MOLECULAR TAGGING AND INHERITANCE OF THE ROOT-KNOT NEMATODE RESISTANCE IN PEPPER (*Capsicum annuum* L.)

HODA A. S. ELGARHY AND M. H. REFAAT

Department of Genetics, Fac. of Agriculture, Benha University, Egypt

Root-knot nematodes, *Meloidogyne* spp., are cosmopolitan plant-parasitic nematodes in many different climates. All species are obligate sedentary endoparasites which have extensive host ranges cover most of the common crops. Accordingly, crop losses and rapid deterioration of soils are frequently associated with heavily soil infestations. In Egypt, these nematode species are widely distributed in different soil types in various localities and appear to be one of the most couses of failures of crop production such as tomato and pepper (Lamberti, 1979; Houssny and Oteifa, 1956).

While most hot pepper *Capsicum annuum* L. cultivars are resistant to the southern root-knot nematode, *Meloidogyne incognita*, they are usually susceptible to the Javanese 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 *Me*1 to *Me*5 that control resistance to various species of *Meloidogyne*. Two of these genes, *Me*1 and *Me*3, 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 especially useful for this enterprise, because they are not affected by the

environmental conditions (Tatineni *et al.*, 1996).

The random amplified polymorphic DNA (RAPD-PCR) and the inter simple sequence repeat-polymerase chain reaction (ISSR-PCR) technique have 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), *Triticum* (Liu *et al*., 1999) and *Capsicum* (Kumar *et al*., 2001; Geleta *et al.*, 2004; Refaat and Hoda, 2007).

This study was carried out to 1) evaluate the inheritance and screening of plants resistance of F_1, F_2 and BC_1 (derived from crosses of resistant cultivars P_1, P_4, P_5 and P_3 with susceptible local cultivar P_7) by using ISSR-PCR technique. 2) identify the molecular markers linked to the *N* gene (to facilitate marker- assisted selection and to isolate *N* resistance gene) and, 3) develop host plant genetic resistance through breeding and DNA manipulation.

MATERIALS AND METHODS

This research was conducted at the Agricultural Experimental Station of the Horticulture Department, Faculty of Agriculture at Moshtohor, Benha University, during two summer seasons of 2006 and 2007.

- ***Plant Materials***

Seeds of pepper cultivars (P_1 to P_6) that used in this study were obtained from the National Germplasm Resources Laboratory, Beltsville, USA and P_7 from the Germplasm Preservation Laboratory, Faculty of Agric. at Moshtohor, Benha University, Egypt. The cultivars P_1: (PI: 15925601_USA), P_4: (PI: 13582401_ Afghanistan), and P_5: (PI: 13587301_ Pakistan) and P_3: (PI: 16736101_Turkey) are homozygous dominant for the *N* gene (*or alleles of the N gene*), which were found to be high resistance and resistance to the root-knot nematode. The local cultivar P_7: (Aswany) and P_2: (PI: 5927201_USA), P_6: (YellowWax_USA) are homozygous resceive for the *N* gene(*nn*), which expressed susceptibility and high susceptible to root-knot nematode. All cultivars were selected to study the inheritance and screening of the root-knot nematode, *M. incognita* race 2, 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 identified by using the perineal patterns and the host range described 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°C on a 16 h of light regime per day.

- ***Screening tests***

The following procedures were used for nematode inoculation: (1) two seeds were sown in flowerpot (15 cm diameter) containing sterile, moist loamy soil (80% sand, 15% silt, and5% clay), (2) the plants were thinned to one per pot at the second true leaf stage, (3) *M. incognita* race 2 inocula were produced in the growth chamber on susceptible pepper, (4) nematode eggs were extracted from roots and infective juveniles (J_2) were emerged in water, and (5) each plant was inoculated at the second-fourth true leaf stage with 1000 J_2 of the *M. incognita* race 2. The plants were harvested at 8 weeks after the inoculation and evaluated according to the method of Barker *et al.* (1985). The 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.

- ***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 to 18 mer oligonucleotide; 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 (25 µl total volume) were composed of dNTPs (200 µM), $MgCl_2$ (1.5 mM), 1x buffer, primer (0.2 µM), DNA (50 ng), Taq DNA polymerase (2 units). Amplification was carried out in a thermo cycler programmed for 94°C for 3 min (one cycle); followed by 94°C for 30 sec, 40°C for 45 sec and 72°C for 1 min (35 cycles), 72°C for 10 min (one cycle), then 4°C (infinitive).

Amplification products (25 µl) were mixed with 3 µl 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 comparison with the 1 kb 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 "1" stands for the presence of a given band 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 intracellularly 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 knotted root system and susceptibility to other pathogens. Nutrient and water uptake are substantially reduced due to 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 or 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) as shown in Table (2) which 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; 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 1 to 5 scales, 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 are shown in Table (3) and Fig. (1 and 2).

The nine primers used in the present 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, but, the extent of percent polymorphism varied with each primer (90.48 to 100%).

The PCR products of primers *844A, 844B* and *814A* and the analysis of their products are illustrated in Fig. (1A, 1B and 1C) and Tables (3 and 4). This primers produced four positive specific marker which were found in P4, P_1 and P_3 with M.W. of 473, 454, 400 and 672.55 bp they were linked with highly resistance to root knot nematode, respectively.

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

The results of ISSR analysis using primer HB_9 were illustrated in Fig. (1F) and Tables (3 and 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 results of ISSR analysis using primer HB_{11} and HB_{12} were illustrated in Fig. (1G and 1H) and Tables (3 and 4). These primers produced seven positive

specific markers linked with highly resistance to root-knot nematode, which were found in P_1, P4 and two negative specific markers which were found in P_7 and P_6 with *M.W.* 554 and 271bp, respectively.

The result of ISSR analysis using primer HB_{13} was illustrated in Fig. (1I) and Tables (3 and 4); however, three positive specific markers were found in P_2, P_3 and P_5 with *M.W*. 825, 792 and 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 Figs (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 primer *17899A* which was 71.44%. in addition a total of 16 specific marker bands were obtained and ranging 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 to root knot nematode. Each, one of these specific marker bands with molecular size 860.16 bp, in (P_7xP_5), did not appear in either 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 to 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 are in accordance with those of Geleta *et al.* 2004; Kumar *et al.*, 2001.

ISSR analyses were applied for fourteen genotypes for qualitative genetic diversity. Adetula (2006) found that, genetic diversity of hot pepper measured by 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. Generally, the definitions of commercial varieties of pepper, 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; 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 while qualitative morphological traits are affected inversely by the 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 prescreening or grouping of existing and candidate varieties.

SUMMARY

The inheritance of the root–knot nematode, *Meloidogyne incogenita* Race 2, resistance in the crosses segregation ratio in pepper cultivars, *capsicum annuum*, suggested a single dominant gene for the high level of resistant. Although the data on disease expression were recorded on 1 to 5 scale gall index, 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 to root knot nematode. One of these specific marker bands with molecular size 860 bp, in (P_7xP_5), did not appear in either resistant or susceptible parents. In addition, the cross (P_7xp_3) gave only one specific marker band with molecular size 344 bp for resistance to root knot nematode.

REFERENCES

Abad, P., B., M. N. Favery and P. Castagnone-Serena (2003). Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Mol. Plant Pathology, 4: 217-224.

Adetula, O. A. (2006). Genetic diversity

of *Capsicum* using Random Amplified Polymorphic DNAs. African Journal of Biotechnology, 5: 120-122.

Barker, K. R. (1985). Nematode extraction and bioassays. In: An Advanced Treatise on *Meloidogyne*: 2. Methodology. (Eds.: K. R. Barker, C. C. Carter and J. N. Sasser). North Carolina State University, pp 30.

Bernet, G. P., S. Bramardi, D. Calvache, E. A. Carbonell and M. J. Asins (2003). Applicability of molecular markers in the context of protection of new varieties of cucumber. Plant Breeding, 122: 146-152.

Castagnone-Sereno, P., M. Bongiovanni and C. Djian-Caporalino (2001). New data on the specificity of the root-knot nematode resistance gene *Me1* and *Me3*. Plant Breeding, 120: 429-433.

Cooke, R. J., G. M. M. Bredemeijer, M. W. Ganal, R. Peeters and P. Isaac (2003). Assessment of the uniformity of wheat and tomato varieties at DNA microsatellite loci. Euphytica, 132:331−342.

Di Vito, M. and F. Saccardo (1978). Risposta de linee e varieta di Capsicum agli attachi di *Meloidogyne* incognita in serra. Nematol. Mediterr., 6: 83-88.

Doyle, J. J. and L. H. Doyle (1990). Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Ferraz, S. and M. L. Mendes (1992). Onematóide das galhas. Inf. Agropecu., 16: 43-45.

Fery, R. L. and P. D. Dukes (1996). The inheritance of resistance to the southern root –knot nematode in Carolina Hot and Cayenne pepper. J. Amer. Soc. Hort. Sci., 121: 1024-102.

Geleta, M. T., M. T. Labuschagne and C. D. Viljoen (2004). Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. Plant Breeding, 123: 467-473.

Hare, W. W. (1956). Resistances in pepper to *Meloidogyne incognita acrita*. Phytopathology, 46: 98-104.

Hendy, H., E. Pochard and A. Dalmasso (1985). Inheritance of resistance to meliodogyne chitwood (Tylenchida) in 2 lines of *Capsicum annuum* L. study of homozygous progenies obtained by androgensis. Agronomie, 5: 93-100.

Hoey, B. K., K. R. Crowe, V. M. Jones and N. O. Polans (1996). Aphylogenetic analysis of *Pisum* based on morphological characters, and allozyme and RAPD markers. Theor. Appl. Genet., 92: 92-100.

Houssny, H. H and B. A. Otefeifa (1956). Preliminary field tests for evaluating some tomato varieties for resistance to root-knot nematodes. *Meliodogyne* spp. Pl. Dis. Rept., 40: 974-976.

Jain, A., S. Bhatia, S. S. Banga, S. Prakash and M. Lakshmikuran (1994). Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationships to heterosis. Theor. Appl. Genet., 88: 116-122.

Kumar, D. L., M. Kathirvel, G. V. Rao and J. Nagaraju (2001). DNA profiling of disputed chilli samples (*Capsicum annuum* L.) using ISSR-PCR and FISSR-PCR marker assays. Forensic Science International, 116: 63-68.

Lamberti, F. (1979). Economic importance of *Meloidogyne* spp. in subtropical and Mediterranean climates. In: Root-knot nematodes (*Meloidogyne* species): Systematics, biology and control (Eds.: F. Lamberti, C. E. Taylor). Academic Press, London, pp. 342-357.

Law, J. R., P. Donini, R. M. D. Koebner, J. C. Reeves and R. J. Cooke (1998). DNA profiling and plant variety registration. III: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. Euphytica, 102: 335-342.

Lefebvre, V., B. Goffinet, J. C. Chauvet, B. Caromel and P. Signoret (2001). Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RAPD and phenotyhpic data. Theor. Appl. Genet., 102: 741-750.

Liu, Z. Q., Y. Pei and Z. J. Pu (1999). Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. Plant Breeding, 118: 119-296.

Lu, Z. X., K. Sossey-Alaoui, G. L. Reighard, Wm. V. Baird and A. G. Abbott (1999). Devolopment and characterization of a co dominant marker linked to root-knot nematode resistance, and its application to peach rootstock breeding. Theor. Appl. Genet., 99: 115-122.

Milligan, S. B., J. Bodeau, J. Yaghoobi, I. Kaloshian, P. Zabel and V. M. Williamson (1998). The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine -rich repeat family of plant genes. The Plant Cell, 10: 1307-1319.

Nei, M. and W. H. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., 76: 5269-5273.

Peixoto, J. R. (1995). Melhoramento de pimentão (*Capsicum annuum* L.) visando a resistência aos nematóides do gênero *Meloidogyne* spp. Doctoral thesis, Universidade Federal de Lavras, Lavras, MG, Brazil.

Refaat, M. H. and Hoda A. S. Elgarhy (2007). Relationship between hybrid performance and genetic diversity based on ISSR-PCR markers in pepper (*Capsicum annuum*. L.). Annals of Agric. Sci., Moshtohor, 45: 1565-1579.

Rohlf, F. J. (1993). NTSYS-PC numerical taxonomy and multivariate analysis system, version 1.70. Exeter Software, Setauket, New York.

Sasser, J. N. and C. C. Carter (1985). Overview of the international Meloidogyne project 1975-1984. In: An Advance Treatise on *Meloidogyne*. Vol. 1. Biology and Control. (Eds.: J. N. Sasser and C.C Carter) North Carolina State University Graphics, Raleigh, pp. 19-24.

Singh, R. K., R. K. Sharma, A. K. Singh, V. P. Singh and N. K. Singh (2004). Suitability of mapped sequence tagged microsatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. Euphytica, 135: 135-143.

Tatineni, V., R. G. Cantrell and D. D. Davis (1996). Genetic diversity in elite cotton Germplasm determined by morphological characteristics and RAPDs. Crop Sci., 36: 186-192.

Taylor, A. L. and J. N. Sasser (1978). Biology, Identification and Control of Root-Knot Nematodes (*Meloidogyne* species). University Graphics, Raleigh, NC, USA, pp. 111.

Thies, J. A. and R. L. Fery (2000). Characterization of resistance conferred by the *N* gene to *Meloidogyne arenaria* races 1 and 2, *M. hapla* and *M. javanica*. J. Amer. Soc. Hort. Sci., 125: 71-75.

Toby, A. J., T. M. Abberton, T. M. Michaelson-Yeates and J. W. Foster (1999). Relationships between genetic distance measured by RAPD-PCR and heterosis in inbred lines of white clover (*Trifolium repens* L.). Euphytica, 107: 159-165.

Tommasini, L., J. Batley, G. M. Arnold, R. J. Cooke and P. Donini (2003). The developments of multiplex simple sequence repeat (SSR) markers to complement distinctness, uniformity and stability testing of rape (*Brassica napus* L.) varieties. Theor. Appl. Genet., 106: 1091-1101.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V.

Tingey (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Williamson, V. M. and R. S. Hussey (1996). Nematode pathogenesis and resistance in plants. Plant Cell, 8: 1735-1745.

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

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 pepper cultivars reaction to root-knot nematode resistance in parents, F_1, F_2, Bc_1 and Bc_2 segregations derived from some pepper crosses.

Cross	Pepper cultivars	Frequencies of root gall index due to nematode infection					Total No. of plants	Response to root-knot Nematode		#χ^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), χ^2 = Chi-square value.

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%	90.5	100.0	94.4	92.9	90.5	100.0	100.0	100.0	100.0	96.5
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_7xP_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_7xP_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_7xP_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_7xP_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_7xP_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_7xP_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	$P7_S xP5_{HR}$
844B	1	746.57	High Susceptible	$P7_S xP6_{HS}$
	1	619.97	High Resistance	$P7_S xP4_{HR}$
	2	504.69		
	1	682.34	High Susceptible	$P7_S xP2_{HS}$
17898A	2	759.46	High Resistance	$P7_S xP4_{HR}$
	1	616.01	High Susceptible	$P7_S xP2_{HS}$
17899A	1	939.55	High Resistance	$P7_S xP4_{HR}$
HB9	1	436.69	High Resistance	$P7_S xP5_{HR}$
	2	366.67		
	3	381.48	High Resistance	$P7_S xP4_{HR}$
	4	344.44	Resistance	$P7_S xP3_R$
HB11	2	684.85	High Resistance	$P7_S xP5_{HR}$
	1	791.94	High Susceptible	$P7_S xP2_{HS}$
HB12	1	761.08	High Susceptible	$P7_S xP6_{HS}$
HB13	1	900.0	High Resistance	$P7_S xP5_{HR}$

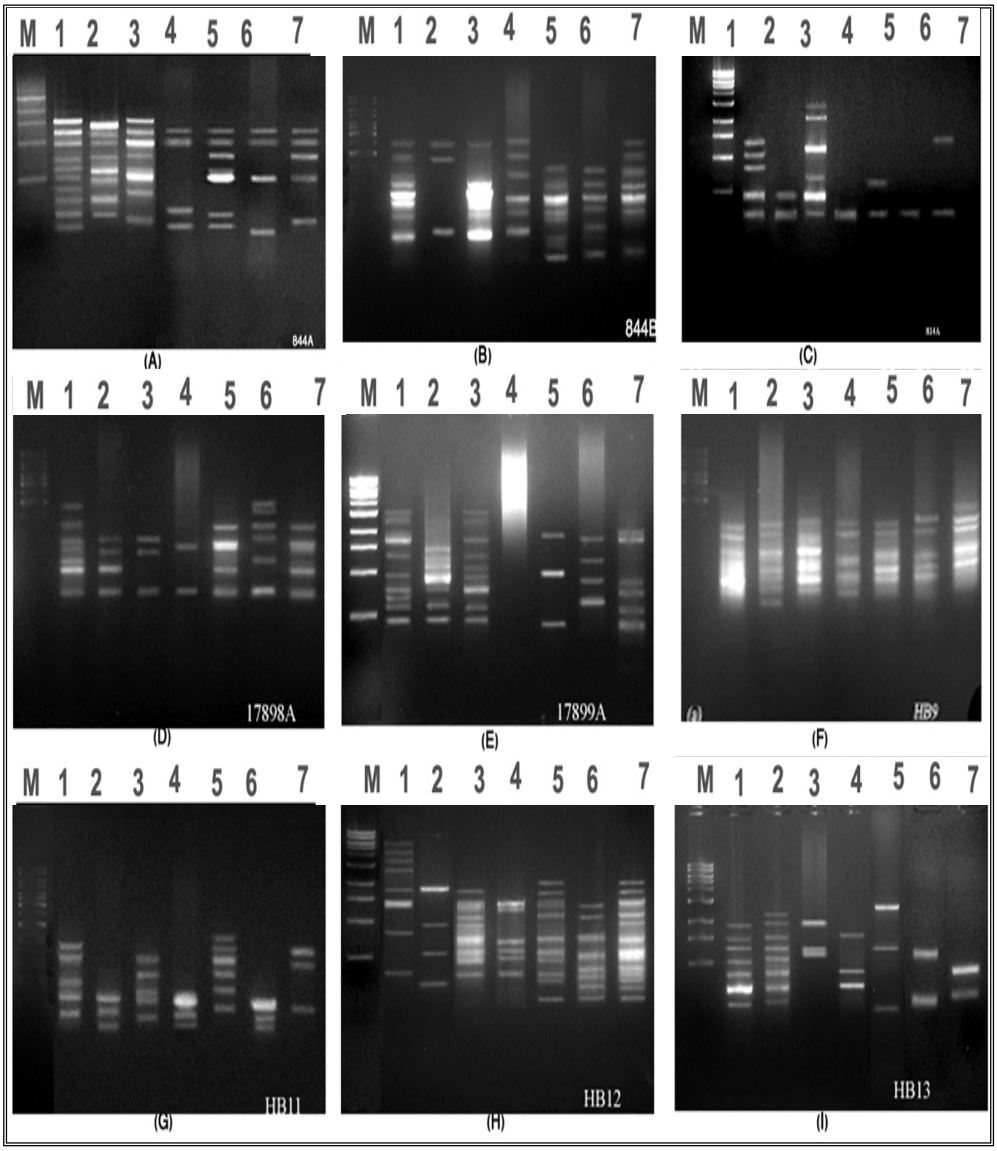

Fig (1): DNA polymorphism of the seven parental pepper genotypes (P_1, P_2, P_3, P_4, P_5, P_6 and P_7) using ISSR-PCR with nine primers.

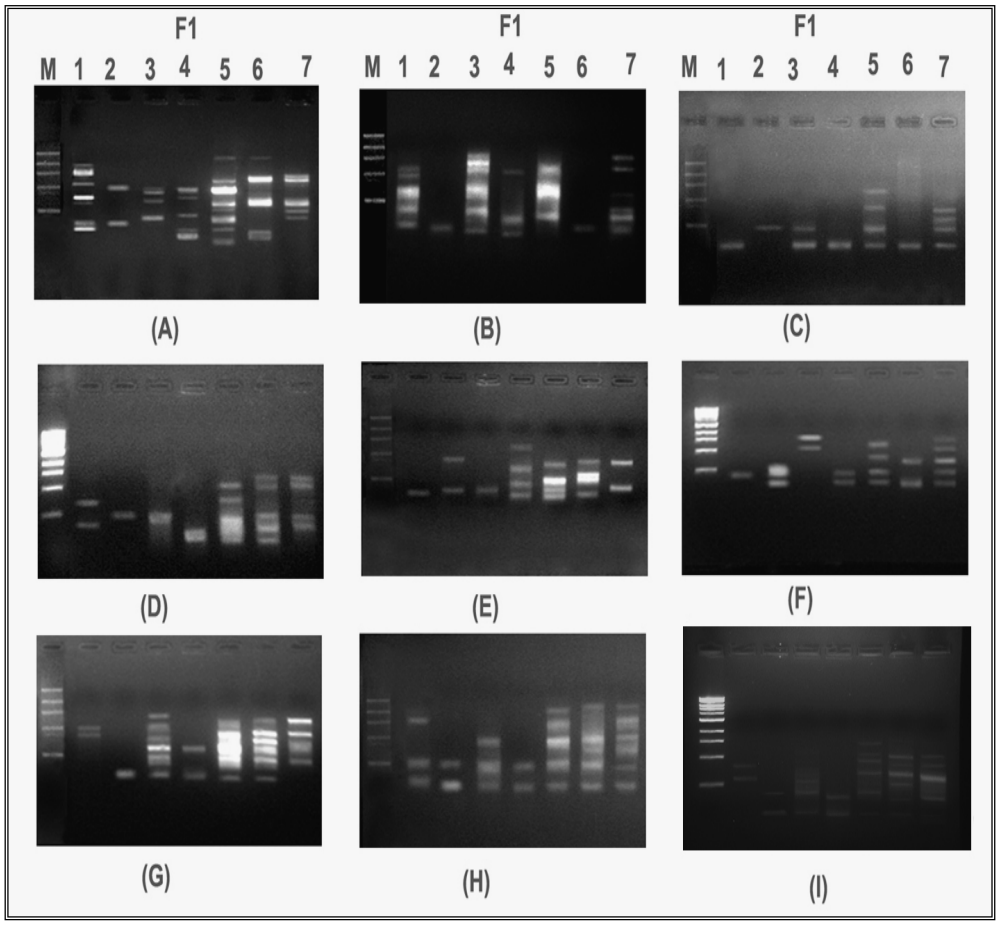

Fig. (2): DNA polymorphism of the seven F_1 crosses pepper (1):P_7xP_5, (2):P_7xP_1, (3):P_7xP_6, (4):P_5xP_4, (5):P_7xP_4, (6):P_7xP_3 and (7):P_7xP_2 using ISSR-PCR with nine primers.