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DNA POLYMORPHISM FOR EVALUATION OF FOUR PEPPER CULTIVARS FOR RESISTANCE TO *Meloidogyne incognita* AND *Rotylenchulus reniformis* NEMATODES

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ABSTRACT

The reaction of four pepper cultivars (California Wonder, Long Green , Hot Green and strain-9852-17), and their plant susceptibility to inoculum relation with either *Meloidogyne incognita* or *Rotylenchulus reniformis* was studied under greenhouse conditions during 2002 and 2003 seasons. Data showed that strain-9852-17 cv. was rated as highly resistant (HR) to root-knot nematode, *M. incognita* and reniform nematode *R. reniformis*. While, California Wonder and Long Green cultivars were rated as susceptible (S) for *M. incognita* and *R. reniformis*. Whereas, Hot Green pepper cv. was rated as slightly resistant (SR) to *M. incognita* and *R. reniformis*. It was noticed that phenolic compounds had a high concentration in un-infected pepper than inoculated by either tested nematode. The percent age of phenol reduction was higher when the pepper infected with *M. incognita* than *R. reniformis*. On the other hand, this evidence was conformed at the molecular level where there are the same differences between the four tested cultivars (California Wonder, Long Green, Hot Green and strain-9852-17) by using RAPD analysis. Amplification of genomic DNA from four pepper cultivars indicated that some bands were common to all cultivars but variations were also existing. The differences were not only in the major bands but also in some minor bands. In primer OP-A10 showed total of five specific bands. One band can be used as a marker on position 950-bp in variety (strain-9852-17) that has highly resistant to *M. incognita* and *R. reniformis*. Also, in primer OP-k11 showed total of eight specific bands. Three bands absent can be used as a marker in cultivar (strain-9852-17) on position 250-bp , 300-bp and 400-bp that have highly resistant and one band on position 1500-bp in one cultivar (Hot Green) which ranked as slightly resistant to both nematode species. Whereas, in primer OP-B5 showed total of five specific bands can be used as a marker in one cultivar strain-9852-17 in three position compared with those of susceptible remaining cultivars to *M. incognita* and *R. reniformis* (California Wonder and Long Green) which probably have carrying a resistance gene to nematodes in this position.

Keywords: Genome mapping, *Meloidogyne incognita*, pepper cultivars, *Rotylenchulus reniformis*.

INTRODUCTION

Pepper, *Capsicum annuum* L is one of the most important and widely consumed vegetable crops in Egypt. It can be planted in Egypt during summer season in open fields or under un-heated plastic houses in winter season. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, *M.arenaria*, *M.javanica*, and *M. hapla* are major pests in bell pepper, *Capsicum annuum* in USA and throughout the world (Amin & Budai, 1993; Di Vito *et al.*, 1985; Sasser and Frankman, 1987; Thies *et al*, 1997; Thomas, *et al*, 1995). However, pepper is highly susceptible to root knot

nematodes, *Meloidogyne* spp. At least one or two *Meloidogyne* species are likely to be present in any one area, which can result in significant yield losses. It is clear that resistant cultivars without nematicides treatment yield as much as high yielding susceptible ones treated with nematicides. Since nematicides are very expensive and of serious environmental damages, biological control is an alternative solution of plant parasitic nematodes with the use of the resistant cultivars (Amin and Abd Al-Wahab, 2001). It is regarded as environmentally acceptable practices for nematode management (Ibrahim *et al.*, 1998). The planting of resistant crops can be a practical and appropriate management strategy for use by small-scale farmers as a protective method against plant parasitic nematodes.

Because of economic importance of agricultural losses to nematodes, genetic resistance to these nematodes has been the focus of research for nearly five decades. Pepper cultivars have been found to vary in their reaction to *Meloidogyne* spp. Their majorities are susceptible to infection with *M. incognita*, *M. arenaria* and *M. hapla*. Only limited information is available on the relationships between root-knot nematode populations and pepper growth. Philips and Richard (1993) stated a new cayenne pepper cultivar has been developed, that is so hot and it kills soil borne nematodes. The pepper cultivar called Charleston hot was also reported to reduce root-knot nematodes by 95% in field trails.

In Egypt, Fatma (1989) found that all the ten tested pepper cultivars were ranked as susceptible to *M. incognita*. In Hungary, Amin and Budai (1993) found eight pepper cultivars were resistant out of 44 cultivars.

In plant pathology, phenolic compounds are considered to be the most important group of chemical compounds in disease resistance. Although, there is seldom correlation between resistant plant and the level of total or special phenolics (Levin, 1976). Many of phenol compounds act as phytoalexins, accumulating in plant tissue after the infection and normally occur in the plants as glycosides which are non-toxic. The compounds decomposed to free phenols by the action of a glycosidase, available in the plant cells or in the nematode's secretion. Hung and Rohde (1973) found that tomatoes resistant to *Meloidogyne* spp had a high levels of phenolic compound. In the contrary results, Amin and Abd El-Wahab (2001) noticed that resistant olive cultivars to three nematode genera gave the lowest value of total phenols than susceptible ones.

Information about resistance of *C. annuum* to *Meloidogyne* species is limited. Martin (1948) observed resistance to *Meloidogyne* species in a pungent line of *C. annuum* and subsequently released the root-knot nematode resistant cayenne pepper 'Carolina Hot' (Martin and Crawford, 1958). Fery *et al*, (1986) selected 'Carolina Cayenne' resistant to *M.incognita* races 1,2,3 and 4. Hare (1957) demonstrated that resistance to *M. incognita* was conditioned by a single dominant gene which was designated the N gene. Resistance to *M. incognita* 'Carolina Cayenne' is also conferred by the N gene and an additional recessive gene (Fery and Duckes, 1996). Recently, 'Charleston Bell' and 'Carolina Wonder' the first bell pepper cultivars with resistance to *M. incognita* (Fery *et al.*,1998). Thies and Fery (2000) concluded that the N gene confers resistance to *M. arenaria* races 1,2 and *M.*

javanica in *C.annuum*, but the N gene does not condition resistance to *M. hapla*. The resistance and susceptibility of pepper to root-knot nematode, *Meloidogyne incognita* and *Rotylenchulus reniformis* were studied by many researchers. Recently, striking sequence similarities have been noted among R genes that confer resistance to fungi, bacteria, nematodes, viruses, and insects in both monocotyledonous and dicotyledonous hosts (Milligan *et al.*, 1998; Molly *et al.*,2000 ; Rossi *et al.*, 1998)

The first specific goal of this research was to focus on DNA polymorphism of four pepper cultivars (California Wonder, Long Green; Hot Green and strain-9852-17) by using random amplified polymorphic DNA (RAPD). The second goal was to study the relation between phenolic compounds and resistant four pepper cultivars. The third goal was to study the susceptibility of four pepper cultivars to infection with *Meloidogyne incognita* and *Rotylenchulus reniformis* under greenhouse conditions.

MATERIALS AND METHODS

1-Pepper cultivars

Four pepper cultivars were tested for their susceptibility to the root-knot nematode, *Meloidogyne incognita* and reniform nematode, *Rotylenchulus reniformis* under greenhouse conditions. Of them three hybrid cultivars was, viz. Long Green (sweet pepper from Japan), Hot Green (hot pepper from Japan) and hot pepper line strain-9852-17 from Taiwan, in addition to the true breeding cv. California Wonder, all of them were evaluated for their susceptibility to infection with either *M. incognita* or *R. reniformis*. Mass raring culture of the root-knot nematode, *Meloidogyne incognita* and *R. reniformis* were propagated on tomato and pigeon bean, *Cajanus indicus* plants respectively under greenhouse conditions.

2-Plant screening tests and nematode inocula

Pepper *Capsicum annuum* L. cultivars to be screened, were grown from seeds in a mixture of steam-sterilized sand and clay (1:1v/v) in 15-cm-d. plastic pots containing 2000-g soil. A suspension of 2000 juvenile-stage was prepared by Cobb sieve technique and dispersed uniformly into five holes of the soil near the stem of the plant to be screened. Each pepper cultivar was replicated six times. After nematode inoculation the pots were arranged in the glasshouse bench in a complete randomized block design at temperature ranging from 25-30°C. Plants were allowed to develop for 50 days after inoculation. After this time, they were removed from their containers. The plant shoots were excised, and soil was gently washed from the root system. The number of galls as well as egg-masses on the root system was determined. Root systems were rated individually galls/egg-masses index (GI/EI) on the scale 0-5 according to Taylor and Sasser (1978) for the number of galls/egg-masses present in the root system inoculated with *M. incognita*. Indexing for resistance of pepper to root-knot and reniform nematodes was done on a (0-5) scale according to Hadisoeganda and Sasser, (1982). The stages of *R. reniformis* were recorded on root and in soil.

The root systems were preserved in a formalin-acetic acid-alcohol (F.A.A) solution till their examination under stereoscopic microscope. Roots were stained in boiling lactophenol-acid-fuchsin solution for 2-3 minutes, cleared in lactophenol for at least 24 hours (Franklin,1949). Roots were examined for the number of immature stages, females and egg-laying females under a stereoscopic microscope at 40-x magnification.

3-Plant growth and phenolic compounds determination.

Data obtained in this study were recorded instantly on individual plant basis on growth characters shoot length and weight; root length and weight. Total yield including, total number and weight of harvested fruits throughout the season) and fruit characters i.e., weight, length and diameter were also recorded. The phenolic content in roots was determined according to the procedure described by Snell and Snell (1953). Means between treatments were compared using New LSD (Waller and Duncan, 1969).

4-DNA fingerprinting.

4-a- Total genomic DNA isolation.

Isolation of DNA was carried out according to the protocol described by Walbot (1988). Additional chloroform – isoamyl alcohol extraction and ethanol precipitation were performed to improve the purity of DNA. RNase treatment was directly conducted before amplification step.

4-b-Poymerase chain reaction (PCR).

All polymerase chain reactions were performed in a reaction mix containing 20 ng genomic DNA, 0.5 unit Tog polymerase , 200 Um each of dATP, dcTP , dGTP and dTTP (sigma), 10 pU random primer and appropriate amplification buffer. The amplification was assembled on ice, over laid with a drop of mineral oil. The reaction was performed in thermal cycles with the following temperature condition: 94C° for three min. followed by 45 cycles of 92 C° for 30 sec. . 35 C° for 60 sec. And 72 C° for two min. (for dematuration, annealing and extension, respectively). Reaction was finally incubated at 72 C° for 10 min., and further 10min. at 62 C°. The RAPD products were analyzed by electrophoresis in 2% agarose in TAE buffer stained with ethidium bromide and photographed under UV light. Ten random, 10-ml primers (Operon Technologies) were screened for RAPD markers. Three primers (OP-A10, OP-B5 and OP-K11) which gave clear and consistent amplification products were used (Table 1). The amplified products bands were scored as present (+) or absent (-).

Table 1: Sequences and amplified products of three primers (Operon) used to generate RAPD markers in pepper cultivars.

Primer	Sequence	GC%
OP-K 11	AATGCCCCAG	60%
OP-B5	AGCGCCCTTC	70%
OP-A10	GTGATCGCAG	60%

RESULTS

1- Resistance and susceptibly of four pepper cultivars.

From the results summarized in Tables 2 and 3, it was clear that the pepper strain-9852-17 consider highly resistant cultivar (HR) for *M. incognita* and *R. reniformis* infestation. California Wonder and Long Green pepper cultivas were rated as susceptible (S) for both studied nematodes. On the other hand, Hot Green pepper cultivar was rated as slightly resistant (SR) for *M. incognita* and *R. reniformis*. The highest number of galls were recorded in Long Green pepper (764 galls /root system), whereas Hot Green has 77 galls. Also, the number of immature stages was variable and ranged from zero in strain-9852-17 to 86 stages in Long Green. The number of females were also associated side by side with the resistance and susceptibility of pepper, from zero in strain-9852-17 to 718 females in Long Green pepper cultivar.

Due to the pepper cultivars grown in soil infested by *Rotylenchulus reniformis* the number of females was ranged from four females in strain-9852-17 to 611 in Long Green cv. Also the rate of build up were between zero for strain-9852-17 cv. and 7.0 for Long Green cv. (Table 3)

2-Plant growth and fruit characters between pepper cultivars.

Data presented in Table (4) show that there were significant differences between infected with *Meloidogyne incognita* and non-infected California Wonder pepper cultivar for all studied characteristics except for shoot weight. Also, there were significance different in the fruit characteristic for Long Green cv. Concerning Hot Green cv., data showed significant differences for shoot and root length. On the contrary, the differences between infected and non-infected plants of the strain-9852-17 were not significant compared with all studied characteristic (Tables 4 and5).

Comparison between *M. incognita* and *R. reniformis* on growth of yields and fruit characters is presented in Tables 4 and 5. Generally, *M. incognita* was more effective than *R. reniformis* on pepper plant growth and yields, which resulted from highly significant reduction on, yield weight and number of fruits. Data showed that there were highly significant in the total yield per plant based on weight and number of fruits between nematode infested plant pepper cv. California Wonder and healthy plant by either *M. incognita* or *R. reniformis*. In spite of pepper cultivars cv. Long Green (Susceptible) had more population on root system than California Wonder (Susceptible), the last one was more affected with either nematode species. The other pepper cultivars (resistant cultivars) showed that, there were no significant difference between infested and healthy ones on growth criteria, total yields and fruits characteristics.

As shown in (Table 5) there were significant differences between infected with *Rotylenchulus reniformis* and non-infected plants of California Wonder cv. for all studied characteristic except for root length and the fruit length.

Table 2: Susceptibility of four-pepper cultivars to *Meloidogyne javanica* infestation

Cultivars	No. of Galls	Total Number of					Gall/egg-masses Index	R Factor	Index
		Immature stages	Females	Egg-masses	Nematode in soil	Nematodes/ pot			
California Wonder	262.0	45.0	248.0	208.0	7033.0	7326.0	5/5	7.0	S
Long Green	764.0	86.0	718.0	651.0	5981.0	6785.0	5/5	6.0	S
Hot Green	77.0	21.0	66.0	50.0	1882.0	1969.0	4/4	1.9	SR
strain-9852-17	0.0	0.0	0.0	0.0	0.0	0.0	0/0	0.0	HR
$LSD_{0.05}$	144.4	18.3	140.5	128.1	1812.8	-	-	-	-
$LSD_{0.01}$	202.5	25.7	197.0	179.6	2541.1	-	-	-	-

S = susceptible SR = slightly resistant HR = highly resistant

Each number presented the mean of sex replicates.

Table 3: Susceptibility of four-pepper cultivars to *Rotylenchulus reniformis* infestation

Cultivars	Total Number of					R Factor	Egg-masses Index[1]	Index
	Un-swollen females	Females	Egg-masses	Nematode in soil	Nematodes/ pot			
California Wonder	48.0	257.0	150.0	5718.0	6023.0	6.0	5.0	S
Long Green	80.0	611.0	545.0	6223.0	6914.0	7.0	5.0	S
Hot Green	32.0	57.0	42.0	1571.0	1659.0	1.7	4.0	SR
Strain-9852-17	2.0	4.0	2.0	0.0	6.0	0.0	1.0	HR
$LSD_{0.05}$	16.3	127.9	91.7	1985.6	-	-	-	-
$LSD_{0.01}$	22.9	179.3	128.6	2783.7	-	-	-	-

Each number presented the mean of sex replicates.

[1]Egg-masses Index (EI) , based on number of egg-masses were, 0=none ; 1=1-2; 2=3-10 ; 3=11-30; 4=31-100; and 5 ?100. The ranges of EI's were used to estimate the degree of resistance as follows: 0-1.0 EI = Highly resistant (HR); 1.1-3.0 EI = very resistant (VR); 3.1-3.5= moderately resistant (MR), 3.6-4.0= Slightly resistant (SR) and 4.1-5.0= Susceptible (S).

Table 4: Effect of *Meloidogyne incognita* infestation on growth, total yields and fruits characteristics of four-pepper cultivars

Cultivars		Vegetative Growth				Total yield /plant		Fruit Characters		
		Shoot		Root		On weight basis (g)	On Number basis	Weight (g)	Length (cm)	Diameter (cm)
		Length (cm)	Weight (g)	Length (cm)	Weight (g)					
California Wonder	Infected	41.0	31.4	23.9	21.8	347.0	14.0	24.8	9.7	3.6
	Non-infected	49.5	38.4	32.8	26.9	405.0	17.8	25.8	9.9	3.9
Long Green	Infected	33.5	29.5	28.8	15.5	249.8	20.5	12.3	9.5	1.8
	Non-infected	38.3	38.0	34.3	19.0	260.0	21.3	12.2	10.1	2.0
Hot Green	Infected	32.5	25.3	36.0	17.3	196.3	22.0	8.9	4.0	1.1
	Non-infected	40.0	32.9	45.8	21.3	205.0	22.8	9.0	4.2	1.2
Strain-9852-17	Infected	29.0	33.3	36.3	24.5	231.0	21.5	10.5	8.9	1.6
	Non-infected	28.8	31.8	37.8	26.5	233.0	21.8	9.9	9.0	1.8
New LSD $_{0.05}$		5.6	9.1	7.9	5.1	23.3	1.8	0.9	0.5	0.4
New LSD $_{0.01}$		7.5	12.7	10.5	6.7	30.8	2.4	1.2	0.6	0.5

Table 5: Effect of *Rotylenchulus reniformis* infestation on growth, total yields and fruits characteristics of four-pepper cultivars

Cultivars		Vegetative Growth				Total yield /plant		Fruit Characters		
		Shoot		Root		On weight basis (g)	On Number basis	Weight (g)	Length (cm)	Diameter (cm)
		Length (cm)	Weight (g)	Length (cm)	Weight(g)					
California Wonder	Infected	33.5	29.0	26.0	19.0	370.1	15.3	24.4	9.6	3.4
	Non-infected	49.5	38.4	32.8	26.8	405.0	17.8	25.8	9.9	3.9
Long Green	Infected	28.8	31.5	29.0	17.4	248.8	20.8	12.0	9.6	1.3
	Non-infected	38.3	38.0	34.3	19.0	260.0	21.3	12.2	10.1	2.0
Hot Green	Infected	34.3	21.9	36.8	16.6	186.3	20.3	9.2	4.1	0.9
	Non-infected	40.0	32.9	45.8	21.3	205.0	22.8	9.0	4.2	1.2
Strain-9852-17	Infected	27.8	31.1	35.0	26.9	212.5	21.0	10.1	8.7	1.7
	Non-infected	28.8	31.8	37.8	26.5	233.0	21.8	9.9	9.0	1.8
New LSD $_{0.05}$		6.8	7.8	9.5	7.6	22.0	2.0	0.9	0.5	0.3
New LSD $_{0.01}$		8.9	10.3	12.5	10.0	28.9	2.6	1.8	0.7	0.4

There were significant differences in shoot and fruit length and its diameter for Long Green cv. and in shoot weight, root length, total yield per plant on number bases, and fruit diameter for Hot Green cultivar. No significant differences were noticed between infected and non-infected strain-9852-17 pepper cultivars (HR) for all characters.

3-Phenolic compound between four pepper cultivars.

In (Table 6) data indicated that susceptible pepper cultivars had high levels of free and total phenolic compound in comparison with resistant ones. Also, phenolic compound had a higher concentration in healthy pepper cultivars than infective ones. On the other hand and in generally, *R. reniformis* stimulate a high level of free and total phenolic compound than *M. incognita* (Fig.1). Correspondingly, the percent of phenolic compound reduction due to nematode infestation was higher in case of *M. incognita* infestation than *R. reniformis* compared with healthy plants (Fig. 1). It is clear to notice that the percentage of free phenol reduction was higher in susceptible cultivars than resistance ones (Fig. 1).

Table 6: Free, conjugate and total phenol in root of four pepper cultivars affected by *Meloidogyne javanica* and *Rotylenchulus reniformis* infestation.

Pepper Cultivars	Non-Infected plant	*Rotylenchulus reniformis*		*Meloidogyne javanica*	
		Infected	% Reduction	Infected	% Reduction
Free phenol					
California Wonder	70[1]	41	41	22	69
Long Green	35	19	46	19	46
Hot Green	19	26	-37	22	-16
Strain-9852-17	20	20	00	15	25
Conjugate phenol					
California Wonder	20	09	55	18	10
Long Green	65	16	75	18	72
Hot Green	126	54	57	03	98
strain-9852-17	24	17	29	21	13
Total phenol					
California Wonder	90	50	44	40	56
Long Green	100	35	65	37	63
Hot Green	145	80	45	25	83
strain-9852-17	44	37	16	36	18

[1] **Phenolic compound in µg/g of fresh roots of pepper.**

4-RAPD polymorphism between four pepper cultivars.

Amplification of genomic DNA from four pepper cultivars (California Wonder, Long Green; Hot Green and strain-9852-17) indicated that some bands were common to all cultivars but some minor variation were also existing. The differences were not only in the major bands but also in some minor bands.

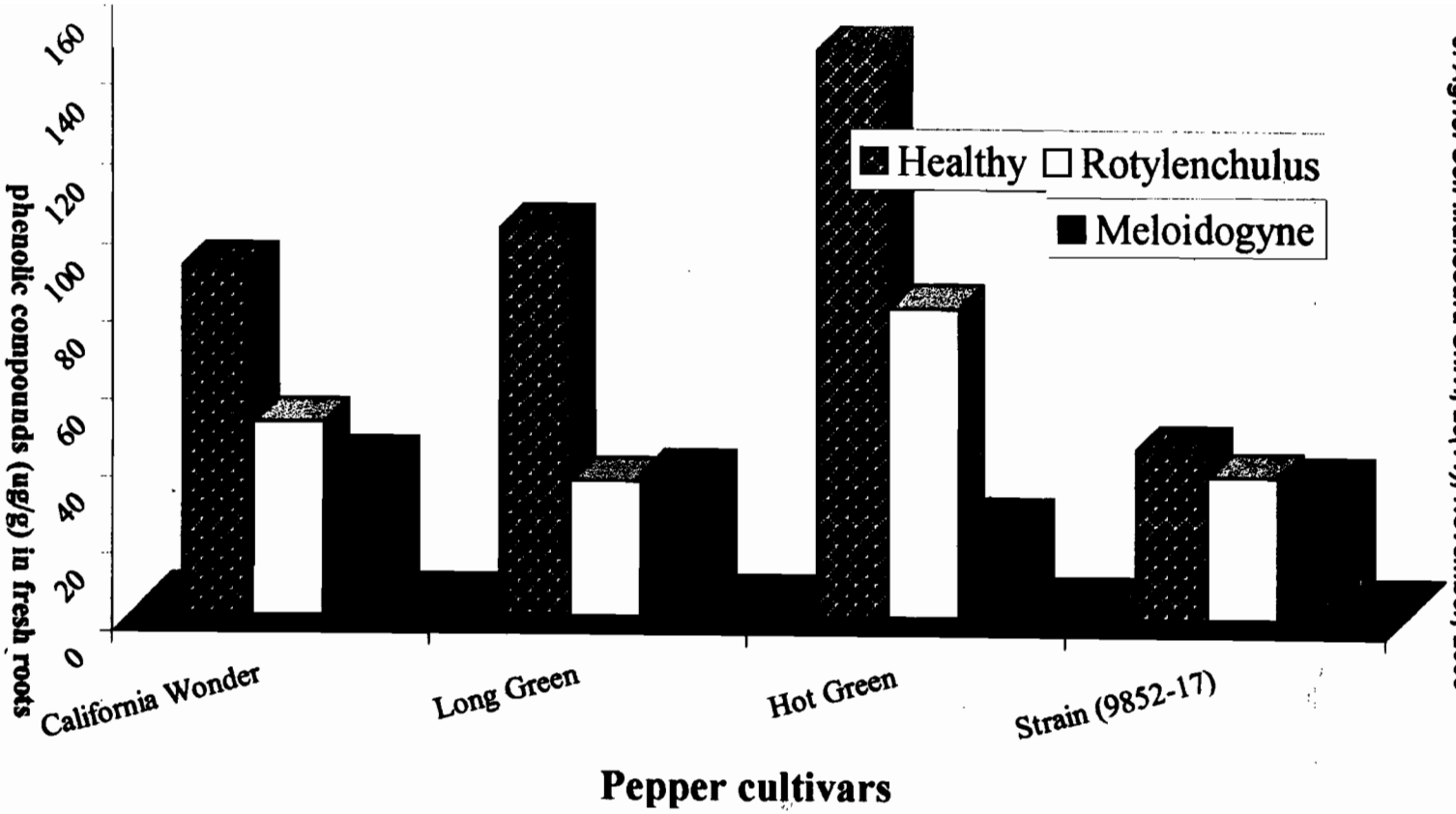

Fig.1.The variation between phenolic compound in four pepper cultivars infested with nematode species.

The reproducibility of RAPD amplification is known to be highly influenced by experimental conditions. It is therefore essential to optimize the PCR conditions to obtain reproducible results before going on routine analysis. Investigating each factor individually, such as genomic DNA quality and concentrations, primer annealing and extension, temperature and denaturation time and temperature is a prerequisite. Series of preliminary experiments were conducted to select the suitable primers and the optimal condition for RAPD analysis (Table 7 and Fig. 2 [a, b and c]).

Table 7: Random DNA primers selected to distinguish random amplified polymorphic DNA (RAPD) fingerprints of four pepper cultivars.

Primers	DNA bands		Size of polybands (pb) mw	Presence or absence of polymorphic bands			
	Total[1]	Polymorphic		1	2	3	4
OPK11	8	4	1500	-	-	+	-
			800	+	+	+	+
			500	+	+	+	+
			450	+	+	+	+
			400	+	+	+	-
			350	+	+	+	+
			300	+	+	+	-
			250	+	+	+	-
OPB5	5	3	500	+	+	+	-
			400	+	+	+	-
			350	+	+	+	-
			300	+	+	+	+
			200	+	+	+	+
OPA10	5	2	950	-	-	-	+
			900	+	+	+	+
			800	+	-	+	+
			700	+	+	+	+
			500	+	+	+	+

[1] **Total number of bands (polymorphic and non – polymorphic), (+) present, (-) absent of band, 1,2,3,4 : four peper cultivars (California Wonder, Long Green, Hot Green , and strain-9852-17.**

Ten mer primers were screened tested for genomic DNA amplification for each cultivar. Three primers (OP-A10, OP-B5 and OP-K11) gave clear and consistent amplification products and were used as markers for the four pepper cultivars. Amplification of genomic DNA from four pepper cultivars (California Wonder, Long Green; Hot Green and strain-9852-17) indicated that some bands were common to all cultivars but some molecular minor variations were also existing. The differences were not only in the major bands but also in some minor bands. Data in (Table 7) illustrates the total bands, number of polymorphic bands and their distribution among the amplification products of the different cultivars. The primers OP-A10 showed one presence monomorphic (950-bp) band only in strain-9852-17 pepper cv. of the total of five bands (Fig 2. a). The primer OP-B5 bands with Mw 350, 400 and 500-bp were absence only in strain-9852-17 cv and present in all other examined cultivars. While, primer OP-K11 provided maximal number of polymorphic bands (8 bands). The primer OP-K11 showed one monomorphic

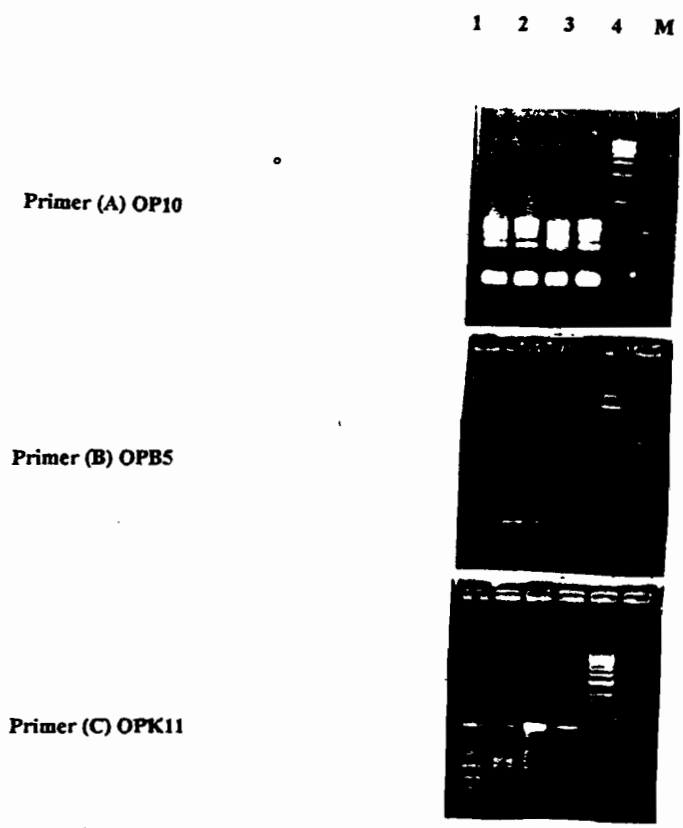

Fig. 2.(A,b,c) Random amplified polymorphic DNA (RAPD)patterns obtained from individual genomic DNA samples of pepper cultivars. Marker lane (M) are one Kb DNA ladders: Lane 1 (California Wonder); Lane 2 (Long Green); Lane 3 (Hot Green) and Lane 4 (Strain (9852-17)) pepper cultivars.

with presence Mw (1500-bp) band only in Hot Green pepper cv. of the total of eight bands and bands with Mw 250-bp , 300-bp and 400-bp were absence only in strain-9852-17 pepper cv. The amplified products for these primers were scored in additional resistant and susceptible pepper plant. Only two of these amplification products, a 950-bp fragment ($OP\text{-}A10_{950}$) amplified by OP-A10 (GTGATCGCAG), may be related with resistance in strain-9852-17 and a 1500-bp fragment ($OP\text{-}K11_{1500}$) amplified by OP-K11 (AATGCCCCAG), may be related with slightly resistance in Hot Green pepper cv. (Fig. 2, a and c).

DISCUSSION

From the previous data that shown in (Tables 2 and 3), it was clear that the pepper strain-9852-17 consider highly susceptible cultivar (HS) for both *M. incognita* and *R. reniformis* infestation according to the scale of Hadisoeganda and Sasser (1982). Hot Green pepper cultivar was rated as slightly resistant (SR) for both studied nematodes. On the other hand, California Wonder and Long Green pepper cultivars were rated as susceptible (S) for both nematodes. These results were similar to those obtain by Ibrahim *et al.*, (1998) who reported that pepper cvs. California Wonder, Sweet Long and Anaheim were rated as susceptible, whereas Red Hot Short cv. was resistant to. *M. arenaria*. Jacob and Kurain (1979) and Lindrsey and Margaret (1982) found no resistant pepper cultivars against *M. incognita*. On the other hand, results of the present study agreed with the findings of Abul-Hasan (1985), who found that out of 31 cultivars only two were found to be resistant against *M. incognita*. The results summarize that between pepper cultivars, there are some pepper cultivars rated as resistant to nematode infestation (Amin and Budai, 1993, Di Vito, *et al.*, 1985, Sasser and Frankman, 1987, Thies *et al.*, 1998 and Thies and Fery, 2000).

The resistance N gene conferring resistance to the nematodes and other pathogens like fungi, bacteria viruses and insects (Bent *et al.*, 1994, Milligan *et al.*, 1998, Molly *et al.*, 2000, Parker *et al.*, 1997, Rossi *et al.*, 1998). Thies and Fery (2000) demonstrated that N gene, which confers resistance to *M. incognita* in *C. annuum*, also confers resistance to *M. arenaria* race 1 and 2 and *M. javanica*. The results of these studies agreed with this finding, which indicated that *C. annuum* cultivars resistance to *M. incognita* were also resistance to *R. reniformis*.

The relation between pepper cultivars and nematode infestation and their relation to plant phenolic compounds response and DNA fingerprinting are useful for study the resistance and susceptibility of plant on molecular and biochemical bases. Therefore, more study on pepper plant warrants further investigation. It's clear that resistant plant infested with nematodes stimulate free phenol more than susceptible one. The percentage of phenol reduction in susceptible plant is higher than resistant plant. This finding agreed with Singh and Choudhurg (1973), who found that phenolic content was found to be directly related to nematodes resistance, being highest in immune cultivars followed by resistant, tolerant and susceptible cultivars. On the other hand, strain-9852-17 and Hot Green pepper cvs gave the lowest value of free phenols and a high level of conjugate phenol. These

results are conformable to their resistant to infestation with either tested nematode. On the contrary this efficacy California Wonder and Long Green cvs are highly susceptible to both nematode infestation.

Increasing the activity of such chemicals are, in part responsible for synthesis auxins, hormones, and many other compound that involved in the defense mechanisms of specific incompatibility of plant to nematode infestation (Veech and Endo, 1970). Levin (1976) noticed that, there is a seldom correlation between plant resistance and the level of total or special phenolics. This notice agreed with the present results, where the inoculated plant has more amounts of phenols than un-inoculated ones. Also, resistant cultivars have more percent of phenols than susceptible cultivars. These results coincide with those of Al-sayed and Montasser (1986). They concluded that glutamic acid could be utilized as helpful materials that may induce and/or increase resistance in plant.

Concerning the fingerprint by using OP-A10, OP-B5 and OP-K11 primers, data showed highly influence by experimental condition. There are many differences in the major bands and in some minor bands between pepper cultivars. It's clear that pepper strain-9852-17 cv has only one band out of five bands. This presence band might be associated with resistance in either tested nematode in position Mw OP-$A10_{950}$ bp that rated as highly resistant to *M. incognita* and *R. reniformis*. Band with Mw OP-$K11_{1500}$ is inseparable in cultivar Hot Green which are rated as slight resistant (SR) to *M. incognita* and *R. reniformis*. Bands with OP-$B5_{350}$, OP-$B5_{400}$ and OP-$B5_{500}$ bp are absent in strain-9852-17 which is rated as resistant to both nematodes. On the other side, Mw OP-$K11_{1500}$ may be carrying a resistance gene against *M. incognita* and *R. reniformis*. Fery *et al.* (1986) selected the resistant cultivar to four races of *M. incognita*. In 1998, Fery, *et al.* selected the first bell pepper cultivars resistant to *M. incognita*. The precise detail of recognition of infection by the host, however, and the downstream events that result in resistance to infection, remain largely unknown (Innes, 1998). In conclusion, the resistant plants infested with nematodes stimulate free phenol more than susceptible one. The percentage of phenol reduction in susceptible plant is higher than in resistant one. Nematode resistant pepper will allow successful production of bell peppers in soils that are heavily infested with nematodes and useful in IPM programs.

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تعدد المظاهر فى الـ (د ن ا) لتقييم مقاومة أربعه أصناف من الفلفل لنيماتودا تعقد الجذور و نيماتودا القطن الكلوية

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تم دراسة حساسية أربعه أصناف من الفلفل (California Wonder و Long Green و Hot Green و strain-9852-17 للإصابة بكل من نيماتودا تعقد الجذور *Meloidogyne incognita* و نيماتودا القطن الكلوية *Rotylenchulus. reniformis* و ذلك تحت ظروف البيوت الزراعية فى موسم ٢٠٠٢/٢٠٠٣ م. و قد دلت النتائج على أن الصنف strain-9852-17 عالي المقاومة لكل من نوعى النيماتودا بينما الصنف Hot Green قليل المقاومة لكل من نوعى النيماتودا المختبرة , كان كل من California Wonder و Long Green حساس لكل من نوعى النيماتودا.

أظهرت النتائج التحليل الكيماوى لمركبات الفينول المرتبطه و الحرة لجذور نباتات الفلفل السليمة و المصابة لكل الأصناف المختبرة أن مركبات الفينول كانت عالية التركيز فى أصناف الفلفل السليمة و الغير مصابه بالنيماتودا مقارنه بأصناف الفلفل المصابه.على الجانب الاخر و جد أن نسبة الانخفاض فى كميه الفينولات بالجذور أعلى عند أصابه أصناف الفلفل بنيماتودا تعقد الجذور عن الاصابه بنيماتودا القطن الكلوية.و قد لوحظ أن الأصناف المقاومة للاصابه تحتفظ بكميه أعلى من الفينولات و بعدم تحللها مقارنه بأصناف الفلفل الحساسة و التى أنخفضت فيها الفينولات بمعدلات أكبر بعد الاصابه. فى نفس الوقت تم دراسة الاساس الجزيئى من خلال البصمة الوراثية لمدى تشابه و إختلاف توزيع حزم الماده الوراثيه مع مستوى كل من الأصناف الحساسة و المقاومة لكل من نيماتودا تعقد الجذور و نيماتودا القطن الكلوية و قد تبين تشابه التركيب الوراثي فى غالبيه الخطوط و اختلافها البسيط فى بعض المواقع و الخطوط الرئيسية و الصغيرة و ذلك يدل على أنها من تراكيب وراثيه واحده واختلافها فى بعض الخطوط يبن أن هذه المواقع من المورثات ربما تحمل جين المقاومة للنيماتودا. كما ظهر ذلك عند استعمال البادئ OP-A10 عند الموقع 950-bp فى صنف الفلفل strain-9852-17 عالي المقاومة و البادئ OP- K11عند الموقع -1500 bp فى صنف الفلفل Hot Green قليل المقاومة.