Characterization of peanut mutants and molecular markers associated with resistance to pod rot diseases and aflatoxin contamination by RAPD and ISSR

(Received: 15. 06. 2007; Accepted: 25. 06. 2007)

Clara R. Azzam*, S. A. Azer, M. M. A. Khaleifa*** and M. F. Abol-Ela******
*Cell Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.
**National Center for Radiation Research and Technology, Cairo, Egypt.
***Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.
****Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt.

ABSTRACT

Ten peanut mutants: RT-6, RT-7, RT-8, RT-9, RT-10, RT-11, RT-12, RT-13, RT-14 and RT-15 and their parent variety (Giza 5) were evaluated for their reaction against pod rot pathogens, invasion by aflatoxigenic fungi and aflatoxin contamination under greenhouse and field conditions during 2004 and 2005 summer seasons. All peanut mutants exhibited significant decrease in percentages of pod rot diseases, occurrence of aflatoxigenic fungi and aflatoxin contamination compared to Giza 5. Mutants RT-10, RT-12 and RT-7 were highly resistant against all categories of pod rot diseases and had the lowest levels of aflatoxin B_1 and/or B_2 under soil infestation with aflatoxigenic fungi: Aspergillus flavus and A. parasiticus, separately or in mixture in greenhouse and came free from any contamination with aflatoxin under field conditions compared to Giza 5 and the other mutants. The mean values of pod yield m^{-2} in the entire mutants were significantly higher compared to parent variety, Giza 5, except the mutant RT-15 which did not differ significantly from the parent variety in pod yield. RT-9 increased significantly but the mutants RT-14 and RT-15 had significant decrease in 100-pod weight compared to Giza 5. The fancy pods percentage (FP %) increased significantly in the two mutants RT-8 and RT-11, while, the total sound mature kernels percentage (TSMK %) in all mutants did not differ significantly from the original variety (Giza 5). The oil content of all mutants decreased significantly than Giza 5. Several molecular markers associated with pod rot resistance/susceptibility in mutants and Giza 5 were obtained by the RAPD primers. The ISSRs did not reveal any marker (positive or negative) associated with pod rot resistance/susceptibility in the mutants and their parent variety. RAPD and ISSR combined data indicated that the three most closely related mutants were RT-7, RT-10 and RT-12.

Key Words: *Peanut, Arachis hypogaea, mutants, pod rot diseases, Aflatoxin, molecular markers, RAPD, ISSR.*

INTRODUCTION

Peanut, (*Arachis hypogaea* L.) is a main world source of edible oil and protein. In Egypt, peanut is one of the export and locally consumed crops. Soil borne fungi can attack peanut pods, when environmental conditions are favorable during their development in soil, after harvesting and during storage (Satour *et al.*, 1978). They

cause serious losses in peanut yield in Egypt; therefore growing peanuts in these soils becomes unprofitable (Hassan and Frederick, 1995).

Aflatoxigenic fungi (*Aspergillus flavus* Link and *A. parasiticus* Spear) are commonly associated with peanut pods during their development in the soil. Preharvest aflatoxin contamination is one of the most challenges facing the peanut production in the world and has a marked consideration in Egypt (Mahmoud, 2004).

Pod rot diseases are widely spread and peanut cultivars differ greatly in their reaction to these diseases. No cultivars were found to be completely resistant to aflatoxin contamination following seed invasion, while there were significant differences in their ability to allow invasion and aflatoxin production (Mahmoud *et al.,* 2006).

Recently, there was a considerable research on possible genetic resistance in groundnuts for seed infection and aflatoxin contamination in the field. Mutation breeding was used for improving plant characters and increasing genetic variability in a variety of crop species including peanuts (Azer *et al.,* 2002; Azzam and El- Sawy, 2005 and Khaleifa *et al.,* 2006).

DNA molecular markers have been integrated in the breeding programs of different field crops and are expected to play a very important role in 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) became one of the widespread DNA techniques, a quick method for developing genetic maps (Van de Ven *et al.,* 1993) and to determine DNA fragments to discriminate peanut cultivars (Guohao *et al.,* 2003; He *et al.,* 2003 and Guo *et al.,* 2005).

Inter simple sequence repeats (ISSR) is an alternative technique to study polymorphism based on the presence of microsatellites through-out genomes (Raina *et al.,* 2001; Wang, 2002 and Pharmawati *et al.,* 2005)

Comparisons of PCR-based markers have shown their efficiency in plant breeding (Mogg and Bond, 2003). As a result of these advantages and their universality, ISSR markers are more and more requested. Gostimskii *et al.* (2005) demonstrated that they are promising for identifying cultivars and determining the genetic purity of strains. Wolfe (2005) reported that ISSR markers were originally devised for differentiating among closely related plant cultivars, but became useful for studies of natural populations of plants, fungi, insects, and vertebrates.

The present study aimed to identify some peanut mutants resistant to pod rot diseases and aflatoxin contamination under artificial and natural infection and to find molecular genetic markers associated with resistance and susceptibility to pod rot diseases and aflatoxin contamination using RAPD and ISSR techniques.

MATERIALS AND METHODS

Greenhouse evaluation

Ten peanut mutants; RT-6, RT-7, RT-8, RT-9, RT-10, RT-11, RT-12, RT-13, RT-14 and RT-15, which were previously selected by Azer *et al.* (2002) and their parent variety; Giza 5 (G5) were evaluated for their reaction against pod rot pathogens and invasion by aflatoxigenic fungi and aflatoxin contamination.

Reaction of peanut genotypes against pod rot pathogens

Fungal inocula of the main pod rot causal pathogens; *Rhizoctonia solani* the causal of dry brown lesion, *Fusarium moniliforme* the

causal of pink discoloration as well as *Macrophomina phaseolina* and *Sclerotium rolfsii* the main causal pathogens of general breakdown pod rot (previously isolated from diseased peanut pods and confirmed their pathogenic capabilities by the authors) were prepared to soil infestation under pot experiment using sorghum- coarse sand- water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for two hr at 1.5 air pressure. The medium in glass bottles inoculated separately using agar discs were used for soil infestation.

Surface sterilized seeds of the ten peanut mutants and their parent (G5) were sown 5 seeds per pot (50 cm diam.) containing soil infested with inocula (2% w/w) of a mixture of causal pathogens; *R. solani, F. moniliforme, M. phaseolina* and *S. rolfsii*. Pots containing sterile non infested soil were used as a control. Four replicates were used for each peanut genotype and the pots were arranged in a randomized complete block design.

At harvest, percentages of diseased pods were determined according to Satour *et al.* (1978). They were 1- *Rhizoctonia* pod rot (pods with dry brown lesion), 2- *Fusarium* pod rot (pods with pink discoloration) and 3- complex pod rot (pods with general breakdown resulting from many fungi *i.e. Puthim* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii*).

Susceptibility of peanut genotypes against aflatoxigenic fungi invasion and aflatoxin contamination

Fungal inocula of the aflatoxigenic fungi strains; *Aspergillus flavus* (FIs2) and *A. parasiticus* (PIs2) isolated from peanut kernels by Mahmoud (2004) were prepared for soil infestation under pots experiment and added at the rate of 2% (w/w) separately and/or in mixed to pots containing sterilized soil. None infested potted soil, served as control. Five surface sterilized seeds of each peanut mutant and the parent variety were sown per pot. At harvest, plants were air dried for 7 days and threshed. Resulted pods were used for isolation and determination of the frequency of *A. flavus* and *A. parasiticus* and detection of aflatoxin contamination.

Aflatoxigenic fungi, associated with the pod samples of peanut genotypes, were isolated after harvest and identified according to Maren and Johan (1988). The frequency of invasion by aflatoxigenic fungi was recorded and calculated based on the percentage of infected samples. The extraction of aflatoxins was conducted according to A.O.A.C. (1998) and determined according to Singh *et al.*, (1991).

Field evaluation

Field experiments were conducted in a randomized complete block design with four replicates during summer seasons of 2004 and 2005 at Nubaria district. Each replicate was 21 m^2. The field soil was sandy loam, heavily infested with fungi causal pathogens. Well sized seeds of the mutants and their parent variety were sown at the first week of May of each season. The kernels were sown in rows spaced 40 cm, with 20 cm between hills. Overhead sprinkler system was used for irrigation and the recommended fertilizer levels and agronomic practices were used as usual in the reclaimed sandy soils.

Pod yield, pod grade and kernel quality

The pod yield of each plot was recorded, and then pod yield m^{-2} was estimated. The pod grade and kernel quality were recorded according to the Cooperative Grading Service Criteria (USDA, 1998). One kilogram of pods from each plot was used to determine fancy pods percentage, FP% [pods riding a 14 mm (36/64 inch) slotted screen]. The fancy pods were used to determine 100 pod weight (g). Five hundred grams randomly selected fancy

pods from each plot were shelled to determine shelling percentage and total sound mature kernels percentage (TSMK %). All kernels were weighed in determining shelling percentage, whereas, for TSMK%, only full mature kernels irrespective of their size (excluding the aborted and shrank ones) were considered. The total sound mature kernels were taken to record 100 kernel weight (g) and large kernels percentage, LK% [kernels riding a 8 mm 20/64 inch) slotted screen]. The oil percentages were determined according to Southcombe (1962).

Pod rots incidence and other criteria were determined as previously mentioned in the greenhouse experiment.

Statistical analysis

The data were statistically analyzed by ANOVA and means were separated by Fisher's protected least significant difference (LSD) at $P \leq 0.05$ level according to Gomez and Gomez (1984).

Molecular genetic markers

For DNA extraction, leaf tissues of each peanut mutant and G5 variety (un-infected) were collected from 7 days old germinated seedlings. DNA was extracted from 100 mg of young leaves according to Junghans and Metzlatt (1990) and the concentration and purity were determined by spectrophotometer.

The extracted DNA was used to perform polymerase chain reaction (PCR) using RAPD and ISSR primers according to Williams *et al* (1990). Thirteen arbitrary 10-mer primers (Operon Technologies, Inc) and 12 ISSR primers were used. The codes and sequences of the used primers are shown in Table (1).

RAPD and ISSR-PCR reactions were optimized and mixtures (25 µl total volume) were composed of dNTPs (200 µ M), Mg Cl_2 (1.5 mM), 1 buffer, primer (0.2 µM), DNA (50 ng), Taq DNA polymerase (2 units). Amplification was carried out in a Thermo Cycler (Perkin Elmer) programmed for 94^{o}C for 3 min (one cycle), followed by 94^{o}C for 30 sec, 36^{o}C for 1 min and 72^{o}C for 2 min (36 cycle), 72^{o}C for 10 min (one cycle), then 4^{o}C (infinitive) with RAPD primers, while with ISSR primers amplification was programmed for 94^{o}C for 3 min (one cycle), followed by 94^{o}C for 30 sec, 40^{o}C for 45 sec and 72^{o}C for 1 min (35 cycle), 72^{o}C for 10 min (one cycle), then 4^{o}C (infinitive). Amplification products (15 µl) were mixed with 3 µl loading buffer and separated on 1.2% agarose gel and stained with 0.5 µg/ ml ethidium bromide with a constant electric current (100 volts) for 25 minutes at room temperature. Bands were visualized, photographed and scored using gel documentation system (UV transilluminator) manufactured by Alpha Ease FC (Alphimager 2200), U.S.A., DNA fragment sizes were determined using the 100 bp DNA Ladder marker.

RAPD and ISSR data analysis

The data of RAPD and ISSR analyses were entered in a computer file as binary matrices. Similarity coefficients were calculated according to Dice matrix (Nei and Li, 1979). Construction of the dendrogram tree was performed using the unweighted pair group method based on arithmetic mean (UPGMA) as implemented in the SPSS Program Version 10.

Table (1): List of the RAPD and ISSR primers and their sequences.

RAPD		ISSR	
Codes	**Sequence `5→`3**	**Codes**	**Sequence `5→`3**
**OP-A1	CAGGCCCTTC	**I-18	$GTGC(TC)_7$
**OP-A9	GGGTAACGCC	*I-28	$(GT)_6CG$
**OP-B10	CTGCTGGGAC	[x]HB8	$(GA)_6GG$
**OP-B12	CCTTGACGCA	[x]HB9	$(GT)_6GG$
**OP-B13	TTCCCCCGCT	*HB10	$(GA)_6CC$
*OP-B19	ACCCCCGAAG	*HB12	$(CAG)_3GC$
*OP-C6[a]	GAACGGACTC	*HB15	$(GTG)_3GC$
**OP-C7[a]	GTCCCGACGA	**814.1	$(CT)_8TG$
[x]OP-Q5	CCGCGTCTTG	**844B	$(CT)_8GC$
[x]OP-Q11	TCTCCGCAAC	[x]17898[a]	$(CA)_6AC$
[x]OP-Q13	GGAGTGGACA	[x]17898[b]	$(CA)_6GT$
**OP-V2	AGTCACTCCC	**UBC830	$(GA)_8C$
**OP-V6	ACGCCCAGGT		

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

RESULTS AND DISCUSSION

Greenhouse experiments

Reactions to pod rot pathogens in artificially infested pots

Data presented in Table (2) indicated that, all peanut mutants exhibited significant decrease in percentages of the three categories of pod rots diseases and significant increase in the apparently healthy pods compared to the parent variety.

In general, pods with breakdown rot had the highest disease incidence, followed by dry brown lesion, whereas pink discoloration was the least one in all evaluated genotypes.

Mutant RT-10 was the highest resistant one against all categories of pod rots diseases and gave the highest percentage of apparently healthy pods, followed by mutants RT-12 and RT-7. On the other hand, the parent variety (G5) was the highest susceptible one for all categories of pod rots and gave the lowest percentage of apparently healthy pods, followed by mutants RT-15 and RT-9. However, the other mutants were intermediate in this respect. These results are similar to those reported by Khaleifa *et al*. (2006) who reported that, mutants RT-10, RT-12 and RT-7 were the most resistance ones for damping-off and root rot diseases of peanut while Giza 5 was the highest susceptible one in this regard.

Reaction to aflatoxigenic fungi and aflatoxin contamination

Data presented in Table (3) illustrate that occurrence of aflatoxigenic fungi was higher in seeds than in shells for all genotypes. *A. flavus* recorded the highest frequency compared to *A. parasiticus* in all genotypes whether infestation was separately or in a mixture with *A. parasiticus*.

Data also showed that mutant RT-10 recorded the least percentage of aflatoxigenic fungi on both seed and shell under infested soil with *A. flavus* and/or *A. parasiticus*, separately or in a mixture, followed by mutants RT-12 and RT-7, while G5, RT-15, RT-9 and RT-14, showed the highest percentage of aflatoxigenic fungi on both seed and shell. These results are in harmony with Mahmoud *et al.* (2006).

Table (2): Evaluation of some peanut genotypes against pod rots disease complex under greenhouse conditions.

Genotypes	% Dry brown lesion	% Pink discoloration	% General breakdown	%Apparently healthy pods
RT-6	10.66	3.02	14.07	72.25
RT-7	8.31	2.27	8.89	80.53
RT-8	7.61	2.82	12.99	76.58
RT-9	12.32	3.22	16.54	67.92
RT-10	4.72	0.50	8.88	85.90
RT-11	9.90	1.31	9.82	78.97
RT-12	8.37	1.75	7.82	82.06
RT-13	7.92	1.59	13.13	77.37
RT-14	14.42	2.43	13.08	70.07
RT-15	14.36	2.54	20.60	62.50
Giza 5	16.18	5.69	23.75	54.39
L.S.D at 5%	2.39	2.37	2.41	2.31

None of the studied mutants and their parent variety showed complete resistance to aflatoxin contamination under soil infestation with *A. flavus* separately and/or in mixture with *A. parasiticus* (Table 3).

Aflatoxin B_1 was higher than B_2 in all genotypes; both were higher in the mixture of *A. flavus* and *A. parasiticus* than each alone. A fairly low or undetectable level of aflatoxin B_1 and B_2 was observed in the harvested kernels of some peanut mutants.

Under infested soil with *A. flavus*, there was no aflatoxin B_1 contamination in mutants RT-7 and RT-10, as well as aflatoxin B_2 in mutants RT-10 and RT-12. Whereas, only mutant RT-7 came free from any contamination with aflatoxin B_1 and RT-7, RT-10, RT-12 and RT-13 came free from any contamination with aflatoxin B_2 under infested soil with *A. parasiticus*. Under infested soil with *A. flavus* in a mixture with *A. parasiticus*, aflatoxin B_1 was not detected in mutants RT-7 and RT-10 as well as aflatoxin B_2 in mutant RT-12. On the other hand, Giza 5 recorded the highest content of aflatoxin B_1 and B_2 contaminations in all cases separately or in a mixture, followed by mutants RT-15, RT-9, RT-14 and RT-6. These results are in harmony with those reported by Hasan *et al.* (2002). The variable amount of aflatoxin in contaminated peanut mutants and their parent variety may be due to the environmental factors and/or nature of the fungal strains (Anderson *et al.,* 1995). Furthermore, the difference in concentration of aflatoxin extracted from seed of various cultivars might be due to genetic and/or biochemical composition of the seed (Holbrook *et al.,* 2000).

Table (3): Occurrence of A. flavus and A. parasiticus in shells and seeds and aflatoxin content in seeds of some peanut mutants and their parent variety (Giza 5) under artificial inoculation conditions.

Mutants	*A. flavus*				*A. parasiticus*				*A. flavus + A. parasiticus*					
	% OF		AC		% OP		AC		% OF		% OP		AC	
	Se	Sh	B_1	B_2	Se	Sh	B_1	B_2	Se	Sh	Se	Sh	B_1	B_2
RT-6	46.7	20.0	117	55	23.3	13.3	83	25	56.7	23.3	43.3	10.0	143	64
RT-7	20.0	13.3	ND	13	0.0	10.0	ND	ND	23.3	6.7	26.7	16.7	ND	9
RT-8	33.3	20.0	72	51	3.3	0.0	29	16	46.7	26.7	50.0	33.3	63	48
RT-9	53.3	30.0	158	109	43.3	20.0	73	46	50.0	30.0	56.7	40.0	166	113
RT-10	0.0	6.7	ND	ND	10.0	0.0	8	ND	20.0	0.0	6.7	0.0	ND	12
RT-11	30.0	20.0	19	11	13.3	0.0	13	6	30.0	23.3	40.0	20.0	36	22
RT-12	16.7	6.7	10	ND	6.7	10.0	11	ND	33.3	0.0	16.7	10.0	19	ND
RT-13	20.0	10.0	23	9	0.0	10.0	17	ND	70.0	46.7	36.7	30.0	38	14
RT-14	46.7	33.3	125	76	36.7	23.3	96	51	76.7	63.3	50.0	36.7	135	89
RT-15	63.3	50.0	191	123	40.0	33.3	117	86	80.0	40.0	43.3	50.0	205	137
Giza 5	70.0	46.7	213	167	56.7	26.7	145	95	80.0	66.7	73.3	60.0	237	180

%OF= % Occurrence of A. *flavus* % OP=% Occurrence of A. *Parasiticus* AC= Aflatoxin content (ppb)
(y)=Each value is the mean of three replicates (3 plates/replicate, five seeds or shell pieces per dish).
Se= Seed, Sh= Shell, ND= Not detected.

Field evaluation

Yield, pod grade and kernel quality

Data of field evaluation (combined overall the two seasons) are given in Table (4). The mean values of pod yield m^{-2} for all the mutants were significantly higher than G5, except the mutant RT-15 which showed pod yield almost equal to that of parent variety. Also, the results showed that the percentage increases in pod yield m^{-2} ranged between 16.50% (RT-14) to 90.0% (RT-6) compared to the pod yield of G5. Hussein *et al*. (1991) selected many mutant lines with higher yielding ability and were significantly superior to their corresponding parent (Giza 4).

Concerning 100 pod weight, the results in (Table 4) indicated that mutant RT-9 had significantly higher value than its parent, G5. The results of fancy pods (FP) showed that no significant differences were detected among the mutants and the control variety (G5), except the two mutants RT-8 and RT-11, which showed significant increases in FP%. Kale *et al.* (2000) selected seven new peanut mutant lines characterized with a large pod size. Also, Branch (2001) released Georgia Valencia peanut mutant line which has a significant larger pod size with 25% more fancy pods than the Georgia Red (the parent variety). The results in Table (4) showed that the shelling percentage and total sound mature kernels percentage (TSMK %) in all mutants did not differ significantly from the control.

The large kernels percentage (LK %) of the three mutants: RT-11, RT-13 and RT-14 were significantly higher than the parent

variety. Meanwhile, the three mutants: RT-6, RT-9 and RT-15 were significantly lower in LK% compared to G5, while the rest of the mutants were approximately equal to G5 variety in LK%. Pathirana (1991) selected mutants of peanut characterized with large kernel size. The mean value of 100-kernel weight (Table 4) was significantly greater in all peanut mutants than G5 variety. The increase of 100-kernel weight over G5 ranged from 2.59 g for the peanut mutant RT-12 to 8.00 g for the mutant RT-9.

The oil content of all mutants (Table 4) showed significant decrease than their parent. The decrease in oil percentage in all mutants relative to G5 ranged from 6.02% (RT-9) to 1.28% (RT-13 and RT-14).

Table (4): Pod yield m^{-2}, pod grade, kernels quality and oil content of ten mutants and the parent variety Giza 5.

Mutants	Pod yield m^{-2} (g)	FP %	100-Pod weight (g)	Shelling %	TSMK %	LK %	100-Kernel weight (g)	Oil %
RT-6	944	73.52	243	71.57	96.89	53.80	96.03	50.99
RT-7	894	72.94	253	71.65	98.56	61.31	98.00	53.75
RT-8	907	83.19	246	71.27	96.95	61.00	94.61	53.43
RT-9	765	72.19	261	72.00	98.32	53.98	100.00	49.49
RT-10	670	72.29	254	71.73	97.96	62.36	95.85	53.61
RT-11	712	78.17	252	71.67	96.89	74.06	95.22	52.85
RT-12	681	73.09	243	71.43	96.96	61.96	94.59	53.01
RT-13	695	72.95	237	71.33	96.85	72.48	94.67	54.23
RT-14	579	73.50	229	72.13	97.88	66.43	98.00	54.23
RT-15	472	72.98	226	71.45	96.88	58.00	95.47	53.59
Giza 5	497	72.58	238	72.07	98.73	62.35	92.00	55.51
LSD at 5%	48	1.31	17.50	NS	NS	1.92	1.57	1.10

FP%: Fancy pods % TSMK%: Total sound mature kernels % LK%: Large kernels %

Reaction against pod rot diseases under natural infestation conditions

Data presented in Table (5) showed the reaction of ten mutants and the parent variety G5 against pod rot diseases under naturally infested field conditions in two successive seasons (2004 and 2005). In general, incidence of pod rot diseases was higher in season 2004 than season 2005 with few exceptions. General breakdown percentage was the highest pod rot incidence, followed by dry brown lesion while, the pink discoloration was the least in all peanut genotypes with few exceptions.

Mutant RT-10 was the highest resistant genotype against all categories of peanut pod rot incidences and gave the highest percentage of apparently healthy pods followed by mutants RT-7, RT-12, RT-13, RT-11 and RT-6 in a descending order. However, G5 was the highest susceptible one, it showed the highest percentage of incidence of all pod rot incidences and gave the lowest percentage of apparently healthy pods followed by mutants RT-9, RT-15, RT-8 and RT-14. The present results demonstrated that all peanut mutants and their parent variety G5 varied in their susceptibility to infection with pod rot disease complex under greenhouse and field conditions. These results are in agreement with Hasan *et al*. (2002).

Table (5): Evaluation of peanut genotypes against pod rot diseases complex under field conditions.

Mutants	2004				2005			
	B	P	G	A	B	P	G	A
RT-6	7.17	2.87	10.14	79.82	4.72	2.36	7.08	85.84
RT-7	2.59	0.49	6.07	90.85	2.23	0.74	4.83	92.20
RT-8	5.73	2.21	17.45	74.61	6.27	2.35	15.30	76.08
RT-9	7.88	3.93	24.47	63.72	9.72	4.05	17.00	69.23
RT-10	2.10	1.48	4.55	91.87	1.70	1.70	2.98	93.62
RT-11	5.33	1.17	10.81	82.69	1.61	2.57	9.00	86.82
RT-12	3.88	0.35	6.49	89.28	5.53	0.00	4.53	90.12
RT-13	3.85	2.52	6.48	87.15	2.51	0.00	8.66	88.83
RT-14	9.20	1.12	15.13	74.45	8.45	0.65	11.69	78.21
RT-15	10.12	4.25	13.41	72.22	9.88	4.32	16.67	69.13
Giza 5	11.24	2.76	18.63	67.37	12.71	4.42	18.23	64.64
L.S.D at 5%	1.85	1.83	3.09	2.76	1.85	1.26	2.81	2.67

B= % Dry brown lesion, P= % Pink discoloration, G= %General breakdown, A= %Apparently healthy pods

Occurrence of A. flavus and A. parasiticus and seed aflatoxin content under natural soil infestation

Tables (6 and 7) present data of *A. flavus* and *A. parasiticus* occurrence in seed and shell and aflatoxin content in seed only of peanut genotypes under natural infestation of field conditions in the seasons 2004 and 2005. The aflatoxigenic fungus, *A. flavus* was more invasive to either pod shells or seeds than *A. parasiticus* and both fungi occurred at a high frequency in seeds compared to pod shells of most mutants and parent variety Giza 5. However, both aflatoxigenic fungi have occurred in a high frequency in pods in both seasons 2004 and 2005. The results also indicated that, mutant RT-10 recorded the least occurrence of aflatoxigenic fungi in both seed and shell, followed by mutants RT-13, RT-12, RT-7 and RT-11, while G5 followed by mutants RT-9 and RT-15 recorded the highest occurrence of aflatoxigenic fungi. Mutants RT-8, RT-6 and RT-14 were intermediate in this respect in season 2004. Th same trend of results was observed in season 2005 with a few exceptions. These results are in harmony with Azer *et al.*, 2002.

Regarding aflatoxin contamination, the obtained results (Tables 6 and 7) also indicated that, aflatoxin B_1 was higher than B_2 in all detected cases of peanut genotypes. In this respect in both seasons, mutants RT-7, RT-10 and RT-12 were free from any contamination while, aflatoxin contamination was the lowest level in mutants RT-8, RT-11 and RT-13 in both seasons. Giza 5 recorded the highest contamination with aflatoxin B_1 and B_2 followed by mutants RT-15, RT-9, RT-14 and RT-6 in both seasons. In season 2004, only mutant RT-13 came free from aflatoxin B_2, while, RT-11 and RT-13 came free from aflatoxin B_2, and B_1, respectively, in season 2005. The present results coincide with those of Mahmoud *et al.* (2006).

Table (6): Occurrence of A. flavus and A. parasiticus in seed and shell and aflatoxin content in seed of peanut mutants and Giza 5 variety under natural infection in field, season 2004.

Mutants	% Occurrence of aflatoxigenic fungi of pod rot categories (y)																AC	
	B				P				G				A					
	A. f		*A. p*		*A.*		*A. p*		*A. f*		*A. p*		*A.*		*A. p*			
	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	B1	B2
RT-6	26.7	13.3	13.3	0.0	30.0	16.7	10.0	0.0	30.0	20.0	20.0	3.3	20.0	10.0	20.0	13.3	89	65
RT-7	10.0	6.7	6.7	6.7	20.0	0.0	10.0	6.7	20.0	10.0	0.0	0.0	6.7	0.0	10.0	0.0	ND	ND
RT-8	26.7	10.0	26.7	13.3	6.7	0.0	10.0	6.7	30.0	30.0	30.0	10.0	10.0	10.0	0.0	3.3	28	17
RT-9	40.0	20.0	30.0	20.0	26.7	13.3	20.0	10.0	50.0	26.7	23.3	16.7	30.0	20.0	16.7	10.0	122	85
RT-10	13.3	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	10.0	3.3	10.0	0.0	0.0	0.0	ND	ND
RT-11	30.0	10.0	16.7	6.7	20.0	6.7	0.0	0.0	30.0	0.0	0.0	0.0	20.0	6.7	3.3	0.0	36	18
RT-12	13.3	0.0	6.7	0.0	16.7	13.3	10.0	0.0	20.0	0.0	20.0	0.0	6.7	0.0	0.0	0.0	ND	ND
RT-13	16.7	13.3	0.0	10.0	13.3	0.0	0.0	10.0	16.7	0.0	0.0	0.0	13.3	0.0	10.0	0.0	18	ND
RT-14	33.3	20.0	13.3	0.0	13.3	6.7	13.3	6.7	40.0	23.3	26.7	13.3	20.0	13.3	16.7	3.3	94	77
RT-15	46.7	23.3	23.3	10.0	26.7	20.0	20.0	6.7	50.0	20.0	33.3	16.7	30.0	16.7	20.0	6.7	136	95
Giza 5	53.3	26.7	30.0	13.3	40.0	26.7	20.0	13.3	50.0	30.0	30.0	20.0	36.7	20.0	30.0	20.0	157	130

B= Dry brown lesion, P=Pink discoloration, G=General breakdown, A= Apparently healthy pods
AC=Aflatoxin content (ppb), *A. f= A. flavus*, *A. p= A. parasiticus* Se= Seed, Sh= Shell
(y)= Each value is the mean of three replicates (3 plates / replicate, five seeds or shell pieces per dish), ND =Not detected.

Table (7): Occurrence of A. flavus and A. parasiticus in seed and shell and aflatoxin content in seed of peanut mutants and Giza 5 variety under natural infection in field, season 2005.

Mutants	% Occurrence of aflatoxigenic fungi of pod rot categories (y)																AC	
	B				P				G				A					
	A. f		*A. p*		*A. f*		*A. p*		*A. f*		*A. p*		*A. f*		*A. p*			
	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	Se	Sh	B1	B2
RT-6	20.0	13.3	16.7	10.0	20.0	10.0	0.0	6.7	20.0	30.0	10.0	10.0	20.0	10.0	6.7	6.7	53	32
RT-7	10.0	10.0	10.0	10.0	6.7	0.0	0.0	0.0	10.0	0.0	0.0	0.0	20.0	10.0	0.0	0.0	ND	ND
RT-8	26.7	20.0	16.7	6.7	16.7	10.0	20.0	6.7	30.0	20.0	16.7	6.7	0.0	10.0	6.7	0.0	16	5
RT-9	30.0	20.0	13.3	10.0	30.0	16.7	20.0	6.7	30.0	20.0	10.0	16.7	20.0	13.3	10.0	10.0	94	72
RT-10	0.0	10.0	10.0	6.7	0.0	0.0	6.7	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	ND	ND
RT-11	20.0	10.0	10.0	10.0	20.0	20.0	10.0	0.0	30.0	20.0	0.0	0.0	16.7	13.3	6.7	0.0	19	ND
RT-12	10.0	3.3	6.7	0.0	20.0	10.0	10.0	0.0	20.0	10.0	0.0	6.7	13.3	10.0	6.7	0.0	ND	ND
RT-13	10.0	10.0	0.0	6.7	10.0	6.7	0.0	0.0	0.0	10.0	6.7	10.0	10.0	6.7	0.0	0.0	ND	10
RT-14	26.7	20.0	16.7	16.7	26.7	13.3	20.0	10.0	40.0	20.0	16.7	10.0	20.0	10.0	13.3	10.0	82	65
RT-15	36.7	20.0	13.3	20.0	30.0	16.7	20.0	6.7	36.7	30.0	13.3	16.7	26.7	20.0	20.0	6.7	119	87
Giza 5	40.0	26.7	20.0	16.7	36.7	20.0	16.7	10.0	40.0	30.0	26.7	20.0	26.7	20.0	10.0	16.7	145	126

B= Dry brown lesion, P=Pink discoloration, G=General breakdown, A= Apparently healthy pods
AC=Aflatoxin content (ppb), *A. f= A. flavus*, *A. p= A. parasiticus* Se= Seed, Sh= Shell
(y)= Each value is the mean of three replicates (3 plates / replicate, five seeds or shell pieces per dish), ND= Not detected.

In this study, the extent of aflatoxigenic fungi and aflatoxin contamination varied in harvested pods of peanut genotypes under natural and artificial inoculation. Furthermore, aflatoxigenic fungi colonization and aflatoxin contamination were greater under artificial than natural inoculation. Data also showed that, under field conditions there was no clear

correlation between the occurrence of aflatoxigenic fungi in kernels of peanut genotypes and their aflatoxin contamination, with some exceptions. This may be due to that not all Egyptian isolates of *A. flavus* and *A. parasiticus* were able to produce aflatoxin in peanut pods (Mahmoud, 2004).

Fingerprinting of peanut mutants and the parent variety, Giza 5

RAPD-PCR was used to evaluate the genetic diversity of the ten peanut mutants from their parent variety (G5) using thirteen arbitrary primers. Of these primers, three did not reveal any DNA amplification, whereas ten successfully amplified DNA fragments for all genotypes. Eight primers generated polymorphic banding patterns, while the remaining two primers generated monomorphic ones and were not scored. A total of 71 fragments were visualized across the ten investigated mutants and their parent variety (G5). Number of bands ranged from 6 (primer OP-A9) to 14 (primer OP-C7) across all genotypes. Level of polymorphism varied from one primer to another. Primer OP-A9 showed the highest level of polymorphism (83.3%), while primer OP-V6 showed the lowest level (57.1 %). The resulted amplified fragments are shown in Fig.(1), only for four primers that presented the highest and the lowest degree of polymorphism. The similarity index and consensus trees of the ten peanut mutants and their parent variety under investigation were developed based on their banding patterns with the 8 RAPD primers, as shown in Table (8) and Fig. (2).

The lowest genetic similarity (0.58 and 0.62) were observed between the parent variety (G5) and each of RT-10 and RT-12, respectively, while the highest genetic similarity were scored between RT-11 and RT-9 (0.93), followed by G5 and RT-6 (0.91).

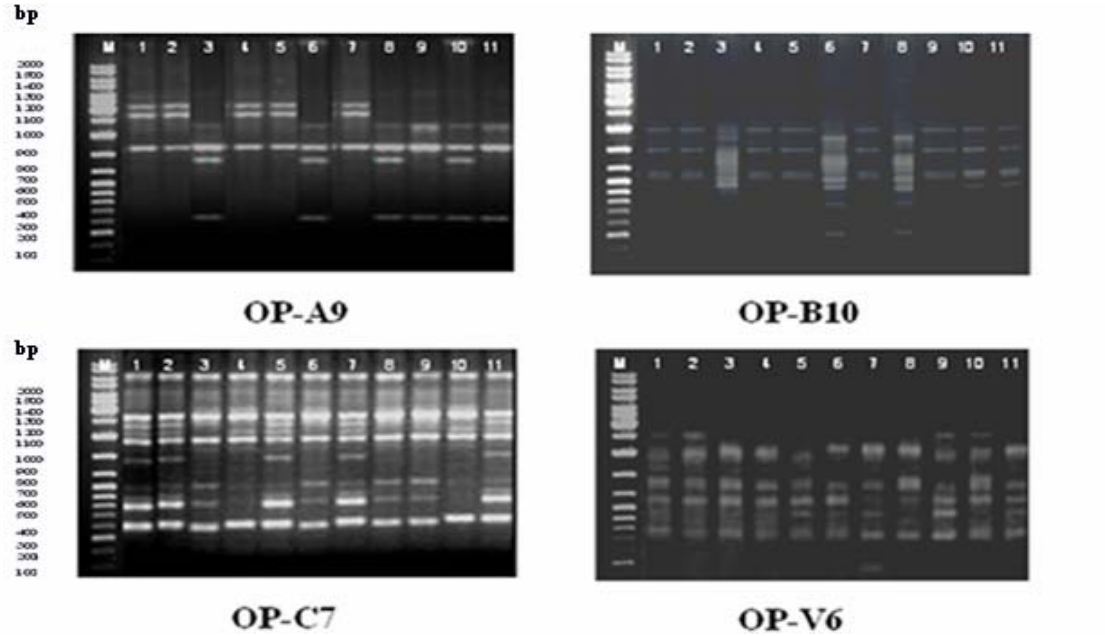


Fig. (1): RAPD-PCR polymorphism of DNA for ten peanut mutants and their parent variety using random primers.

Table (8): Similarity index for ten peanut mutants and their parent variety Giza 5 as calculated on the basis of their banding patterns with RAPD primers.

Genotypes	G5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14
RT6	0.91									
RT7	0.67	0.69								
RT8	0.71	0.78	0.68							
RT9	0.80	0.88	0.72	0.84						
RT10	0.58	0.68	0.82	0.71	0.76					
RT11	0.80	0.89	0.76	0.79	0.93	0.75				
RT12	0.62	0.65	0.88	0.63	0.67	0.90	0.70			
RT13	0.74	0.77	0.77	0.78	0.74	0.74	0.75	0.75		
RT14	0.71	0.73	0.69	0.79	0.72	0.70	0.67	0.67	0.81	
RT15	0.83	0.81	0.71	0.69	0.80	0.66	0.78	0.66	0.79	0.84

Cluster analysis classified the eleven genotypes into two main subclusters (Fig. 2). The first main subcluster consisted of three peanut mutants (RT-10, RT-7 and RT-12), while the second main subcluster is divided into two sub-clusters, the first consisted of three peanut mutants (RT-14, RT-15 and RT-13), whereas, the second is divided into two sub-sub- clusters, one of them consisted of mutant RT-8 and the other consisted of the parent variety, RT-6, RT-9 and RT-11.

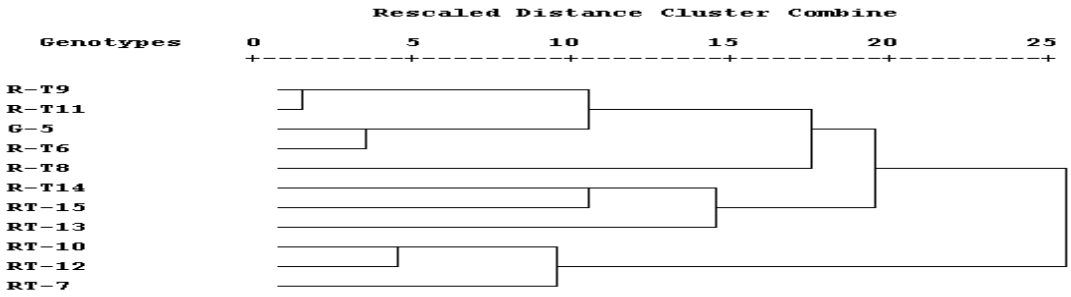
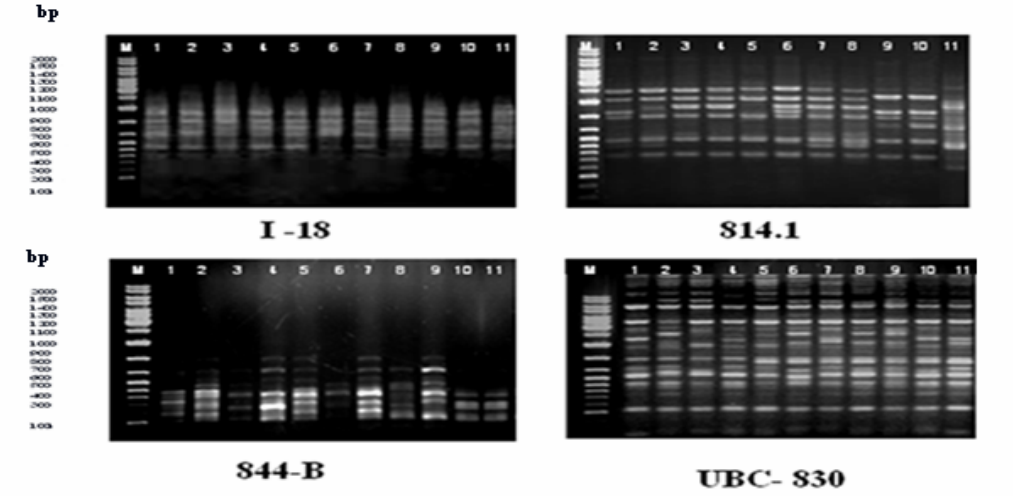


Fig. (2): Dendrogram of the genetic distances among the ten peanut mutants and their parent variety (Giza 5) based on RAPD analysis.

ISSR-PCR was used to evaluate the genetic diversity of the ten peanut mutants from their parent variety (G5) using twelve ISSR primers. The resulted amplified fragments are shown in Fig.(3). Of the twelve primers, four did not reveal any DNA amplification, whereas eight successfully amplified DNA fragments for all genotypes; four primers (Fig. 3) generated polymorphic banding patterns and the remaining four primers generated monomorphic ones and were not scored. A total of 77 fragments were visualized across the ten investigated mutants and their parent variety (G5). Number of bands ranged from 8 (primer I-18) to 23 (primer UBC-830) across all investigated mutants and the control.

Level of polymorphism varied from one primer to another. Primer 814.1 showed the highest level of polymorphism (92.3%), while primer UBC-830 showed the lowest level (56.5 %).

The similarity index and consensus tree of the ten peanut mutants and their parent variety under investigation were developed based on their banding patterns with the 4 ISSR primers, as shown in Table (9) and Fig. (4). The results of genetic similarity are shown in (Table 9). The lowest genetic similarity (0.67 and 0.68) were observed between the mutant RT-15 and each of RT-8 and RT-13, respectively, while the highest genetic similarity index was scored between RT-10 and RT-7 (0.93) followed by RT-9 and RT-6 (0.92). Cluster analysis classified the eleven genotypes into two main subclusters (Fig.4). RT-15 was located alone in the first main subcluster and the second is divided into two sub-clusters, the first consisted of G5 and RT-14, while the second is divided into two sub-sub- clusters, one of them consisted of mutants RT-7, RT-10 and RT-12 and the second consisted of the rest of peanut mutants and their parent variety.



M=100bp DNA Ladder 1= Giza 5 2= RT-6 3= RT-7 4= RT-8 5= RT-9
6= RT-10 7= RT-11 8= RT-12 9= RT-13 10= RT-14 11= RT-15

Fig. (3): ISSR-PCR polymorphism of DNA for ten peanut mutants and their parent variety (Giza 5) using random primers.

Table (9): Similarity index for ten peanut mutants and their parent variety Giza 5 as calculated on the basis of their banding patterns with ISSR primers.

Mutants	G5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14
RT6	0.81									
RT7	0.76	0.82								
RT8	0.73	0.88	0.80							
RT9	0.84	0.92	0.85	0.85						
RT10	0.78	0.78	0.93	0.76	0.84					
RT11	0.81	0.90	0.88	0.82	0.87	0.81				
RT12	0.77	0.83	0.84	0.81	0.81	0.83	0.86			
RT13	0.77	0.85	0.83	0.83	0.88	0.80	0.88	0.76		
RT14	0.83	0.75	0.72	0.69	0.78	0.71	0.75	0.71	0.79	
RT15	0.77	0.72	0.73	0.67	0.72	0.71	0.81	0.77	0.68	0.73

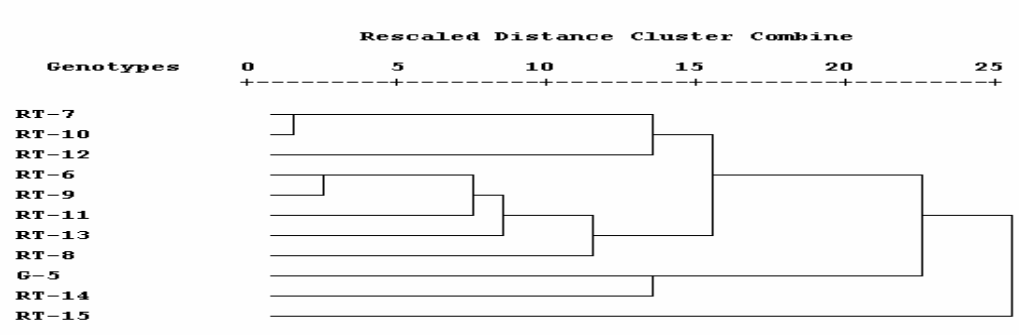


Fig. (4): Dendrogram of the genetic distances among the ten peanut mutants and the parent variety (Giza 5) based on ISSR analysis.

Genotypes distribution on the consensus tree according to the banding patterns of ISSR shuffled from that based on RAPD (banding patterns) consensus tree due to the coverage of each technique to different parts of the genome; so that it is better to use a combination of the two techniques of the banding patterns to cover as much as possible of the genome, that will increase the validity of the consensus tree. The results of the similarity index and consensus tree of the combined data for the ten peanut mutants and their parent variety (G5) (Table 10) and Fig. (5) showed that the lowest genetic similarity (0.63 and 0.67) was observed between the parent variety and each of RT-10 and RT-12, respectively, while the highest genetic similarity (0.91 and 0.90) was scored between RT-9 and each of RT-11 and RT-6, respectively.

Table (10): Similarity index for ten peanut mutants and their parent variety Giza 5 as calculated on the basis of their banding patterns with RAPD and ISSR primers.

Mutants	G5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14
RT6	0.88									
RT7	0.70	0.73								
RT8	0.71	0.82	0.72							
RT9	0.81	0.90	0.76	0.84						
RT10	0.63	0.71	0.85	0.73	0.79					
RT11	0.81	0.89	0.80	0.80	0.91	0.77				
RT12	0.67	0.71	0.87	0.69	0.71	0.88	0.75			
RT13	0.75	0.80	0.79	0.80	0.79	0.76	0.80	0.76		
RT14	0.75	0.74	0.70	0.76	0.74	0.70	0.70	0.68	0.80	
RT15	0.81	0.78	0.72	0.68	0.77	0.68	0.79	0.69	0.75	0.80

Based on the combined data of RAPD and ISSR, the consensus tree divided genotypes into two main clusters; the first included mutants RT-7, RT-10 and RT-12 in one of the two main clusters in the phenogram. The other cluster was divided into two main sub-clusters; the first one included RT-14 and RT-15, while the second was divided into two main sub-sub-clusters, which consisted of RT-8 and RT-13 in the first one and the other was divided again and consisted of Giza 5 alone and mutants RT-9, RT-11 and RT-6 are located in the second one. The results indicated that the three most distantly related

mutants are RT-7, RT-10 and RT-12 compared to the parent variety, with a similarity index of 0.70, 0.63 and 0.67, respectively. According to RAPD, ISSR and RAPD+ISSR analysis, these three mutants are resistant to pod rot diseases and are located always in the other main cluster or subcluster, in contrast with the susceptible ones, (Figs.2, 4 and 5). Although the genetic distances between the resistant mutants are very close.

Raina *et al.* (2001) reported that it was possible to identify accessions, particularly those of divergent origins, by RAPD and/or ISSR fingerprints and marker-based genetic improvement in *A. hypogaea*. None of the 486 RAPD and 330 ISSR amplification products were found to be commonly shared among 13 species of section *Arachis* and one species each of sections *Heteranthae*, *Rhizomatosae*, and *Procumbentes*. Dendrograms constructed from RAPD, ISSR, and RAPD + ISSR data showed overall similar topologies.

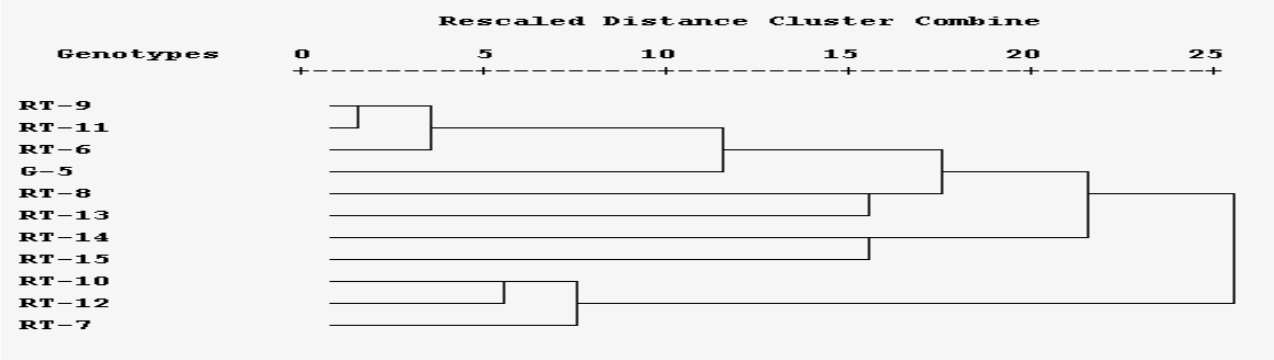


Fig. (5): Dendrogram of the genetic distances among the ten peanut mutants and their parent variety (Giza 5) based on RAPD and ISSR analysis.

RAPD and ISSR markers related to pod rot resistance/susceptibility

The data under greenhouse and field conditions, indicated that mutants RT-10, RT-12 and RT-7 were the highly resistant mutants against all categories of pod rots diseases and had the lowest or undetectable levels of aflatoxin B_1 and/or B_2 under soil infestation with aflatoxigenic fungi *i.e. A. flavus* and *A. parasiticus***,** in greenhouse and came free from any aflatoxin contamination under field conditions compared to the parent variety and the other mutants. However, G5 was highly susceptible against all categories of pod rot diseases and recorded the highest contamination with aflatoxin B_1 and B_2. The presence/absence of a certain DNA fragment in the electrogram of a resistant/susceptible genotype may be considered as a molecular marker that indicates the resistant/susceptible genotype. In this respect, several molecular markers related to pod rot resistance/susceptibility in peanut mutants and the parent variety, Giza 5, were obtained (Table 11).

Fragments of 200 and 800 bp were present only on the electrogram in the three resistant mutants RT-7, RT-10 and RT-12, while they were absent from the DNA fragments amplified from all other peanut mutants and their parent variety using primer OP-A9. While fragments of 1650 and 2000 bp were present only on the electrogram of the three susceptible mutants (RT-6- RT-8 and RT-9) and their parent variety (G5) and were not observed for other mutants with the same primer; these fragments could be considered as positive markers for susceptibility.

Table (11): Molecular genetic markers generated from different RAPD primers and their relations to pod rot resistance/susceptibility in peanut mutants and their parent variety Giza 5.

Primer	Marker/pb	Reaction*
OP-A9	(+) 200 (+) 800	Resistance
	(+) 1650 (+) 2000	Susceptibility
OP-B10	(+) 450 (+) 550	Resistance
	(+) 650 (+) 850 (+) 1000	Susceptibility
OP-C7	(-) 1000	Resistance
OP-V6	(-) 600	Susceptibility

(+) positive marker (-) negative marker * resistance or susceptibility to pod rot diseases

Primer OP-B10 revealed fragments of 450 bp and 550 bp which were present only in the DNA fragments of the three resistant mutants RT-7, RT-10 and RT-12, and also revealed fragments of 650, 850 and 1000 bp which were present only in the DNA fragments of the three susceptible mutants (RT-6, RT-8 and RT-9) and their parent variety (G5) and were not observed for other mutants with the same primer. A band of 1000 bp was generated on the electrogram of all peanut mutants and their parent variety (G5) except the three resistant mutants when tested against primer OP-C7. On the other hand, a fragment of 600 bp, when tested against primer OP-V6 was present; this fragment could be considered a negative marker for pod rot resistance in all peanut genotypes except the parent (G5), the susceptible variety.

Although a high degree of polymorphism was revealed by using ISSR primers, it failed in generating molecular markers related to either resistance or susceptibility of root rot disease in peanut.

Molecular genetic markers can help in selecting the tolerant/ susceptible genotypes. For this purpose RAPD-PCR and ISSR-PCR were performed to fingerprint the studied genotypes. The techniques were successful in fingerprinting different mutants and Giza 5 variety 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 and the nature of evolutionary mechanisms underlying the variation measured (Powell *et al.*, 1996). Guohao *et al.* (2003) suggested that it is desirable to isolate and characterize more DNA markers in cultivated peanut for more productive genomic studies, such as genetic mapping, marker-assisted selection and gene discovery. Our results revealed that the chosen RAPD markers are distributed in the peanut genome and may be useful to investigate the genetic diversity among the studied peanut genotypes. 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. He *et al*. (2004) reported that AFLP markers were better than RAPD and ISSR markers in terms of the number of polymorphic bands detected and the experimental stability.

Our results revealed that several molecular genetic markers that may be related to pod rot and aflatoxin contamination resistance/susceptibility in peanut mutants and G5 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 peanut genome. This needs to be confirmed through further studies on molecular markers such as converting these markers into SCAR or using the bulk of segregated F_2 individuals of a cross between contrasting resistant and susceptible parents and 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 pod rot resistance in peanut. Previous investigators used the same technique to generate molecular markers related to resistance or susceptibility to TSWV and/or leaf spots in peanut. Guo *et al*. (2005) have been characterizing and developing DNA polymorphic markers associated with the resistant traits in peanut lines resistant or susceptible to TSWV and/or leaf spots, and generating a segregating population to map/clone the resistance loci/gene(s).

REFERENCES

A.O.A.C. (1998). Official Method of Analysis of Official Analytical Chemists. 16th ed. Kenneth Helrich edit. Published by the Association of Official Analytical Chemists Inc, Virginia, USA.

Anderson, W. F; Holbrook, C. C.; Wilson, D. M. and Matheron, M. E. (1995). Evaluation of preharvest aflatoxin contamination in several potentially resistant peanut genotypes. Peanut Sci., 22: 29-32.

Azer, S. A.; Shahin, A. A. M. and Aziz, N. H. (2002). Radiation-induced mutation of peanut resistant to colonization by toxigenic *Aspergillus flavus*. Egypt. J. Rad. Sci. Appl., 15 (1):97-107.

Azzam, Clara R. and El-Sawy, W. (2005). Variation induced *via* gamma radiation in peanut (*Arachis hypogaea* L.) and its assessment by protein finger printing. Egypt. J. Plant Breed, 9 (2): 237-256.

Branch, W. D. (2001). Registration of Georgia Valancia peanut. Crop Sci., 41: 2002-2003.

El-Adawy, S. S.; Hussein, Ebtissam A. and El-Itriby, Hanaiya A. (2002). Assessment of genotypic variations among maize inbred lines based on simple sequence repeates (SSR). Egypt. J. of Genet. Cytol., 31 (2): 176-182.

Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. 2nd Ed. John Wiley and Sons Ltd., New York, 680pp.

Gostimskii, S. A.; Kokaeva, Z. G. and Konovalov, F. A. (2005). Studying plant genome variation using molecular markers. Genetica, 41(4):480-92

Guo, B.; Holbrook, C.; He, G.; Culbreath, A. and Kvien, C. (2005). Identification and characterization of molecular marker(s) associated with resistance to TSWV and leaf spots in peanuts. http://www. gapeanuts.com /growerinfo /10bg05.pdf.

Guohao, H.; Meng, R.; Newman, M.; Gao, G.; Pittman, R. N. and Prakash, C. S. (2003). Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biology, 3, 3.

Hasan, S. A.; El-Deeb, A. A. and El-Korashy, M. A. (2002). Chemical and

biological control of yellow pod rot of peanuts with reference to nodulation, oil, N content and yield. Egypt. J. Appl. Sci., 17: 62-82.

Hassan, A. M. and Frederick, M. S. (1995). Peanut Health Management. The Amer. Phytopathol. Soc. Press., 117 pp.

He, G.; Meng, R.; Newman, M.; Gao, G.; Pittman, R. N. and Prakash, C. S. (2003). Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biol., 3: 3.

He, X.; Liu, O.; Wang, Y. and Zhai, H. (2004). Analysis of genetic diversity among sweetpotato landraces in China. Agricultural Sci. in China, 3(12): 888-895

Holbrook, C. C.; Wilson, D. M.; Matheron, M. E.; Hunter, J. E.; Knauft, D. A. and Gorbet, D. W. (2000). *Aspergillus* colonization and aflatoxin contaminations in peanut genotypes with reduced linoleic acid composition. Plant Dis., 84:448-450.

Hussein, H. A.; El-Sharkawy, A. M. and Hussein, E. H. (1991). Mutation breeding experiments in peanut. Plant Mutation Breeding for Crop Improvement, IAEA (2): 199-204.

Junghans, S. and Metzlatt, M. (1990). A simple and rapid method for the preparation of total plant DNA. Biotechniques, 8:176.

Kale, D. M.; Badigannavar, A. M. and Murty, G. S. S. (2000). Forty years of mutation research in groundnut at Trombay: Accomplishments and potentials. Proceeding of the DAE-BRANS Symposium on use of Nuclear Techniques in Crop Improvement, Mumbia (India). Bhabha Atomic Research Center, 184-193.

Khaleifa, M. M. A.; Azzam, Clara R. and Azer, S. A. (2006). Biochemical markers associated with disease resistance to damping-off and root-rot diseases of peanut mutants and their productivity. Egypt. J. Phytopathol., 34, (2):53-74.

Mahmoud, E. Y. (2004). Integrated control of pod rots diseases of peanut. Ph.D. Thesis, Fac. of Agric, Ain - Shams Univ. Egypt, 154 pp.

Mahmoud , E. Y.; Draz, Eetmad E. I. and Abol-Ela, M. F. (2006). Evaluation of some peanut cultivars for the susceptibility of infection by damping-off, root and pod rot diseases and occurrence of aflatoxigenic fungi. J. Agric. Sci. Mansoura Univ., 31 (12):7589-7604.

Maren, A. K. and Johan, I. P. (1988). A laboratory guide to the common *Aspergillus* species and their teleomorph. Commo-nwealth Scientific and Industrial Res. Org. Division of Food Processing. 116 pp.

Michelmore, R. W.; Paran, I. and Kesseli, R. V. (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis. A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Nat. Acad. Sci., 88:9828-9832.

Mogg, R. J. and Bond, J. M. (2003). A cheap, reliable and rapid method of extracting high-quality DNA from plants, Molecular Ecology Notes, 3: 666.

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

Pathirana, R. (1991). Increasing efficiency of selection for yield in gamma irradiated population of groundnut and sesame through yield component analysis.Plant Mutation Breeding for Crop improvement, IAEA(2):299-316.

Pharmawati, M.; Yan, G. and Finnegan, P. M. (2005). Molecular variation and fingerprinting of leucadendron cultivars (Proteaceae) by ISSR markers. Annals of Botany., 95 (7):1163 – 1170.

Powell, M. M.; Morgante, C.;Andes, M.; Vogel, J.; Tingey, S. and Rafeiliski, A.

(1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellites) markers. Molecular Breeding, 2: 225-238.

Raina, S. N.; Rani, V.; Kojima, T.; Ogihara, Y.; Singh, K. P. and Devarumath, R. M. (2001). RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome, 44 (5):763-772.

Saiki, R. K.; Gelfand, D. H.; Stoffel, S.; Scharf, S. J.; Higuchi, R.;Horn, G. T.; Mullis, K. B. and Erlich, H. A. (1993). Primer-directed enzymatic amplification of DNA with a thermo-stable DNA polymerase. Science 239: 487-491.

Satour, M. M.; Abd-El-Sattar, M. A.; El-Wakil, A. A.; El-Akkad, E. A. and El-Ghareeb, L. A. (1978). Fungi associated with stem and pod rot diseases of peanut in Egypt. 10th Annual Meeting of American Peanut Res. Educ. Assoc. (APREA), Gainesville, Florida (Abstract.).

Singh, K.; Frisvard, J. C.; Thrane ,U. and Mathur S. B. (1991). An illustrated manual on identification of some seed borne *Aspergilli, Fusaria, Penicillia* and their mycotoxins. Copenhagen, Denmark.

Southcombe, J. E. (1962). Chemistry of the oil industries, Constable and Co. Ltd. London, pp:144.

U. S. D. A. (1998). Farmers stock peanuts inspection instructions. USDA. Washington DC.

Van de Ven, W. T. G.; Duncan, N.; Ramsay, G.; Phillips, M.; Powell, W. and Waugh, R. (1993). Taxonomic relationships between *V. faba* and its relatives based on nuclear and mitochondrial RFLPs and PCR analysis. Theor. Appl. Genet. 86:71-80.

Wang, J. B. (2002). ISSR markers and their applications in plant genetics. Yi Chuan.24(5):613-6.

Williams, J. G. K.; Kubelik, A. R. ; Livak, K. J.; Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18:6531-6535.

Wolfe, A. D. (2005). ISSR techniques for evolutionary biology methods. Enzymol., 395:134-144.

الملخص العربي

توصيف طوافر الفول السوداني والواسمات الجزيئية المرتبطة بالمقاومة لأمراض أعفان الثمار و التلوث بالافلاتوكسينات باستخدام الـ ISSR و RAPD

كلارا رضا عزام*، صبحى عطا الله عازر، ممدوح محمد عبد الفتاح خليفة***، محمد فتحى أبو العلا******

* قسم بحوث الخلية، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية، جيزة ، مصر.

** المركز القومي لبحوث وتكنولوجيا الإشعاع، القاهرة، مصر

*** معهد بحوث أمراض النباتات، مركز البحوث الزراعية، جيزة ، مصر.

**** المركز الاقليمى للأغذية و الأعلاف، مركز البحوث الزراعية، جيزة ، مصر.

تم تقييم عشرة طوافر فول سوداني هم RT-6, RT-7, RT-8, RT-9, RT-10, RT-11, RT-12, RT-13, RT-14 and RT-15 الناتجة من التشعيع بأشعة جاما بالإضافة إلى الصنف الأب (جيزة ٥) لمقاومتهم للمسببات المرضية المسببة لأعفان الثمار ، ومدى اصابتهم بالفطريات المفرزة للافلاتوكسين بالإضافة للتلوث بالافلاتوكسين، تحت ظروف الصوبة والحقل ، في النوبارية ، في الموسميين الصيفيين ٢٠٠٤، ٢٠٠٥ . أظهرت طوافر الفول السوداني المختبرة انخفاضا واضحا في نسب الإصابة بأمراض أعفان الثمار وتواجد الفطريات المفرزة للافلاتوكسين *Aspergillus flavus* and *A. parasiticus* و التلوث بالافلاتوكسينات تحت ظروف الصوبة والحقل مقارنة بالصنف الأب (جيزة ٥). كانت الطوافر RT-10, RT-12 and RT-7 هي الأكثر مقاومة لأمراض أعفان الثمار و الأقل تلوثا أو الخالية تماما من التلوث بالافلاتوكسينات B1 and B2 تحت ظروف العدوى الاصطناعية بالفطريات المفرزة منفردة أو مجتمعة أو تحت ظروف العدوى الطبيعية بالحقل. تحت ظروف الحقل ، زاد محصول الثمار للمتر المربع معنويا في الطوافر العشر من الفول السوداني مقارنة بالصنف الأب (جيزة ٥)، باستثناء الطافر RT-15 والتي لم يختلف معنويا معه. زاد وزن الـ ١٠٠ ثمرة معنويا في الطافر RT-9 بينما تناقصت معنويا في الطافرين RT-14 and RT-15 مقارنة بالصنف الأب (جيزة ٥). زادت نسبة الثمار ذات القطر الأكبر من ١٤ مم زيادة معنوية في الطافرين RT-8 and RT-11 في حين لم تتغير معنويا نسبة البذور الكلية الناضجة عن الصنف الأب (جيزة ٥). وعلى العكس، زادت نسبة البذور الكبيرة (أكبر من ٨ مم) معنويا في الطوافر RT-11, RT-13 and RT-14. نقصت نسبة الزيت معنويا في الطوافر العشر من الفول السوداني مقارنة بالصنف الأب (جيزة ٥). استخدمت طريقتان للتكبير العشوائي للحمض النووي هما RAPD و ISSR لإيجاد واسمات وراثية جزيئية علي مستوي الحمض النووي DNA لتمييز المقاومة أو القابلية للإصابة لأمراض أعفان الثمار و التلوث بالافلاتوكسين . أظهرت بعض البادئات وجود بعض الشظايا من الحمض النووي DNA والتي ارتبطت إما إيجابا أو سلبا مع صفة المقاومة أو الإصابة بالمرض. وقد تم حساب التباعد الوراثي بين الطوافر والصنف الأب و بين الطوافر وبعضها وتم رسم شجرة القرابة الوراثية والتي أظهرت أن الطوافر المقاومة لأمراض أعفان الثمار والخالية من التلوث بالافلاتوكسين تقع في نفس المجموعة دائما مع تحليل كل من RAPD و ISSR منفردين أو مجمعين.