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USE OF RAPD ANALYSIS TO DETECT MOLECULAR MARKERS FOR ROOT ROT RESISTANCE/SUSCEPTIBILITY IN SOYBEAN

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ABSTRACT

This investigation aimed to evaluate the reaction of ten soybean cultivars to root rot disease and to find out some molecular genetic markers related to resistance/susceptibility of these cultivars to the root rot. The ten soybean cultivars were tested against each of the three root rot fungi i.e; Fusarium solani, Macrophomina phaseolina and Rhizoctonia solani separately. Healthy survivals were used to evaluate the reaction of the cultivars to the disease compared with their respective controls. Giza 35 and Giza 83 were the most susceptible, while, Giza-111, Crowford, and Forrest were the most resistant, as they recorded the highest percentage of survived seedlings 40-days after sowing. DNA was extracted from leaves of the healthy cultivars and RAPD-PCR was used to determine if there is any relationship between the obtained DNA fragments and resistance/susceptibility of the soybean cultivars to root rot disease. Thirteen, arbitrary chosen, 10-mer RAPD primers were used, six primers succeeded in the amplification of DNA fragments revealing polymorphism. Primers; OP-A11, OP-B03, OP-D16, OP-L12 and OP-L20 were successful in generating either positive or negative molecular markers related to root rot resistance in soybean.

Key words: ***Soybean, Glycine max, RAPD, Molecular markers, Root-rot resistance, F. solani, M. ohasiolina, R. Solani***

INTRODUCTION

Soybean (*Glycine max* L.Merr) is considered one of the main oil crops all over the world. Also, it has a special importance in Egypt a source of oil and protein. Soybean seed contains 20% oils and 40% proteins (Kassem 1982).

Soybean root rot disease complex may cause considerable yield losses depending on the prevailing environmental conditions and susceptibility of cultivars (Sinclair 1982).

Many investigators reported differences among soybean cultivars in their susceptibility to infection with fungi causing root rot disease. In this respect, Hassanin (1992) studied the response of different soybean cultivars against charcoal rot disease caused by *Macrophomina. phaseolina* under

greenhouse conditions. He found that soybean cultivars McCall, Evans, Hardin, Calland, Essex, Cutlar71, Clark, Columbous, Crowford and Kent were more resistant compared with the other tested cultivars. Moreover, Gupal and Jagadeashuer (1997) found variation among 70 genotypes in susceptibility to infection with *Macrophomina phaseolina.*

Ondrej (1994) evaluated 24 soybean varieties for resistance to *R. solani* and *Fusarium* spp. under greenhouse and field conditions, He reported that resistance to root rot diseases caused by both fungi were high in cultivars Rito, Paradis, Alvia and Maple Iale. Cho and Cho (1999) tested 6 soybean cultivars to infection with *F. solani* and reported that cultivar Hartz 6686 was highly susceptible while cultivar PI520733 was highly resistant. Chakraborty *et al* (2003) tested ten soybean genotypes for resistance to *F. oxysporum*, one of the causal agents of root rot disease; they found that different cultivars exhibited varying degrees of susceptibility.

DNA based molecular methods have been integrated in the breeding programs of different field crops and are expected to play a very important role in the future. Polymerase chain reaction (PCR) techniques have been initiated as a novel genetic assay based on selective DNA amplification (Saiki *et al* 1993). Random amplified polymorphic DNA (RAPD) was evolved as an alternative method to restriction fragment length polymorphism (RFLP) because it does not require the use of isotopes like RFLP. Thus, it became one of the most widespread DNA techniques. This analysis is more amenable to automation than conventional techniques, simple to perform, requires only a small amount of DNA and provides a quick method for developing genetic maps (Van de Ven *et al* 1993). The technique was previously used to determine RAPD amplified fragments to discriminate faba bean cultivars tolerant to *Orobanche* and to identify faba bean cultivars resistant to chocolate spot (Omar 2004), it was also used to generate molecular markers related to Powdery mildew resistance in flax (Ashry *et al* 2002), to fingerprint some soybean (*Glycine max*) cultivars resistant to *Etiella zinckenella* (Fahmy and Salama, 2002) and to generate molecular genetic markers related to cotton leaf worm tolerance in soybean (El-Demerdash *et al* 2006).

The present study was initiated to assess the reaction of ten soybean cultivars to root rot, and to obtain some DNA markers related to the disease resistance.

MATERIALS AND METHODS

This investigation was carried out in the greenhouse and laboratories of Cell Research Section, Field Crops Research Institute (FCRI), and Leguminous Diseases Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza during the summer of 2005. Ten soybean cultivars (Giza-21, Giza-22, Giza-35, Giza-82, Giza-83, Giza-111, Crowford, Clark, Catler, and Forrest (referred to as V1 toV10 ,respectively) were used in the present study, they were supplied by Leguminous Crops Research Department, FCRI, ARC, Giza, Egypt.

Reaction of soybean cultivars to root rot fungi

a- Preparation of fungal inoculum:

Corn meal-sand medium (3:1w/w) were autoclaved in 500-ml glass bottles at 121°C for 20 min. The sterilized bottles were then inoculated with descs (5 mm in diameter) of seven-day old cultures of *Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani* separately and incubated at 25±2°C for 15 days.

b- Soil infestation:

Fungal inoculum of each fungus was mixed with the potted sterilized soil at the rate of 5%, 5% and 3% for *F. solani, M. phaseolina* and *R. solani*, respectively. The infested soils were watered daily for one week to enhance growth and distribution of the fungal inoculum. Infested soils were then sown with the seeds of the previously mentioned soybean cultivars separately. Seeds were sown at the rate of 10 seeds per pot (30 cm in diameter). Three pots were used as replicates for each treatment of each cultivar. Un-inoculated control soil were sown with the same rate.

c- Disease assessment:

Percentages of survivals were determined 40-days after sowing. Reactions of the cultivars were recorded according to the following scale.

Resistant (R) = <25 % reduction in the healthy surviving seedlings.

Moderate-susceptible (MS) = 26-35% reduction in the healthy surviving seedlings.

Susceptible (S) = 36- 45 % reduction in the healthy surviving seedlings.

Highly susceptible (HS) = >45 % reduction in the healthy surviving seedlings.

Reduction % of surviving seedlings was calculated as follows:

$$\text{Reduction \%} = \frac{\text{number of surviving seedlings in infected soil - number of surviving seedlings in control}}{\text{number of survived seedlings in control}} \times 100$$

d- Statistical analysis:

Completely randomized design with three replicates was used. When the estimated F value was significant for treatments, LSD value was calculated and used to compare means of different cultivars, fungus treatment and cultivar X fungus interaction.

Molecular genetic studies

DNA extraction and purification was achieved according to Doyle and Doyle (1990). Leaf samples from un-infected seedlings were used for DNA extraction, Thirteen arbitrary 10-mer primers listed in Table (1) with their universal names and sequences were used; ten out of them succeeded in DNA amplification. Six primers marked with (**) generated polymorphism, whereas the other four generated monomorphic banding patterns (*). The amplification was carried out in a DNA thermal-cycler apparatus (Perkin Elmer) programmed as follows: One cycle at 94°C for 2 min, 35 cycles each of 94°C for 1 min; 35°C for 30 sec.; and 72°C for 2 min and one cycle at 72°C for 5 min, then, 4°C infinite. PCR products were separated on agarose gels 1.2% stained with ethidium bromide with a constant electric current (100 volts) for 25 minutes at room temperature. Gels were visualized under UV lamp, photographed and scanned with AlfaEase Video Densitometer, USA, at a wave length of 577 nm, the instrument's software for densitometry assessments and data analysis were used, bands were scored as present (+) or absent (-).

Table 1. List of RAPD-primers universal names and sequences.

No.	Primer	Sequence `5→`3	No.	Primer	Sequence `5→`3
**1	OP-A11	`5CAA TCG CCG T`3	*8	OP-C15	'5GAC GGA TCA G 3'
*2	OP-A18	'5AGG TGA CCG T 3'	**9	OP-D16	`5AGG GCG TAA G`3
*3	OP-B01	`5 GTT TCG CTC C`3	[1]10	OP-E01	`5CCC AAG GTC C`3
[1]4	OP-O02	'5ACG TAG CGT C 3'	**11	OP-L12	`5GGG CGG TAC T`3
**5	OP-B03	`5CAT CCC CCT G `3	**12	OP-L20	`5TGG TGG ACC A`3
[1]6	OP-B20	`5GGA CCC TTA C `3	**13	OP-O19	`5GGT GCA CGT T`3
*7	OP-C13	`5AAG CCT CGT C `3			

[1] Amplification wasn't detected *Primers revealed monomorphism **Primers revealed polymorphism

RESULTS

Reaction of soybean cultivars to root rot fungi

Percentage of surviving seedlings to *F. solani* (Table 2) varied among the tested cultivars. Giza-22, Giza-82, Giza-111, Crowford, and Forrest showed non-significant differences between un-inoculated and inoculated treatments and were, therefore, considered resistant, while the other cultivars showed significant reductions and were considered susceptible. The susceptible cultivars showed different degrees of susceptibility and were classified according to their response as susceptible (S) including Giza-21 and Katller, or highly susceptible (HS) including Giza-35, Giza-83 and Clarck.

Table 2. Mean percentage of surviving seedlings of soybean cultivars in un-inoculated and inoculated soil with *F. solani*.

Code	Cultivar	Soil		Difference	Reduction%	Response
		Un-infested	infested			
V1	Giza-21	83.30	53.00	30.00*	36.01	S
V2	Giza-22	93.30	73.00	23.30	24.97	R
V3	Giza-35	90.00	33.30	56.70*	63.00	HS
V4	Giza-82	86.60	66.60	20.00	23.09	R
V5	Giza-83	90.00	40.00	50.00*	55.56	HS
V6	Giza-111	93.30	86.60	6.70	7.18	R
V7	Crowford	80.00	60.00	20.00	25.00	R
V8	Clark	86.60	46.70	39.90*	46.67	HS
V9	Kattler	86.70	53.30	33.40*	36.52	S
V10	Forrest	100.00	76.70	23.30	23.30	R
	LSD 5%	23.40				

* The difference between treatments is significant (P≤ 0.05%)

The reduction percentage of healthy surviving seedlings to *M. phaseolina* varied among the studied cultivars (Table 3). Giza-111, Crawford, Clark, and Forrest showed insignificant differences between the infested and non-infested soils and were, therefore, considered resistant cultivars. The other cultivars showed significant differences and variable degrees of susceptibility. Giza-21, Giza-35, and Giza 83 showed the highest susceptibility (HS), Giza-82 was susceptible (S), while, Giza-22 and Katller were moderately susceptible (MS)

Table 3. Mean percentage of surviving seedlings of soybean cultivars in un-inoculated and inoculated soil with *M. phaseolina*.

Code	Cultivar	Soil		Difference	Reduction%	Response
		Un-infested	infested			
V1	Giza-21	83.30	33.40	49.90*	59.90	HS
V2	Giza-22	93.30	66.70	23.60*	28.51	MS
V3	Giza-35	90.00	36.70	53.30*	59.22	HS
V4	Giza-82	86.60	53.30	33.30*	38.44	S
V5	Giza-83	90.00	36.70	53.30*	59.22	HS
V6	Giza-111	93.30	83.30	10.00	10.72	R
V7	Crowford	80.00	73.30	6.70	8.38	R
V8	Clark	86.60	76.30	9.30	11.43	R
V9	Kattler	86.70	60.00	26.70*	30.80	MS
V10	Forrest	100.00	90.00	10.00	10.00	R
	LSD 5%	16.92				

* The difference between treatments is significant (P≤ 0.05%)

Differences in the reduction% of healthy surviving seedlings in infested and non-infested soils with *R. solani* (Table 4) showed significant differences among cultivars. Giza-22, Giza-111, Crowford, Clark, and Forrest showed non-significant differences and were considered resistant whereas, variable degrees of susceptibility was observed in the other cultivars. Giza-35 and Giza-83 showed the highest susceptibility (HS), whereas, Giza-21, Giza-82 and Kattler were moderately susceptible (MS)

Table 4. Mean percentage of surviving seedlings of soybean cultivars in un-inoculated and inoculated soil with *R. solani*.

Code	Cultivar	Soil		Difference	Reduction%	Response
		Un-infested	infested			
V1	Giza-21	83.30	56.70	26.60*	31.93	MS
V2	Giza-22	93.30	83.40	10.10	10.82	R
V3	Giza-35	90.00	33.30	56.70*	63.00	HS
V4	Giza-82	86.60	63.30	23.40*	26.99	MS
V5	Giza-83	90.00	36.70	53.30*	57.22	HS
V6	Giza-111	93.30	90.00	3.00	3.23	R
V7	Crowford	80.00	63.40	16.60	22.65	R
V8	Clark	86.60	76.70	9.90	11.43	R
V9	Kattler	86.70	60.00	26.70*	30.80	MS
V10	Forrest	100.00	93.30	6.70	6.70	R
	LSD 5%	17.50				

* The difference between treatments is significant (P≤ 0.05%)

Table (5) summarizes the obtained results for the three pathgens. Data revealed that cultivars Giza-25 (V3) and Giza-83 (V5) were the most susceptible for the three pathogens, while Giza-111 (V6), Crowford (V7), and Forrest (V10) were the most resistant. These cultivars were used in the subsequent molecular studies to generate DNA markers for root rot resistance/susceptibility in soybean.

Table 5. Summary of response of the ten soybean cultivars to the three pathogens involved in root rot disease.

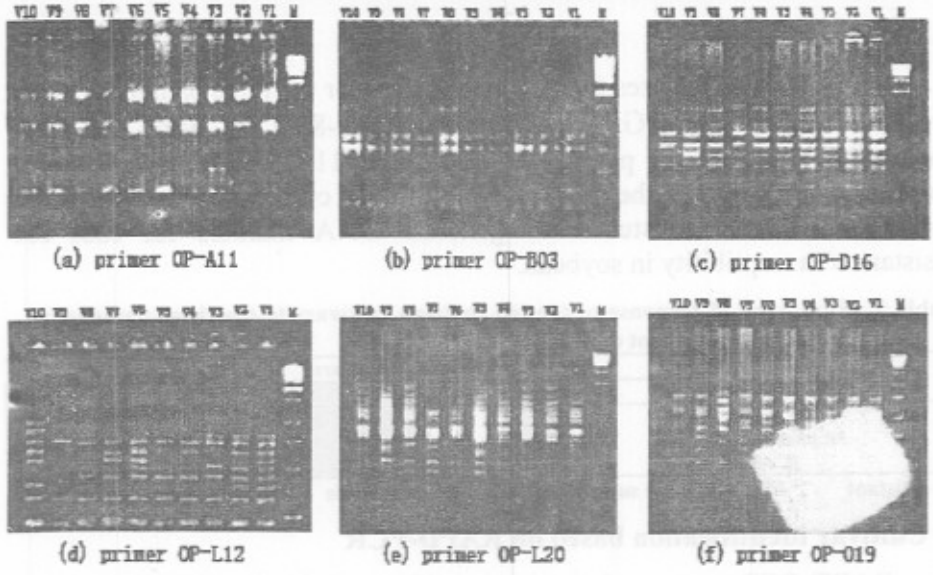
Pathogen	Cultivars									
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
F. solani	S	R	HS	R	HS	R	R	HS	S	R
M. phaseolina	HS	MS	HS	S	HS	R	R	R	MS	R
R. solani	MS	R	HS	MS	HS	R	R	R	MS	R

R: resistant MS: moderate susceptible S: susceptible HS: highly susceptible

a- Cultivar identification based on RAPD-PCR

RAPD-PCR was used to evaluate the genetic diversity of the ten soybean cultivars using thirteen arbitrary primers. The resulted amplified fragments are shown in Fig.1 (a to f) and their densitometric analyses are illustrated in Table (6 a to d). Of the thirteen primers, three did not reveal any DNA amplification, whereas ten successfully amplified DNA fragments for all genotypes. Six primers generated polymorphic banding patterns, while the remaining four primers generated monomorphic ones and were not scored. A total of 71 fragments were visualized across the ten investigated cultivars. Number of bands ranged from five (primer OP-B03) to 19 (primer OP-L20) across cultivars

Level of polymorphism varied from one primer to another. Thus, primer OP-L20 showed the highest level of polymorphism (84.21%), while primer OP- B03 and OP-D16 showed the lowest level (60.00 %).



(a) primer OP-A11 (b) primer OP-B03 (c) primer OP-D16

(d) primer OP-L12 (e) primer OP-L20 (f) primer OP-019

Figure 1. DNA fragments generated by different Primers

Table 6. Densitometric analysis for RAPD fragments generated from testing the ten soybean cultivars against different primers

(a) Primer OP-A11											
Band no.	W (bp)	V1	V2	V 3	V 4	V 5	V 6	V 7	V 8	V 9	V 10
1	1410	+	+	+	+	+	+	+	+	+	+
2	1030	+	+	+	+	+	+	+	+	+	+
3	700	-	+	-	-	-	-	-	-	-	-
4	510	-	-	+	-	-	-	-	-	-	+
5	400	-	-	+	-	+	-	-	-	-	-
6	270	+	-	+	+	+	+	+	+	+	+

(b) Primer OP-B03											
Band no.	MW (bp)	V1	V2	V 3	V 4	V 5	V 6	V 7	V 8	V 9	V 10
1	1340	+	+	-	-	-	+	-	+	-	+
2	890	+	+	+	+	+	+	+	+	+	+
3	770	+	+	+	+	+	+	+	+	+	+
4	340	-	-	-	-	-	+	+	-	-	+
5	230	+	+	+	-	-	+	-	-	+	+

(c) Primer OP-D16											
Band no.	MW (bp)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
1	2119	+	+	+	+	+	+	-	+	+	+
2	1690	+	+	+	+	+	+	+	+	+	+
3	1280	+	+	-	+	-	+	-	-	-	+
4	1210	+	+	+	+	+	+	+	+	+	+
5	1140	-	-	-	-	-	+	+	-	-	-
6	980	+	+	+	+	+	+	+	+	+	+
7	920	-	-	-	+	-	-	-	+	+	+
8	720	+	+	+	+	+	+	+	+	+	+
9	660	-	-	+	-	+	-	-	-	-	-
10	600	+	+	+	+	+	+	+	+	+	+
11	500	+	+	+	+	+	-	-	+	+	+
12	460	+	+	+	+	+	-	+	+	-	-
13	410	-	-	-	-	-	-	-	+	+	-
14	360	-	-	-	-	-	+	+	-	-	+
15	300	+	+	+	+	+	+	+	+	+	+

(d) Primer OP-L12											
Band no.	MW (bp)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
1	1160	-	-	-	-	-	-	-	-	-	+
2	980	-	-	-	-	-	-	-	-	-	+
3	840	-	-	+	+	-	-	-	-	-	-
4	760	+	+	+	+	+	+	+	+	+	+
5	630	+	+	-	+	-	+	+	-	-	+
6	600	-	-	-	-	-	+	+	-	-	+
7	510	+	+	+	-	-	+	-	-	+	-
8	440	+	+	+	+	+	+	+	+	+	+
9	390	+	+	-	-	-	-	-	-	-	+
10	340	+	+	+	+	+	+	+	+	+	+
11	200	+	+	+	+	+	+	+	+	+	+

(e) Primer OP-L20											
Band no.	MW (bp)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
1	2400	+	+	-	-	-	+	+	-	-	+
2	2210	-	-	-	-	-	-	-	-	+	-
3	1860	+	+	+	+	+	-	+	+	+	-
4	1780	+	+	+	+	+	-	-	+	+	-
5	1400	+	+	+	+	+	-	+	-	-	-
6	1260	+	+	+	+	+	+	+	+	+	+
7	1190	-	-	-	-	-	-	-	+	+	-
8	1090	+	+	+	+	-	-	+	-	+	+
9	830	+	+	+	+	+	+	+	+	+	+
10	780	-	-	+	-	+	+	+	+	+	-
11	680	+	+	+	+	+	+	+	-	+	+
12	610	-	-	-	+	-	+	+	-	+	+
13	580	-	-	-	-	-	+	-	-	-	+
14	510	+	+	+	+	+	-	+	-	+	+
15	460	+	-	+	+	+	+	+	+	+	-
16	380	-	-	+	+	+	+	+	+	+	+
17	300	+	+	-	-	-	-	-	-	+	-
18	260	+	+	+	+	+	+	+	+	+	+
19	200	-	-	-	-	-	+	+	-	-	+

(f) Primer OP-O19											
Band no.	MW (bp)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
1	1900	+	+	+	+	+	+	+	+	+	+
2	1790	-	-	-	-	-	-	-	+	-	-
3	1510	+	+	+	-	-	-	-	+	+	+
4	1330	+	+	-	+	+	+	+	-	+	+
5	1150	+	+	-	-	-	-	-	+	-	-
6	1090	+	-	-	-	-	-	-	-	-	-
7	1030	+	+	+	+	+	+	+	-	-	+
8	920	+	+	+	+	+	+	+	+	+	+
9	770	-	-	-	-	-	-	+	-	+	+
10	650	-	-	+	+	+	+	+	+	-	-
11	510	+	-	+	+	+	+	+	-	+	+
12	440	+	+	+	+	+	+	+	+	+	+
13	340	-	-	-	+	-	-	-	-	-	-
14	220	+	+	+	+	+	+	+	+	+	+
15	160	-	-	-	+	-	-	-	+	-	-

b- DNA molecular markers related to root rot resistance/susceptibility

The presence/absence of a certain DNA fragment in the electrophoregram of a resistant/susceptible cultivar may be considered as a molecular marker that indicates the resistant/susceptible cultivar. In this work several molecular markers related to root rot resistance/susceptibility in soybean were obtained (Table 7).

A fragment of 400 bp was present only in the two susceptible cultivars Giza-35 (V3) and Giza-83 (V5) while, it was absent from the DNA fragments amplified from all other cultivars using primer OP-A11. Primer OP-B03 revealed a fragment of 340 bp which was present only in the DNA fragments of the three resistant cultivars Giza-111 (V6), Crowford (V7) and Forrest (V10). A fragment of 360 bp was generated from the DNA extracted from the three resistant cultivars Giza-111 (V6), Crowford (V7) and Forrest (V10) when tested by primer OP-D16 that was not recorded for any other cultivars. On the other hand, a fragment of 660 bp was present only on the electrophoregram of the two susceptible cultivars Giza-35 (V3) and Giza-83 (V5) and was not observed for other cultivars, this fragment could be considered as a marker for susceptibility. Similarly a band of 600 bp was generated for the three resistant cultivars when tested against primer OP-L12 and one (200 bp) was generated for the same cultivars using primer OP-L20 in addition to a negative one (1780 pb) which was present on the electrophoregram of all cultivars except the three resistant cultivars, this fragment could be considered a negative marker for root rot resistance in soybean. Although a high degree of polymorphism was revealed by using primer OP-O19, it failed in generating molecular markers related to either resistance or susceptibility of root rot disease in soybean.

Table 7. Molecular genetic markers generated from different primers and their relations to root rot resistance/susceptibility in soybean

Primer	Marker/pb	Reaction*
OP-A11	(+) 400	Susceptibility
OP-B03	(+) 340	Resistance
OP-D16	(+) 360	Resistance
	(+) 660	Susceptibility
OP-L12	(+) 600	Resistance
OP-L20	(+) 200	Resistance
	(-) 1780	Resistance

(+) positive marker (-) negative marker * resistance or susceptibility to the three pathogens

DISCUSSION

Root rot is the major soil-born disease of soybean. The causal organisms of root rot disease (*R. solani, F. solani and M. phasiolina*) frequently occur together in the same field during the growth stages of soybean. The obtained results showed that cultivars Giza-111, Crowford, and Forrest were resistant to the three tested fungi in seedling stage. On the other hand; cultivars Giza-35 and Giza-83, were found to be the most susceptible to the three pathogens.

The wide range of resistance and/or susceptibility to root rot fungi suggested the presence of polygenic (multiple gene action) in soybean plants responsible for such degrees of resistance and/or susceptibility. Omar 1984 in faba bean, Omar *et. al* 1988 in lentil, Salem *et. al* 1991 in chickpea reported the presence of variable resistance/susceptibility levels in different legume crops. In soybean Gowily *et al* (1994), Gupal and Jagadeashuer (1997), Cho and Cho (1999) and Chakraborty *et al* (2003) found variations in susceptibility of cultivars against soil born fungi causing root rot disease and that cultivars exhibited varying degrees of susceptibility.

Molecular genetic markers can help in selecting the tolerant/ susceptible genotypes, for this purpose RAPD-PCR was performed to identify the studied genotypes and to generate molecular genetic markers for root rot resistance/susceptibility in soybean. The technique was successful in fingerprinting different soybean cultivars and revealed high degrees of polymorphism. Several factors may affect the estimates of genetic relationships i.e., number of markers used, distribution of markers in the genome (genome coverage) and the nature of evolutionary mechanisms underlying the variation measured (Powell *et al* 1996). Our results revealed that the chosen RAPD markers are fully distributed in the soybean genome and could be useful to investigate the genetic diversity among the studied soybean cultivars. Link *et al* (1995) reported that RAPD data are useful for classification of germplasm and identification of divergent heterotic groups in faba bean. Bagheri *et al.* (1995) and Hoey *et al.* (1996) found that RAPDs could be useful in cultivar identification in *Pisum sativum* (L). El-Adawy *et al.*, (2002) revealed that RAPD was more useful than SSR in classifying maize inbred lines and generating a dendrogram more fitted to their pedigree, Omar (2004) successfully used RAPD-PCR to identify ten Egyptian faba bean cultivars differing in resistance to chocolate spot disease. El-Demerdash *et al* (2006) reported that RAPD successfully identified two mutant lines in soybean. Our results revealed that several

molecular genetic markers that may be related to root rot resistance/susceptibility in soybean cultivars were generated. Some primers revealed more than one marker for the trait; the increase in number of markers generated might be due to the great coverage of RAPD markers to the soybean genome. This needs to be confirmed through more accurate molecular markers such as converting theses markers into SCAR or using the bulk of segregated F_2 individuals of a cross between contrasting resistant and susceptible parents test the validity of these markers according to the analysis proposed Michelmore *et al* (1991). In all cases our results represent a preliminary step towards the use of molecular markers in assisting breeding programs directed for root rot resistance in soybean. Previous investigators used the same technique to generate molecular markers related to salt tolerance in maize (Abdel-Tawab *et al* 1997) and in flax (Ashry 1998). Filho *et al* 1999 generated RAPD and SCAR makers related to frog eye leaf spot disease in soybean, RAPD-PCR markers were also useful in priming F_2 segregated population conferring differences in resistance to powdery mildew in flax (Ashry *et al* 2002). Omar (2004) deduced RAPD markers for chocolate spot resistance in faba bean.

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استعمال اختبار RAPD للكشف عن معلمات جزيئية للمقاومة أو القابلية للإصابة بمرض عفن الجذور في فول الصويا

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أجريت هذه الدراسة في كل من صوب ومعامل قسمي بحوث أمراض المحاصيل البقولية بمعهد بحوث أمراض النباتات وقسم بحوث الخلية بمعهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية خلال الموسم الصيفي 2005 بغرض تقييم مجموعة من أصناف فول الصويا المنزرعة في مصر من حيث مقاومتها أو قابليتها للإصابة بالفطريات المسببة لعفن الجذور. استخدمت في الدراسة عشرة أصناف من فول الصويا اختبرت للمقاومة لكل من فطريات Fusarium solani, Macrophomina phaseolina or Rhizoctonia solani كل علي حدة. قدرت النسبة المئوية للنباتات الحية بعد ٤٠ يوم من الزراعة وبناء علي ذلك تم حساب درجة المقاومة أو القابلية للإصابة بالمرض.

أظهرت الأصناف المختبرة فروقا معنوية وكان الصنفان جيزة ٣٥ و جيزة ٨٣ أشد الأصناف قابلية للإصابة بمرض عفن الجذور في حين كان كل من الأصناف جيزة -١١١ وكراوفورد و فورست هم الأكثر مقاومة للإصابة.

تم استخلاص الحمض النووي DNA من أوراق العشرة أصناف المختبرة والتي لم تتعرض للعدوي لإيجاد واسمات وراثية جزيئية علي مستوي الحمض النووي DNA لتمييز المقاومة أو القابلية للإصابة، أستخدمت طريقة التضاعف العشوائي للحمض النووي RAPD باستعمال ثلاثة عشر بادئا عشوائيا ، نجحت عشرة منها في إجراء تضاعف لقطع من الحمض النووي DNA، وأعطت أربعة منها شظاياأحادية المظهر بينما نجحت الستة بادئات الأخري في مضاعفة أجزاء متعددة المظهر. وقد أمكن إجراء تعريف وراثي للأصناف المختبرة عن طريق التباينات المظهرية الناتجة من التضاعف العشوائي لأجزاء من الحمض النووي DNA لكل صنف تحت الاختبار كما أظهرت بعض البادئات (OP-A11, OP-B03, OP-D16, OP-L12& OP-L20) وجود بعض الشظايا من الحمض النووي DNA والتي ارتبطت إما إيجابا أو سلبا مع صفة المقاومة أوالإصابة بالمرض.