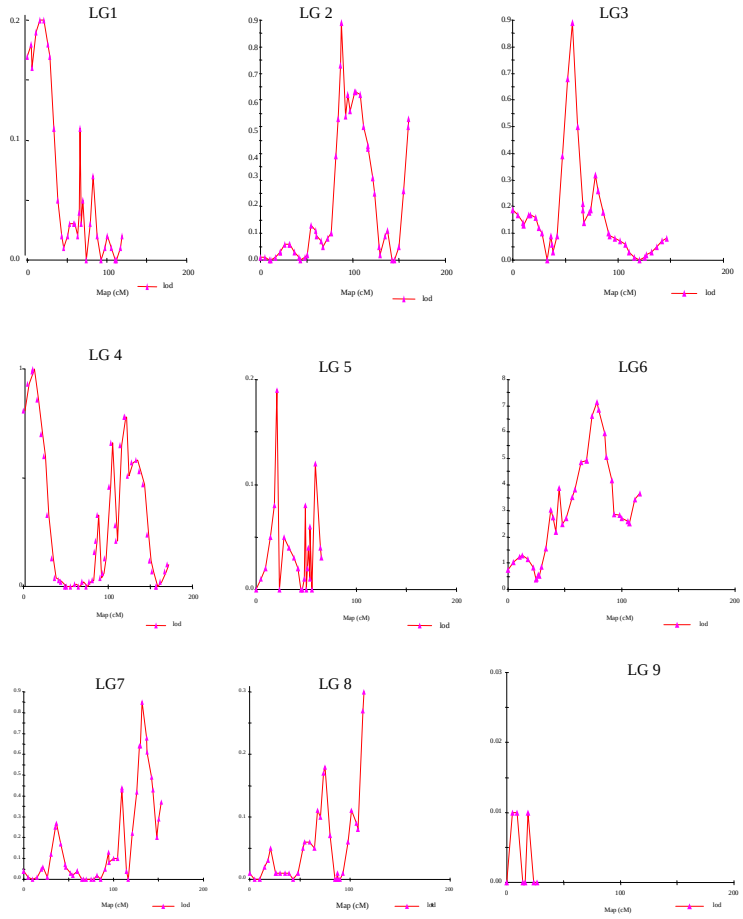

Fig. (3): Interval mapping analysis (Single QTL model) applied on linkage groups of the *Raphanus* re- constructed combined map to detect markers-QTL(s) associations based on the LOD score values. X axes represent the map distance in centiMorgen (cM) and the Y axes represent the LOD score values.

Multiple-QTL Model (MQM) analysis was applied to the trait of cysts count and the marker data in order to identify stable QTL for the BCN resistance. In addition, to facilitate rediscovering of other QTLs in LG6 and/or in other LGs using powerful selected cofactor markers. The MQM analysis was carried out by MAPQTL 4.0 software during two major steps. First, 2 makers were selected as a cofactors; E39M48-268(D) and OPD-04-568, which were located on LG6. Second, the LOD score values of possible QTLs throughout the genome were determined. The MQM results were highly consistent with that obtained from the IM analysis with respect to the number of discovered QTLs. As presented in Fig. (4) one major QTL located at the telomeric region of LG6 was distinguished. The target markers E39M48-268(D) and OPD-04-568 explained 48% and 62.2% of the total phenotypic

variations, respectively (Table 4). Therefore, both markers were suggested to be tightly associated with the QTL of the BCN resistance in the repulsion phase. This QTL explained a total of 83.6% of the phenotypic variance.

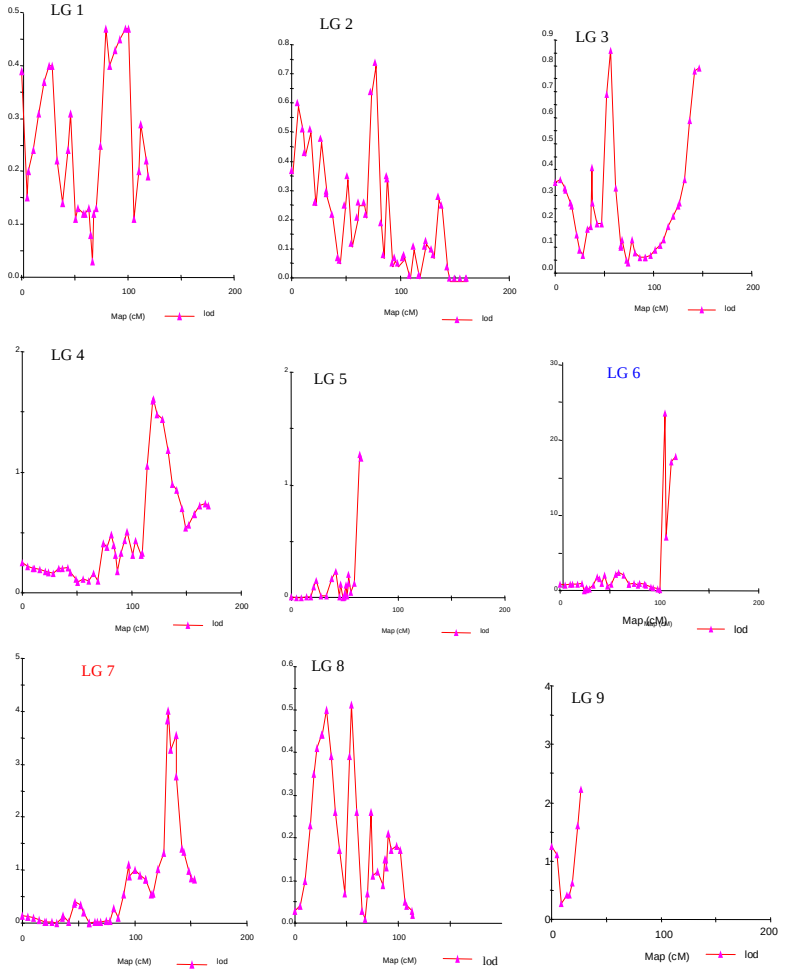

Fig. (4): Localization of putative QTL(s) for BCN resistance gene(s) on *Raphanus* genome using approximate Multiple-QTL Model (MQM) analysis. OPD-04-568 and E39M48-268(D) and E40M50-410 markers at linkage group 6 are used as a cofactors. The X and Y axes represent the Map distance (cM) and the LOD score values, respectively.

Table (4): RAPD and AFLP markers detected to flank with the HsI^{Raph} gene for resistance to the BCN

Markers	LG	LOD	Phenotypic segregation		explained variance%	Additive effect	Dominant effect
			Resistance	Susceptible			
*E39M48-268(D)**	6	7.16	65	176	48	-38.62	-37.1
*OPD-04-568**	6	17.8	53	188	62.2	-48.7	-42.1
Total[a]					83.6		

*** = Markers selected as cofactor.**

[a] = Total phenotypic variances explained by both markers as calculated by MapQTL program.

On the other hand, using tow markers as cofactors permitted to discover another minor QTL at LG7 nearby the AFLP markers E41M48-260, E39M48-260 and E35M59-192 (Fig. 5). The reliability and stability of the minor QTL on LG7 were extensively investigated. The results suggested a random association between these markers and the QTL for the resistance to BCN (Table 5).

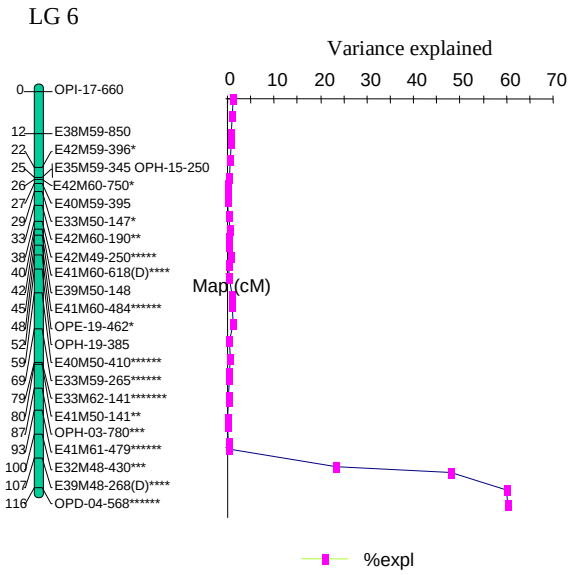

Fig. (5): Approximate Multiple-QTL Model (MQM) for analysis of target linkage group 6 to estimate the percentage of phenotpic variations explained by the linked markers with QTL of BCN resistance trait. OPD-04-563 and E39M48-268(D) markers were selected as cofactores. The asterisks represented the significance level between marker genotype classes revealed by Kruskal-Wallis test.

Table (5): Analysis of the probability of second QTL in linkage group7 detectedusing 2 cofactor markers.

Marker	Kruskal Wallis test			Interval mapping		MQM analysis					
	Phenotypic classes		K*	LOD	% of explained variance	Genotypes			% of explained variance	Additive effect	Dominant effect
	A-[a]	aa[b]				A[c]	H[d]	B[e]			
E41M48-260	20.52	25.81	3.9**	0.64	1.6	36.9	37.0	67.3	6.6	-15.2	-15.1
E39M48-260	19.83	26.1	4.9**	0.64	1.6	36.7	37.1	67.4	6.7	-15.4	-15.0
E35M59-192	21.1	25.7	4.5**	0.61	1.5	36.7	36.7	65.5	6.8	-14.4	-14.4

[a] = Mean number of cysts for the resistance genotypes.
[b] = Mean number of cysts for the susceptible genotypes.
[c] The homozygous genotypes for the resistance parent.
[d] The heterozygous genotypes.
[e] The homozygous genotypes for the susceptible parent.
** significant at P ≤ 0.01

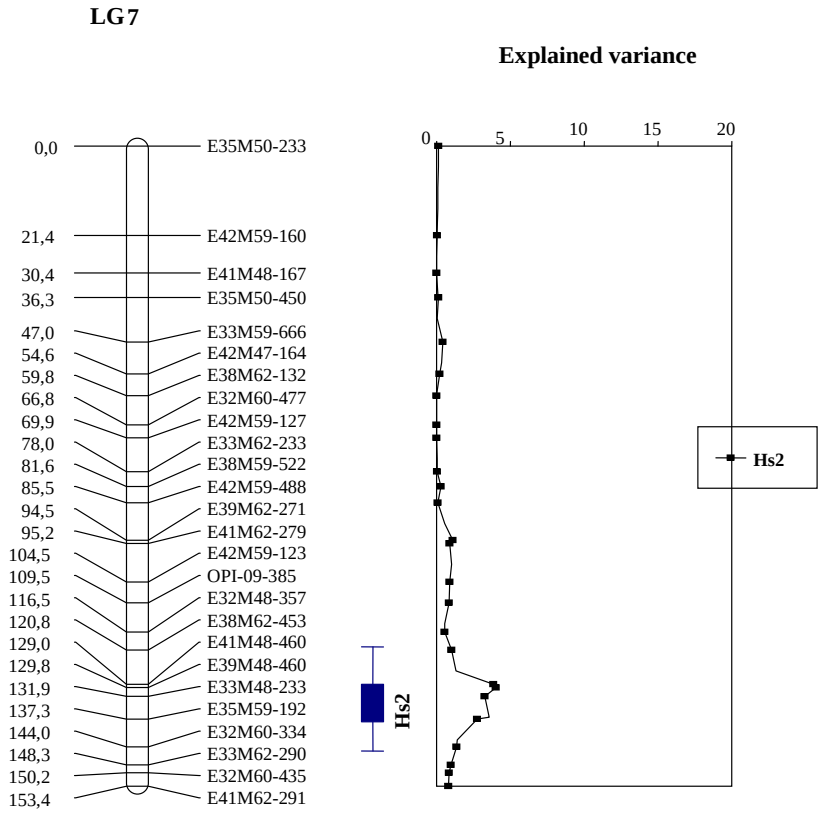

Fig. (6): Probability of second minor QTL located on linkage group7 for the BCN resistance.

DISCUSSION:

BCN resistance trait:

The average cysts count for 'Pegletta' was 4.12, with a standard error of ±0.53, whereas the average cysts count for 'Silettanova' was 135.53, with a standard error of ±7.2. These results reflected the distinct genetic background between the two parents in the BCN resistance which is considered to be important criteria for gene mapping (Paterson *et al.*, 1991). The F_1 plants were quite comparable to the resistant parent both in mean and phenotypic variance. All F_1 plants were appeared in the resistance parent classes. These results indicate the presence of dominance for the BCN resistance gene which was consistent with that reported by Baukolh (1976). The F_2 population showed a clear bimodal shape distribution, suggesting the distribution of at least one gene with major effects, rather than strict quantitative control. Moreover, the phenotypic analysis of the F_2 population indicated that the resistance to the BCN is governed by single major gene with dominance of the allele contributed by the resistance parent (Baukolh, 1976; Lelivelt and Hoogendoorn, 1993).

Efficiency of Bulked Segregant Analysis (BSA):

The resistant and susceptible bulks were constructed by mixing equal amounts of DNA from the individuals with the extreme resistant and susceptible phenotypes. The usage of BSA technique facilitates the identification of molecular markers linked to the BCN resistance

gene. It permitted to remove the randomized genetic background of the unlinked loci and, therefore, allowed to detect the genomic region(s) associated with the BCN resistance (polymorphic DNA fragments between the bulks) (Mansur *et al*., 1993; Michelmor *et al*., 1991). In the present study, the resistance and the susceptible bulks were constructed from the 24 F_2 plants with the extreme phenotype of the resistant and the susceptible parents. We decided to use a large number of the F_2 individuals in each bulk to decrease the probability of false results (false linkage) that will arise from using small number of individuals. Moreover, the large number of individuals may increase the probability that the two pools will not differ for alleles other than the target once of the trait of interest (Chague *et al*., 1996). Nevertheless, we observed neither RAPD nor AFLP candidates clearly distinguished the two bulks (i.e. generated bands present in one bulk only). This perhaps can be attributed to two reasons: 1) one of the two DNA bulks or may be both comprised some recombinant F_2 plants (Ling *et al*., 2000), and 2) the distance between the RAPD and AFLP markers and the target locus was more than 25 cM, therefore, they differentiated the resistant and susceptible bulks based on the band intensity. Michelmore *et al*. (1991) studied the effectiveness of bulked segregant analysis to detect RAPD and RFLP markers linked within 30% window of recombination frequency. The results showed that RAPD markers were always detectable between the bulks when they were 10 cM around the target locus. In addition, RAPD markers were also detected as unequal band intensity by bulk segregant analysis when they were 30 cM around the target locus. For RFLP markers, bulked segregant analysis detected RFLP markers as unequal band intensity when they linked at 20 cM around the gene, whereas the RFLP markers linked at 30 cM around the gene have not been detected by bulked segregant analysis.

The suitable number of individuals to be used in constructing the bulks differs based on two criteria: 1) the type of marker technique (dominant or co-dominant) and 2) the mapping population (e.g., F_2, backcross, DH, RIL population) (Michelmor *et al*., 1991). For instance, a small number of individuals for each bulk are recommended to use when a dominant markers are used with an F_2 population. However, the frequency of false positive increases as the number of individuals used to construct the bulks decreased (Michelmor *et al*., 1991). Ling *et al*. (2000) observed that the RAPD markers OPW14800 and OPC17750 were present in the resistant bulk and absent in the susceptible bulk, when both bulks were constructed from 6 plants of each. By increasing the number of individuals to 15 plants in each bulk, OPW14800 and OPC17750 generated much weaker polymorphic bands from the susceptible bulks than that produced by the resistant bulk. They attributed these results to the recombinant individuals presented in the susceptible bulk as a consequent of increasing the number of individuals in each bulk.

In the present study, two RAPD (OPD-04-568) and AFLP E39M48-268 markers were distinguished between the susceptible and the resistant bulks (based on the band intensity) at a fragment size of 586 and 268 bp, respectively. The results showed that both markers were co-segregated according to the expected segregation ratio of dominant monogenic inheritance. We suggest that these markers are linked to a component of the BCN resistance. In order to clarify the effects of these markers on the phenotypic variance of the resistant trait and to study precisely the genetics of the resistance, a QTL analysis was carried out using the molecular and the quantitative data.

QTL analysis:

To our knowledge, this is the first report attempt to map the BCN resistance gene(s) in oil radish. The QTL analysis was done using the quantitative data collected from the resistance test and the combined map that re-constructed after the exclusion of the RAPD and AFLP markers with much missing genotypes. The missing genotype markers were reported to increase the false marker-QTL associations (Causse *et al.*, 2002; Van Ooijen and Maliepaard, 2001). The non-parametric Kruskal-Wallis test was used for primarily detection of marker-QTL(s) associations. Because of the fact that Kruskal-Wallis test uses the linked and unlinked markers, it is preferable to increase the significant threshold to reduce as possible the false positive (Bryan *et al.*, 2002; Van Ooijen and Maliepaard, 2001). Therefore, we used a significant threshold at P< 0.005 for marker-QTL associations in order to obtain a confidence level of 0.95 throughout the genome. The Kruskal-Wallis test revealed at least 3 different QTLs all are located at the arm of LG6 of the reconstructed combined map. Moreover, there were significant differences between the resistance and the susceptible genotypes of 13 unmapped markers that supported the effect of these markers in the resistance trait. The Kruskal-Wallis test is a simple helpful to screen all marker sets using the background of the quantitative data to perform a primarily knowledge about the marker-QTL(s) associations. However, the test results are unreliable and further investigations using rigorous linkage analysis systems are required, in order to obtain a confidence level of 0.95 throughout the genome.

A more accurate QTL analysis was carried out using the Interval mapping (IM) option of the MAPQTL 4.0 software. The IM is a single-QTL model, searching each single locus (based on the LOD score values) for association with the QTL(s) of the BCN resistance. The results of the IM analysis were in contrast with the Kruskal-Wallis test. The IM analysis suggested the existence of one major QTL spread over a large number of markers assigned at the telomeric region of LG6. The greatest LOD score values were found at one arm of LG6. Moreover, only one unmapped marker (OPI-17-644) out of 13 (revealed from Kruskal-Wallis test) was observed to associate with the QTL of the BCN resistance. The MQM results were similar to that obtained from the IM mapping analysis. As presented in Fig. (IV-5) the markers mapped at the arm of LG6 showed the greatest LOD values. The LOD values of all remained linkage groups were too low in comparison to the LOD values of LG6. These results suggested the existence of one major QTL on LG6 at the telomeric region. The OPD-04-568 and E39M48-268(D) that were previously appointed out from the BSA (have shown differences on the band intensity between the resistance and the susceptible bulks) were observed to explain 48% (E39M48-268(D) and 62.2% (OPD-04-568) from the total phenotypic variance in the MQM analysis. These results reflect the contribution of BSA for mapping the BCN resistance gene (HsI^{Raph}). The two markers (E39M48-268(D) and OPD-04-568) that located near the major QTL showed the most significant differences between the homozygous resistance and susceptible genotypes, 16.6 and 93.8 (77.44 cysts) for E39M48-268(D) and 17.8 and 110.0 (92.2 cyst) for OPD-04-568, respectively. Moreover, E39M48-268(D) and OPD-04-568 segregated according to the expected 1:3 ratio, 65:176:4 (Pegletta : Sillta nova : missing, X^2 = 1.9, P>0.1) and 53:188:4 (Pegletta : Sillta nova : missing, X^2 = 1.7, P>0.1), respectively. These results suggested that both markers flanked the QTL of the BCN resistance

in the repulsion phase. This QTL explained 83% of the total phenotypic variations.

The stability of the minor QTL at LG7 was tested. The differences between marker genotypes were less significant than our threshold for the marker-QTL associations (P<0.005). The LOD values of E41M48-260, E39M48-260 and E35M59-1920 (the markers that associated with the minor QTL at LG7) were 0.64, 0.64 and 0.61, respectively. MQM analysis showed that the variance explained by E41M48-460, E39M48-460 and E35M59-192 were 6.6%, 6.7% and 6.8%, respectively. All these results suggested a random association. Instable QTLs were observed for the blackleg resistance in oilseed rape (Pilet *et al*., 2001). The authors attributed the instability of the detected QTLs to: 1) the genetic background of the crossed parent, 2) the infestation level of the pathogen and 3) the genome coverage by the constructed genetic map.

The present study described the characterization and mapping of BCN resistance gene in oil radish. It represents a step towards isolation and integration of this gene to the economically important related crops. Two DNA markers OPD-04-568 and E39M48-268(D) flanked the resistance gene in the repulsion phase. Both markers can be usefully utilized in marker assisted selection for the BCN resistance in radish. Additional work is required to test the reproducibility of these markers by confirming these markers to a more efficient single locus PCR-based marker (e.g. SCAR markers).

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تحليل مواقع الصفات الكمية (QTL) المقاومة للنيماتودا بنجر السكر فى الفجل

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تعتبر هذه الدراسة هى الأولى من نوعها لتحديد مواقع الصفات الكمية للمقاومة للنيماتودا باستخدام عشيرة الجيل الثانى الانعزالية الناتجة من التهجين بين النوعين الاثنين من أصناف الفجل. تم استخدام عدد ٢٩٠ نبات من عشيرة الجيل الثانى الانعزالية الناتجة من التلقيح الذاتى لأحد نباتات الجيل الأول. أظهرت النتائج أن صفة المقاومة للنيماتودا قد توزعت طبيعياً لصفة بسيطة يتحكم فيها جين واحد سائد، وتم الحصول على عدد ١٦ واسم وراثى (RAPD and AFLP) تظهر اختلافات كمية (على أساس كثافة الـ band) بين الـ BULK المقاوم والـ BULK الحساس. الواسم الوراثى (OPD-04-568)، والواسم الوراثى ((E39M48-268(D) أظهرا اختلافات واضحة فى كثافة الـ band بين الـ BULK المقاوم والـ BULK الحساس، وتم إجراء تحليل مواقع الصفات الكمية (QTL) باستخدام برنامج MAPQTL4 .

كما أظهرت نتائج الاختبارات Multible-QTL Model, Interval Mapping test, Kruskal-Wallis test وجود QTL واحد على المجموعة الارتباطية رقم ٦ مرتبطة بصفة المقاومة للنيماتودا فى الفجل. أما بالنسبة للواسم الوراثى (OPD-4-568) والواسم الوراثى ((E39M48-268(D) اتضح وجود ارتباطاً كبيراً بالصفة، وقد أثرا بنسبة ٦٢%، ٤٨% على التوالى ، وكان مجمل تأثير الـ QLT المكشف حوالى ٨٣% من أجمالى الاختلافات الكلية.