Molecular Tagging and Inheritance of the Root-Knot Nematode Resistance in Pepper (*Capsicum annuum* L.)

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Root-knot nematodes, *Meloidogyne* spp., are obligate, Chit wood and *M. incognita* Chit wood. They cause serious sedentary endoparasites of many plant species. Their damage to tomato and pepper crops (Lamberti, 1979), especially in potential host range encompasses more than 3000 plant tropical, sub-tropical and warm climates. In Egypt, the most important and widely spread in different soil types and locations are the root knot nematodes which are in some cases they consider as limiting factors of tomato and pepper production (Houssny and Oteifa, 1956).

While most hot pepper *Capsicum annuum* L. cultivars are resistant to the root-knot nematode species *Meloidogyne incognita*, they are usually susceptible to the Southern root-knot nematode *M. javanica* (Taylor and Sasser, 1978; Peixoto, 1995). Even though varietal resistance is considered one of the most efficient methods for nematode control (Ferraz and Mendes, 1992), very little emphasis has been placed on breeding peppers for nematode resistance.

The *N* gene and the Me genes have been reported to control resistance to root-knot nematodes in pepper, *Capsicum annuum* (Castagnone-Sereno *et al.* 2001; Hare, 1956; Hendy *et al.* 1985; Feiy and Dukes, 1996). Hendy *et al.* (1985) observed five genes, designated Me1 to Me5 that control resistance to various *Meloidogyne* spp. Two of these genes, Me1 and Me3, confer broad spectrum resistance to *M. incognita*, *M. arenaria*, and *M.javanica* (Hendy *et al.* 1985). Likewise, the N gene confers high resistance to *M. incognita*, *M. arenaria* races 1 and 2, and *M. javanica* (Thies and Fery, 2000). Although each gene system has been individually well characterized, resistance controlled by the two genetic systems has not been compared in a single study; e.g. there is no information about the relationship of the N. and Me gene systems to each other and whether the N and Me genes are allelic. (Thies and Fery, 2000).

The use of molecular markers has been proposed as an alternative procedure (Toby *et al.,* 1999). Molecular markers based on polymorphisms of the DNA are specially useful for this enterprise, because they are not affected by the environment (Tatineni *et al.,* 1996).

The Random Amplified Polymorphic DNA (RAPD-PCR) and Inter Sequence Repeat Polymerase Chain Reaction (ISSR-PCR) technique has been widely used to quantify the genetic variation due to its simplicity and power to detect differences, even among closely related individuals, in species of Brassica (Jain *et al*., 1994), Pisum (Hoey *et al.,* 1996), Wheat (Liu *et al*., 1999) and Pepper (Refaat and Hoda, 2007 and Geleta *et al.* 2004 and Kumar *et al*., 2001).

The objective of this study was to inheritance and screening F_1, F_2 and BC_1 plants' resistance to *M. incognita* race 2 derived from a cross The cultivars P_1, P_4, and P_5 and P_3 (Resistant) with the local cultivar susceptible P_7 by ISSR-PCR to Identification of molecular markers linked to the *N* gene will facilitate marker- assisted selection and the isolation of the *N* resistance gene and will develop host plant genetic resistance through breeding and DNA manipulation which includes: (I) development of molecular tagging and identify and track resistance genes, and (II)development of improved to identify, evaluate and confirm resistance in large host plant populations.

MATERIALS AND METHODS

- This research was conducted at the Agricultural Experimental Station of the Horticulture Department, Faculty of Agriculture at Moshtohor, Benha University, during three summer seasons of 2006 to 2007.
- **Plant Materials:**

Seeds of cultivars used in this study obtained from the National Germplasm Resources Laboratory, Beltsville, U.S.A. and Germplasm Preservation Laboratory, Faculty of Agriculture at Moshtohor, Benha University, Egypt. The cultivars P_1: (PI: 15925601_U.S.A.), P_4: (PI: 13582401_ Afghanistan), and P_5: (PI: 13587301_ Pakistan) and P_3: (PI:16736101_ Turkey) are homozygous dominant for the $\underline{N}$ gene(*or alleles of the* $\underline{N}$ *gene*), which were found to be high resistance and resistance to the Root-Knot Nematode and the local cultivar P_7: (Aswany) and P_2: (PI:

5927201_U.S.A.), P_6:(YellowWax_U.S.A.) are homozygous receive for the *N* gene(*nn*), which expressed susceptible and high susceptible to root-knot nematode, were selected to study the inheritance and Screening of the root-knot nematode resistance in pepper (*Capsicum annuum. L*).

On March 22nd, 2006, seeds of all selected cultivars were sown in the field. The following crosses were made between the parental genotypes: (P_7 X P_1), (P_7 X P_3), (P_7 X P_4) and (P_7 X P_5). Seeds of the F_1's were harvested separately and kept for the next season.

On March 17th, 2007, hybrid seeds of each cross and seeds of the parental genotypes were planted in the field. Crosses between the parental genotypes were repeated and F_1 plants were selfed to obtain F_2 seeds. Backcrosses populations were obtained by crossing each F_1 hybrid with its respective parents

- **Nematode cultures:**

M. incognita race 2 was described based on perineal patterns and the host range by Barker *et al.* (1985). *M. incognita* race 2 culture was maintained on susceptible pepper in flower pots containing a sterile, moist loamy soil (80% sand, 15% silt, and 5% clay) in a growth chamber for 6 weeks at 22-26^{0}C on a 16 h of light regime.

- **Screening Tests:**

The following methods were used for nematode inoculation: (1) two seeds were sown in a 15 cm diameter flowerpot containing a sterile, moist loamy soil (80% sand, 15% silt, and5% clay), (2) plants were thinned to one per pot at the second true leaf stage, (3) *M .incognita* race 2 inoculum was produced in the growth chamber on susceptible pepper, (4) nematode eggs were extracted from roots, and (5) each plant was inoculated at the second-fourth true leaf stage with 1000 J2 of the *M. incognita* race 2. Plants were harvested 8 weeks after the inoculation and evaluated according to the method of Barker *et al.* (1985). Plants were classified as resistant (≤ 2 root gall index) or susceptible (> 2 root gall index) based upon the distribution of F_1, F_2 and BC_1 plants infected with *M. incognita* race 2. In addition, reproduction factors were calculated as the final level of J2 in soil / 1000 J2 (the amount of J2 in each flowerpot that was initially inoculated with *M. incognita* race 2) and statistical analysis was

detected according to Mather (1963).

- **DNA extraction:**

Young leaves were collected in 1.5 ml eppendorf tube, quickly frozen in liquid nitrogen and ground with konte pestles into fine powder. DNA was extracted according to Doyle and Doyle, (1990) mini preparation protocol. The purity of extracted DNA was tested on 1% agarose gel using 0.5x TE (Tris EDTA) buffer and stained with 10 mg/ml ethidium bromide. The gel was exposed to UV-light and photographed. Optimizations of the working dilutions were made using various dilution ratios. Finally, the dilution produced amplification with the ISSR primer and three samples for screening was in ratio of 1:1000 after determining the concentration with a TD-700Fluorometer.

- **ISSR- PCR:**

ISSR-PCR was carried out according to (Williams *et al*., 1990). The primers used were 11 to18 mer olignucleotide; nine primers were selected as potentially useful. The codes and sequences of the used primers are shown in Table (1). PCR reactions were optimized and mixtures (25µl total volume) were composed of dNTPs (200µM), $MgCl_2$ (1.5mM),1x buffer, primer(0.2µM), DNA (50ng),Taq DNA polymerase (2units).Amplification was carried out in a thermo Cycler programmed for 94°C for 3min(one cycle); followed by 94°C for 30sec,40°C for 45 sec and 72°C for 1 min (35 cycle), 72°C for 10 min (one cycle) then 4°C (infinitive).

Amplification products (25µl) were mixed with 3µl loading buffer and separated on 1.3%agarose gel and stained with 0.5 µg/ml ethidium bromide, and visualized under ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 1kb DNA ladder marker.

- **Data analysis:**

The obtained data of ISSR analysis was entered in a computer file as binary matrices where "0" stands for the absence of a band and in each individual sample. Similarity coefficients were calculated according to dice matrix (Nei and Li, 1979; Rohlf, 1993).

RESULTS AND DISCUSSION

Root-knot nematodes of the genus Meloidogyne are economically important pathogens of a wide range of crops (Sasser and Carter, 1985). Infective second-stage juveniles of these obligate endoparasites penetrate the roots of the host and migrate intercellularly to the vascular cylinder (Williamson and Hussey, 1996).

The primary symptom of root-knot nematode infection is the formation of typical root galls on the roots of susceptible host plants. Their invasion of the root system of host plants results in a shallow, knotted root system and susceptibility to other pathogens. Nutrient and water uptake are substantially reduced because of the damaged root system, resulting in weak and poor yielding plants (Abad *et al*., 2003).

Recently, foreign firms have developed fresh market Pepper varieties with resistance to root-knot nematode, and these have become commercially available as an option for nematode management.

In these resistant varieties, nematodes fail to develop and reproduce normally within the pepper root tissues, allowing plants to grow and produce fruit even though nematode infection of roots occurs.

- **Inheritance of the Root-Knot Nematode Resistance:**

The frequency distribution for plant reaction to root- knot nematode resistance in the F_2, BC_1 and BC_2 populations of the crosses (P_7 X P_1), (P_7 X P_3), (P_7 X P_4) and (P_7 X P_5) Table (2), indicated that this character was inherited quantitatively. The plant reaction to root- knot nematode was resistant in F_1 families from crosses of (P_1 X P_7), (P_3 X P_7), (P_4 X P_7) and (P_5 X P_7) and F_2 families from these crosses segregated in a 3 resistant: 1 susceptible ratio; the backcross of F_1 (P_1 X P_7), F_1 (P_3 X P_7), F_1 (P_4 X P_7) and F_1 (P_5 X P_7) to Aswany segregated 1 resistant: 1 susceptible ratio. These segregations clearly indicate that susceptible gene in Aswany parent is controlled by a single recessive gene and allelic to *N* or *Me* reported in cultivars (Fery and Dukes, 1996 and Milligan *et al*., 1998).

Inheritance of the root–knot nematode resistance in the crosses segregation ratio suggested a single dominant gene for the high level of resistant; although the data on disease expression were recorded on a 1 to 5 scale, the results and subsequent observation fitted a single gene model for resistance better than a quantitative pattern.

The results of this test were interesting because they appeared to be a range of reactions to *M. incognita* race 2 among the root-knot nematode resistance genotypes tested. All F_2 families and backcross families from these crosses carrying the *N* gene were highly resistant to *M. incognita*, but differences ($P<0.05$) were observed in resistance conferred by different combination of the *Me* genes. It appears that the *N* gene and the *Me3* gene both confer higher resistance than the *Me1* gene (Thies and Fery, 2000). However, the test must be repeated in order to confirm these results; in addition, allelism tests between the *N* and *Me* genes will be conducted in the near future in order to determine whether these two gene systems are different.

- **Identification of ISSR Markers linked to the N gene for Root-knot Nematode Resistance in Pepper:**

In the present study, the genetic variability among different genotypes of pepper based on ISSR-PCR analysis has been studied.

Nine ISSR primers were used to screen seven genotypes of pepper for nematode resistance. These primers that produce different polymorphic bands and amplified DNA fragments as shown in Tables (3) and Fig.(1,2).

The nine primers used in the study generated a total of 173 amplification products, among which 167 were found to be polymorphic; this resulted in 96.53% polymorphism. All the primers produced polymorphic amplification products, however, the extent of percent polymorphism varied with each primer (90.48 to 100%).

The PCR products of primers 844_A, *844B* and 814A analysis of there products are illustrated in Fig.(1A,1B and 1C) and Tables (3, 4); this primers produced four positive specific marker was found in P_4 , P_1 and P_3 with *M.W.* of 473, 454, 400*bp* and 672.55*bp* were highly resistance to root knot nematode, respectively

Analysis of the PCR products of both primers 17898_A and 17899_A are illustrated in (Tables 3, 4 and Fig.1D,1E). These primers produced four negative specific markers in P_6 , P_2 and P_7 with *M.W.* of 658, 475, 430 and 311*bp*), respectively which can be used as a marker for susceptibility of root knot nematode.

The result of ISSR analysis using primer HB_9 was illustrated in Fig.1F and Tables (3, 4); there were two negative and one positive specific markers in P_2, P_7 and P_4 with *M.W.*459, 694 and 482*bp*, respectively.

The result of ISSR analysis using primer HB_{11} and HB_{12} were illustrated in Fig.(1G and 1H) and Tables (3, 4); this primers produced seven positive specific markers linked with highly resistance to root knot nematode were found in P_1, P4 and two negative specific markers found in P_7 and P_6 with *M.W.* 554 and 271*bp*, respectively.

The result of ISSR analysis using primer HB_{13} was illustrated in Fig.1I and Tables (3,4); however, three positive specific markers were found in P_2, P_3 and P_5 with *M.W.* 825, 792and 613*bp*, respectively.

The results of total amplified fragments (*TAF*), amplified fragments (*AF*), and specific marker (*SM*) for some Six F_1 families pepper crosses based on ISSR-PCR analysis with nine primers were shown in table (3) and fig (2A,2B,2C,2D,2E,2F,2G,2H and 2I).

The nine ISSR primers produced high polymorphisms ranging from 11 (*17898A*, *17899A*, and *HB09* primers) to 28 fragments (*844A* primer) all of these bands were polymorphic 100% except of those of primer *17899A* which was 71.44%. a total of 16 specific marker bands were obtained as they ranged from one (*844A*, *17899A*, *HB12*, and *HB13* primers) to four specific marker bands (*814A* and *HB09* primers) except of primer *844B* which did not exhibit any specific marker bands.

In general, the over all results for evaluation of pepper F_1 progeny reaction to the root knot nematode using specific ISSR markers in table (4) showed that both crosses (P_7xP_5) and (P_7xP_4) exhibited five specific marker bands for highly resistance of root knot nematode each, one of these specific marker bands with molecular size *860.16*bp, in (P_7xP_5), did not appear in both resistant or susceptible parents. In addition, the cross (P_7xp_3) gave only one specific marker band with molecular size 344.44 *bp* for resistance of root knot nematode.

These results indicated that these twenty three positive and fourteen negative ISSR Specific markers linked with root-knot nematode resistance in pepper in accordance with (Geleta *et al.* 2004 and Kumar *et al.*, 2001).

ISSR analyses were applied for fourteen genotypes for qualitative genetic diversity. Adetula, (2006), found that that genetic diversity of hot pepper measured using ISSR markers exhibited highly significant association with geographic origin and plant pathology races or strains

Molecular markers have been successfully used in the genus Capsicum to select parents for hybrid production, for intra-specific or inter-specific classification, and for the analysis of variation. However, commercial pepper varieties possess a low level of molecular polymorphism (Lefebvre *et al.*, 2001). In agreement with this, in the present study ISSR markers detected a low level of polymorphism (8.5%) in the commercial varieties tested. It is general knowledge that the definitions of commercial varieties of pepper, and particularly hybrid varieties, are based on increasingly low variation at the genetic level.

Microsatellite markers have several advantages over other types of markers such as RFLPs, RAPDs, AFLPs, and inter simple sequence repeats (ISSRs). The molecular fingerprinting of a plant variety is of the utmost importance for protecting plant breeders' rights (Cooke *et al.,* 2003; Law *et al.,* 1998 and Lu *et al*.1999).

The biotic stress characters have been widely used for descriptive purposes and are commonly used to distinguish plant varieties; these methods are however questionable because resistance and susceptible traits are strongly affected by environmental conditions. In addition, this approach is inefficient because of the time and cost involved (Singh *et al.*, 2004).

ISSR analysis measures genetic variation mainly in non-coding sequences which probably have a relatively minor impact on the phenotype and qualitative morphological traits on the other hand are affected by environmental conditions and so show considerable variation. Finally, finding a correlation between ISSR and morphological data might depend on the number of ISSR markers and morphological characters available for comparison.

Several researchers have reported the use of molecular markers for registration testing in various crops. RAPD, AFLP, and SSR markers have been extensively used for fingerprinting in rice (Singh *et al., 2004)*, cucumber (Bernet *et al., 2003*), and Pepper (Refaat and Hoda, 2007) and rape **(**Tommasini *et al.*, 2003). These previous results indicate that molecular markers could be used for pre-screening or grouping of existing and candidate varieties.

- **Summary**

The inheritance of the root–knot nematode resistance in the crosses segregation ratio suggested a single dominant gene for the high level of resistant; although the data on disease expression were recorded on a 1 to 5 scale, the results and subsequent observation fitted a single gene model for resistance better than a quantitative pattern. In general, the overall results for evaluation of pepper F_1 progeny reaction to the root knot nematode using specific ISSR markers showed that both crosses (P_7xP_5) and (P_7xP_4) exhibited five specific marker bands for highly resistance of root knot nematode each, one of these specific marker bands with molecular size *860*bp, in (P_7xP_5), did not appear in both resistant or susceptible parents. In addition, the cross (P_7xp_3) gave only one specific marker band with molecular size 344*bp* for resistance of root knot nematode.

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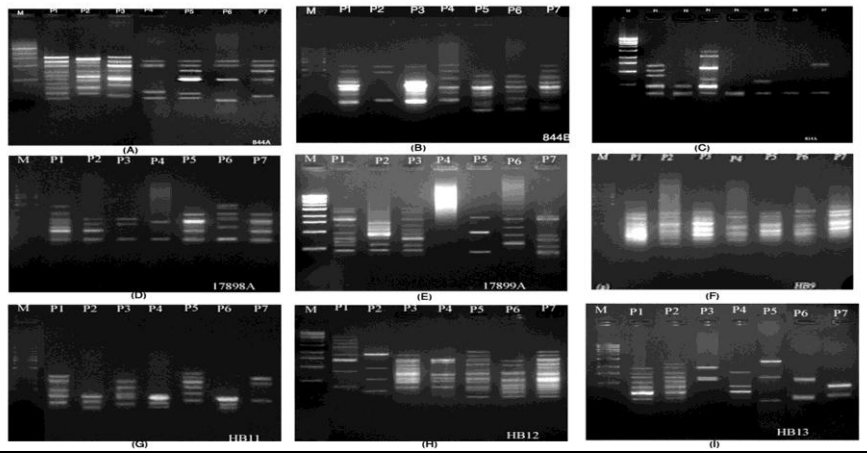

Fig (1): DNA polymorphism of the seven parental pepper genotypes (P_1, P_2, P_3, P_4, P_5, P_6 and P_7) using ISSR_PCR with nine primers.

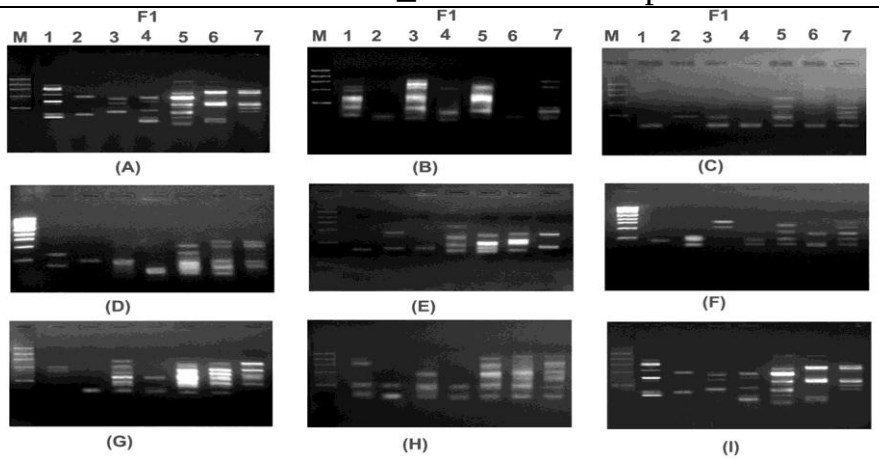

Fig (2): DNA polymorphism of the seven F_1 crosses pepper (1):P_7xP_5, (2):P_7xP_1, (3):P_7xP_6, (4):P_5xP_4, (5):P_7xP_4, (6):P_7xP_3 and (7):P_7xP_2 using ISSR_PCR with nine primers.

Table (1): Name and sequences of the used primers with ISSR molecular markers.

ISSR Primer	Nucleotide sequence 5' to 3'
814_A	$(CT)^8TG$
844_A	$(CT)^8AC$
844_B	$(CT)^8GC$
17898_A	$(CA)^6AC$
17899_A	$(CA)^6AG$
HB_9	$(GT)^6GG$
HB_{11}	$(GT)^6CC$
HB_{12}	$(CAC)^3GC$
HB_{13}	$(GAG)^3GC$

Table (2): Frequency distributions for plant reaction to Root-Knot Nematode Resistance in parents, F_1, F_2, Bc_1 and Bc_2 segregations derived from some Pepper crosses.

Cross	Population	Scale of root gall index under Nematode infection					Total No. of plants	#Response to root-knot Nematode		X^2
		1	2	3	4	5		R	S	
1	P_7^S	-	-	-	10	20	30	-	30	
	$Bc_1(F_1XP_7)$	26	11	3	11	9	60	37	23	*3.266***
	F_1	4	26	-	-	-	30	30	-	
	F_2	38	45	4	14	19	120	83	37	*2.177***
	$Bc_2(F_1XP_1)$	24	22	-	6	8	60	46	14	
	P_1^{HR}	30	-	-	-	-	30	30	-	
2	P_7^S	-	-	-	10	20	30	-	30	
	$Bc_1(F_1XP_7)$	20	16	8	11	5	60	36	24	*2.400***
	F_1	5	25	-	-	-	30	30	-	
	F_2	50	47	6	5	12	120	97	23	*2.177***
	$Bc_2(F_1XP_3)$	16	26	5	2	11	60	42	18	
	P_3^R	30	-	-	-	-	30	30	-	
3	P_7^S	-	-	-	10	20	30	-	30	
	$Bc_1(F_1XP_7)$	24	10	5	11	10	60	34	26	*1.066***
	F_1	4	26	-	-	-	30	30	-	
	F_2	41	50	4	13	12	120	91	29	*0.044**
	$Bc_2(F_1XP_4)$	34	19	-	6	1	60	53	7	
	P_4^{HR}	30	-	-	-	-	30	30	-	
4	P_7^S	-	-	-	10	20	30	-	30	
	$Bc_1(F_1XP_7)$	18	14	5	14	9	60	32	28	*0.266***
	F_1	7	23	-	-	-	30	30	-	
	F_2	42	45	3	12	18	120	87	33	*0.400***
	$Bc_2(F_1XP_5)$	20	25	1	7	7	60	45	15	
	P_5^{HR}	30	-	-	-	-	30	30	-	

#R: Resistant (≤ 2 root gall index), S: Susceptible (> 2 root gall index).

Table (3): Number of total amplified fragments, number of polymorphic bands and percentage of polymorphic bands of seven parental pepper genotypes and F_1 crosses based on ISSR-PCR analysis with nine primers.

Genotypes		ISSR Primers									Total
		844A	**844B**	**814A**	**17898A**	**17899A**	**HB9**	**HB11**	**HB12**	**HB13**	
	TAF	21.0	14.0	18.0	14.0	21.0	18.0	19.0	28.0	20.0	173.0
	MB	2.0	0.0	1.0	1.0	2.0	0.0	0.0	0.0	0.0	6.0
	PB	19.0	14.0	17.0	13.0	19.0	18.0	19.0	28.0	20.0	167.0
	PB%	90.48	100.0	94.44	92.86	90.48	100.0	100.0	100.0	100.0	96.53
P_1	*AF*	8.0	7.0	4.0	6.0	8.0	4.0	5.0	8.0	7.0	57.0
	SM	0.0	1.0	2.0	0.0	0.0	0.0	1.0	5.0	0.0	9.0
P_2	*AF*	6.0	3.0	1.0	3.0	8.0	5.0	3.0	4.0	8.0	41.0
	SM	0.0	0.0	0.0	0.0	2.0	1.0	0.0	0.0	1.0	4.0
P_3	*AF*	5.0	4.0	5.0	2.0	8.0	3.0	5.0	7.0	3.0	42.0
	SM	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	1.0	3.0
P_4	*AF*	2.0	7.0	0.0	1.0	0.0	4.0	3.0	6.0	3.0	26.0
	SM	1.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	3.0
P_5	*AF*	4.0	6.0	1.0	3.0	3.0	5.0	6.0	11.0	3.0	42.0
	SM	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0
P_6	*AF*	2.0	6.0	0.0	5.0	4.0	5.0	3.0	8.0	2.0	35.0
	SM	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	0.0	2.0
P_7	*AF*	2.0	7.0	1.0	3.0	7.0	5.0	3.0	11.0	2.0	41.0
	SM	0.0	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	3.0
$P_{7x}P_5$	*AF*	5.0	1.0	1.0	1.0	1.0	0.0	4.0	2.0	5.0	20.0
	SM	1.0	0.0	0.0	0.0	0.0	2.0	1.0	0.0	1.0	5.0
$P_{7x}P_1$	*AF*	2.0	2.0	2.0	1.0	0.0	1.0	1.0	1.0	2.0	12.0
	SM	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$P_{7x}P_6$	*AF*	4.0	1.0	1.0	2.0	1.0	1.0	4.0	5.0	3.0	22.0
	SM	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	2.0
$P_{7x}P_4$	*AF*	8.0	4.0	2.0	3.0	2.0	2.0	3.0	5.0	6.0	35.0
	SM	0.0	0.0	2.0	1.0	1.0	1.0	0.0	0.0	0.0	5.0
$P_{7x}P_3$	*AF*	5.0	3.0	2.0	1.0	3.0	3.0	1.0	6.0	4.0	28.0
	SM	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0
$P_{7x}P_2$	*AF*	4.0	2.0	4.0	3.0	4.0	4.0	5.0	4.0	4.0	34.0
	SM	0.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	3.0
Total	*AF*	57.0	53.0	24.0	34.0	49.0	42.0	46.0	78.0	52.0	435.0
	SM	2.0	1.0	8.0	3.0	4.0	7.0	5.0	7.0	4.0	41.0

TAF=total amplified fragment, MB= monomorphic bands, PB=polymorphic bands. AF=amplified fragment, SM=specific marker.

Table (4): Evaluation of plant reaction to the root knot nematode by specific ISSR markers in the Pepper Parents and F_1 families.

ISSR Primers	Band Number	MW *(bp)*	Plant Reaction	Parents
844A	3	473.33	**High Resistance**	**P_4**
844B	1	400.0	**High Resistance**	**P_1**
814A	1	672.55	**Resistance**	**P_3**
	2	453.97	**High Resistance**	**P_1**
17898A	2	658.23	**High Susceptible**	**P_6**
17899A	2	475.07	**High Susceptible**	**P_2**
	3	429.60		
	6	311.37	**Susceptible**	**P_7**
HB9	3	459.09	**High Susceptible**	**P_2**
	4	481.82	**High Resistance**	**P_4**
	1	693.25	**Susceptible**	**P_7**
HB11	3	400.0	**High Resistance**	**P_1**
	1	368.56	**High Resistance**	**P_4**
	2	553.66	**Susceptible**	**P_7**
HB12	1	918.79	**High Resistance**	**P_1**
	2	814.45		
	3	771.44		
	4	700.0		
	8	400.0		
	7	270.59	**High Susceptible**	**P_6**
HB13	1	824.96	**High Susceptible**	**P_2**
	1	791.92	**Resistance**	**P_3**
	1	612.82	**High Resistance**	**P_5**

ISSR Primers	Band Number	MW *(bp)*	Plant Reaction	Crosses
844A	1	860.16	**High Resistance**	**P7 xP5 S HR**
814A	1	746.57	**High Susceptible**	**P7 xP6 S HS**
	1	619.97	**High Resistance**	**P7 xP4 S HR**
	2	504.69		
	1	682.34	**High Susceptible**	**P7 xP2 S HS**
17898A	2	759.46	**High Resistance**	**P7 xP4 S HR**
	1	616.01	**High Susceptible**	**P7 xP2 S HS**
17899A	1	939.55	**High Resistance**	**P7 xP4 S HR**
HB9	1	436.69	**High Resistance**	**P7 xP5 S HR**
	2	366.67		
	3	381.48	**High Resistance**	**P7 xP4 S HR**
	4	344.44	**Resistance**	**P7 xP3 S R**
HB11	2	684.85	**High Resistance**	**P7 xP5 S HR**
	1	791.94	**High Susceptible**	**P7 xP2 S HS**
HB12	1	761.08	**High Susceptible**	**P7 xP6 S HS**
HB13	1	900.0	**High Resistance**	**P7 xP5 S HR**