Proceeding Fifth Plant Breeding Conference May 27, 2007 (Giza)

Egypt. J. Plant Breed. 11(2): 829-859 (2007) Special Issue

FINGERPRINTING AND CHARACTERIZATION OF SOME PEANUT MUTANTS AND DETECTION OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO POD ROT DISEASES AND AFLATOXIN CONTAMINATION BY RAPD AND ISSR

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ABSTRACT

Ten peanut mutants i.e. RT-6, RT-7, RT-8, RT-9, RT-10, RT-11, RT-12, RT-13, RT-14 and RT-15, as well as, the parent variety (Giza 5) were evaluated for their reaction against pod rot pathogens and invasion by aflatoxigenic fungi as well as aflatoxin contamination, under greenhouse and field conditions during 2004 and 2005 summer seasons. Under greenhouse and field conditions, all peanut mutants exhibited significant decrease in percentages of pod rot diseases, occurrence of aflatoxigenic fungi and aflatoxin contamination compared to the parent variety (Giza5). Mutants RT-10, RT-12 and RT-7 were highly resistant 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. Aspergillus flavus and A. parasiticus, separately or in mixture in greenhouse and came free from any contamination with aflatoxin under field conditions compared to the parent variety (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 was equal to pod yield of the parent variety. 100-pod weight of RT-7, RT-9, RT-10 and RT-11 were significantly increased, meanwhile the mutants RT-14 and RT-15 had significant decrease in 100-pod weight compared to their parent variety, Giza-5. The fancy pods percentage (FP %) significantly increased in the two mutants RT-8 and RT-11, the total sound mature kernels percentage (TSMK %) in all mutants did not significantly differ from the original variety (Giza-5). In contrast, the large kernels percentage (LK %) of RT-11, RT-13 and RT-14 had significant increase (74.06, 72.48 and 66.43%, respectively), compared to (62.30%) of the parent variety. The oil content of all mutants significantly decreased compared to the parent variety, Giza-5. Leaf tissue for each of the ten mutants and the parent variety G5 (un-infected) was collected and DNA was extracted and used to perform polymerase chain reaction (PCR) using RAPD and ISSR primers [13 arbitrary 10-mer primers (Operon Technologies, Inc), and 12 ISSR primers]. RAPD-PCR was used to evaluate the genetic diversity of the ten peanut mutants, as well as, the parent variety (Giza-5). Out of the thirteen 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. Several molecular markers (positive and negative) related to pod rot resistance/susceptibility in peanut mutants and their parent variety, Giza-5 were obtained by the RAPD primers. Out of the

twelve ISSR primers, four did not reveal any DNA amplification, whereas eight successfully amplified DNA fragments for all genotypes, and four primers succeeded to generate polymorphic banding patterns, while the remaining four primers generated monomorphic ones. ISSR didn't reveal any marker (positive or negative) associated with pod rot resistance/susceptibility in peanut mutants and their parent variety, Giza-5. According to RAPD, ISSR and the combined data of RAPD and ISSR the three most closely related mutants were RT-7, RT-10 and RT-11 which were located very close to each other in the consensus tree.

Key Words: *Peanut, Arachis hypogaea , Pod grade, kerkent quality, Pod rot diseases, Aflatoxin contamination, Molecular markers, RAPD, ISSR, Genetic distance, Cluster analysis.*

INTRODUCTION

Peanut, (*Arachis hypogaea* L.) is a main world source of edible oil and protein. It is estimated that some two-third of total world production of peanut is crushed for oil, the remained is eaten as whole nuts or pasts. Peanut oil is also used in soaps, hair lotions, cosmetics and toilet preparations. The oil is of excellent quality with a high smokepoint, and is mainly used for cooking and salad. In Egypt, peanut is one of the most export and locally direct human consumed crop. Soil borne fungi can attack peanut pods, when environmental conditions are favorable for their infection and growth, during their development in soil after harvesting and during storage (Satour *et al* 1978 and Al-Ahmer *et al* 1989). They cause serious quantitative and qualitative 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 its development in the soil. Peanut pods are a good substrate for growth of *A. flavus* and *A. parasiticus*, and for subsequent aflatoxin production by aflatoxigenic fungi (Xue *et al* 2003 and Mahmoud 2004). Meanwhile preharvest aflatoxin contamination is one of the most challenges facing the peanut producers in many parts of the world (Wilson and Payne 1994) and it has marked consideration in Egypt.

Pod rot diseases are widely spread and the tested cultivars differ greatly in their reaction to diseases, in both quantity and quality of peanut yield (Mahmoud *et al* 2006). Also, no cultivars were completely resistant to aflatoxin contamination following seed invasion with aflatoxigenic fungi, while there were a significant difference in their ability to allow invasion and aflatoxin production (Mahmoud *et al* 2006).

In recent years, there was a considerable research on possible genetic resistance in groundnuts for seed infection and aflatoxin contamination in the field (Mehan *et al* 1986). Many investigators indicated that, gamma radiation is an ionizing radiation which can be used for improving plant

characters and increasing genetic variability in a variety of crop species including peanut (Azer *et al* 2002; Shahin and Azer 2003; Azzam and El-Sawy 2005 and Khaleifa *et al* 2006).

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 in order to discriminate peanut cultivars by Burow *et al* 1996, Garcia *et al* 1996, Raina *et al* 2001, Guohao *et al* 2003, He *et al* 2003 and Guo *et al* 2005.

Inter simple sequence repeat (ISSR) is an alternative technique to study polymorphism based on the presence of microsatellites through-out genomes (Zietkiewicz *et al* 1994; Leroy *et al* 2001, Raina *et al* 2001, Wang 2002 and Pharmawati *et al* 2005)

Comparison of ISSR and other PCR-based markers have shown their efficiency in plant breeding (Mogg and Bond 2003). As a result of these advantages and their universality and easiness of development, ISSR markers are more and more requested. Gostimskii *et al* (2005) reported that markers have been demonstrated to be promising for identifying cultivars and determining the purity of genetic strains of pea. Wolfe (2005) reported that inter-simple sequence repeat (ISSR) markers were originally devised for differentiating among closely related plant cultivars but have become extremely 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 under artificial and natural infection with high yield components, high pod yield, pod grade and kernels quality and to find molecular genetic markers for resistance based on RAPD and ISSR markers.

MATERIALS AND METHODS

Greenhouse evaluation

Ten peanut mutants *i.e.* 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 from mutant generations (Azer *et al* 2002) as well as the parent variety (Giza 5) were

evaluated for their reaction against pod rot pathogens and invasion by aflatoxigenic fungi as well as aflatoxin contamination.

Reaction of peanut genotypes against pod rot pathogens

Fungal inocula of the main pod rot causal pathogens *i.e. 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 which were previously isolated from diseased peanut pods and confirmed their pathogenic capabilities by the authors were prepared to soil infestation under pots experiment using sorghum - coarse sand - water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for two hours at 1.5 air pressure. The autoclaved medium in glass bottles was inoculated separately using agar discs obtained from the periphery of 5 day old colony of each of the tested fungi and incubated at 26° C for two weeks and were then used for soil infestation.

Surface sterilized seeds of the ten peanut mutants and parent variety Giza 5 (entries) were sown as five seeds per pot and each pot (50 cm diam.) containing soil previously infested with inocula at the rate of 2% (w/w) of a mixture of the main pod rots causal pathogens *i.e. R. solani; F. moniliforme; M. phaseolina* and *S. rolfsii*. Four replicates were used for each peanut entry, as well as four pots containing sterile non infested soil were used as control. The pots were arranged in four replicates in a randomized complete block design.

At harvest, percentages of diseased pods were determined. Three categories for apparent symptoms of pod rots beside the apparently healthy pods were adopted according to Satour *et al* (1978). They were a) Rhizoctonia rot, pods with dry brown lesion, b) Fusarium rot, pods with pink discoloration and c) complex rot pod with general breakdown resulting from many fungi.

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 to soil infestation under pots experiment as mentioned before and added at the rate of 2% (w/w) separately and/or mixed to pots (50cm) containing sterilized soil thoroughly with soil surface of each pot. None infested potted soil, served as control. Five surface sterilized seeds of each of the ten peanut mutants and parent variety were sown per each pot. The pots were arranged in four replicates in a complete randomized block design. 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* as well as for detection of aflatoxin contamination.

Aflatoxigenic fungi, associated with the samples of peanut entries pods, were isolated after harvest according to Garren and Porter (1970). Identification of the isolates was carried out based on taxonomic criteria for these fungi as described by Maren and Johan (1988). The frequency of invasion by aflatoxigenic fungi in pod samples was recorded and calculated as follow:

$$\% \text{ invasion by aflatoxigenic fungi} = \frac{\text{Number of infected samples}}{\text{Number of total samples}} \times 100$$

The extraction of aftatoxins was conducted according to A.O.A.C (1998) and was determined according to Singh *et al* (1991).

Field evaluation

Field experiments in a randomized complete block design with four replicates were conducted during summer seasons of 2004 and 2005 at Nubaria district, El- Behara Governorate. Each replicate was 21 m^2. The field soil was sandy loam, heavily infested with the pod rots and aflatoxigenic fungi causal pathogens. Well sized seeds of the ten mutants, as well as, the parent variety, Giza 5 were sown at the first week of May of each season. The kernels were sown by hand in rows spaced 40-cm., with 20-cm between hills. Overhead sprinkler system (Central pivot 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

To estimate the yield of peanut entries, the pod yield of each plot was recorded. The genotypes were harvested 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 14mm (36/64 inch) slotted screen]. The fancy pods were used to determine 100-pod weight (g.). 500 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 shrenked 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, presence of pathogenic and aflatoxigenic fungi and aflatoxin contamination were determined as previously mentioned in greenhouse experiments.

Statistical analysis

The obtained data were statistically analyzed by analysis of variance (ANOVA). Means were separated by Fisher's protected least significant differences (LSD) at $P \leq 0.05$ level (Gomez and Gomez 1984).

Molecular Genetic Markers

For DNA extraction, leaf tissue for each peanut mutant and G5 variety (un-infected) was collected from 5-7 days old germinated seedlings. DNA was extracted from 100mg of young leaves according to Junhans 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 in this study. The universal name and sequences of the used primers are shown in Table (1). Eight out of the arbitrary 10-mer primers succeeded in DNA amplification, while seven ISSR primers succeeded in DNA amplification. The primers marked with (**) generated polymorphism, whereas the other primers generated monomorphic banding patterns (*) and the primers marked with (ˣ) means that amplification wasn't detected.

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

RAPD			ISSR		
No.	Names	Sequences `5→`3	No.	Names	Sequences `5→`3
1**	OP-A1	CAGGCCCTTC	1**	I-18	$GTGC(TC)_7$
2**	OP-A9	GGGTAACGCC	2*	I-28	$(GT)_6CG$
3**	OP-B10	CTGCTGGGAC	3ˣ	HB8	$(GA)_6GG$
4**	OP-B12	CCTTGACGCA	4ˣ	HB9	$(GT)_6GG$
5**	OP-B13	TTCCCCCGCT	5*	HB10	$(GA)_6CC$
6*	OP-B19	ACCCCCGAAG	6*	HB12	$(CAG)_3GC$
7*	OP-C6[a]	GAACGGACTC	7*	HB15	$(GTG)_3GC$
8**	OP-C7[a]	GTCCCGACGA	8**	814.1	$(CT)_8TG$
9ˣ	OP-Q5	CCGCGTCTTG	9**	844B	$(CT)_8GC$
10ˣ	OP-Q11	TCTCCGCAAC	10ˣ	17898[a]	$(CA)_6AC$
11ˣ	OP-Q13	GGAGTGGACA	11ˣ	17898[b]	$(CA)_6GT$
12**	OP-V2	AGTCACTCCC	12**	UBC830	$(GA)_8C$
13**	OP-V6	ACGCCCAGGT			

ˣAmplification wasn't detected. *Primers revealed monomorphism.**Primers revealed polymorphism

RAPD-PCR reactions were optimized and mixtures (25µl total volume) were composed of dNTPs (200 µ M), Mg Cl_2 (1.5 mM), 1x buffer, primer (0.2 µM), DNA (50ng), Taq DNA polymerase (2 units). Amplification was carried out in a Thermo Cycler (Perkin Elmer) programmed for 94°C for 3 min (one cycle), followed by 94°C for 30 sec, 36°C for 1 min and 72°C for 2 min (36 cycle), 72°C for 10 min (one cycle), then 4°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 by comparisons with the 100 bp DNA Ladder marker.

ISSR-PCR reactions were optimized and mixtures (25µl total volume) were composed of dNTPs (200 µ M), Mg Cl_2 (1.5 mM), 1x buffer, primer (0.2 µM), DNA (50ng), Taq DNA polymerase (2 units). Amplification was carried out in a Thermo Cycler (Perkin Elmer) programmed for 94°C for 3 min (one cycle), followed by 94°C for 30 sec, 40°C for 45 sec and 72°C for 1 min (35 cycle), 72°C for 10 min (one cycle), then 4°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 by comparisons with the 100 bp DNA Ladder marker.

RAPD and ISSR Data analysis

The obtained data of RAPD and ISSR analyses were entered in computer file as binary matrices where o stands for the absence of a band and 1 stands for the presence of a band in each individual sample. 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.

RESULTS AND DISCUSSION

Greenhouse experiments

Reaction of peanut genotypes against 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 i.e. dry brown lesion, pink discoloration and general

Table 2. Evaluation of some peanut entries 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 0.05	2.393	2.367	2.410	2.314

breakdown and increased the apparently healthy pods compared to the parent variety (Giza5).

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 peanut entries.

Mutant RT-10 was the highly resistant one against all categories of pod rots diseases and gave the highest percentages of apparent healthy pods followed by mutants *i.e.* RT-12 and RT-7. On the other hand, the parent variety (Giza 5) was the highly susceptible for all categories of pod rots diseases and gave the lowest percentage of apparently healthy pods, followed by mutants *i.e.* 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 found that, mutants RT-10, RT-11, RT-12 and RT-7 were resistant to damping off and root-rot diseases while Giza 5 followed by RT-15 and RT-14 were the most susceptible against both diseases under greenhouse and field conditions. They added that, this resistance may be due to occurrence of some biochemical changes in the resistant mutants, as increasing the activities of oxidative enzymes i.e., peroxidase (PO); polyphenoloxidase (PPO) and catalase as well as increasing the amounts of free and total phenols and reducing total sugars. They detected some protein bands related to resistance and susceptibility to damping off and root-rot diseases in peanut.

Reaction of peanut genotypes to occurrence of aflatoxigenic fungi and aflatoxins

Data presented in Table (3) illustrated that, occurrence of aflatoxigenic fungi *A. flavus* and *A. parasiticus* was higher in seed than shell in all evaluated peanut entries. *A. flavus* recorded the highest frequency compared to *A. parasiticus* in all evaluated peanut entries whether infestation was separately or in a mixture with *A. parasiticus*.

Obtained data also showed that mutant RT-10 recorded the least occurrence (percentage) of aflatoxigenic fungi (*A. flavus* and *A. parasiticus)* in both seed and shell under infested soil with *A. flavus* and/or *A. parasiticus* separately or in a mixture, followed by mutants *i.e.* RT-12 and RT-7, while Giza 5, RT-15, RT-9 and RT-14, showed the highest occurrence of aflatoxigenic fungi on both seed and shell. These results are in harmony with Horn *et al* (1994) and may be due to that *A. flavus* is more aggressive than *A. parasiticus* and peanut seeds are good substrate for its growth and subsequent aflatoxin production (Xue *et al* 2003). The resistance of peanut kernels to *A. flavus* and/or *A. parasiticus* invasion appears to be associated with certain structural and biochemical characters of the pod and seed and there is a possibility that genotypes may have differential effects upon the population of aflatoxigenic fungi in geocarposhere (Nahdi 1989).

Regarding aflatoxin contamination, in general, none of studied mutants and their parent variety showed completely resistance to aflatoxin contamination under soil infestation conditions with *A. flavus* separately and/or in mixture with *A. parasiticus* (Table 3). Aflatoxin B_1 was higher than B_2 in all peanut entries. The mixture of *A. flavus* and *A. parasiticus* recorded the highest content of aflatoxin $B_1$and B_2 followed by *A. flavus* while *A. parasiticus* recorded the lowest content of aflatoxin B_1 and B_2.

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 contamination with aflatoxin B_1 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 of aflatoxigenic fungi separately or in a mixture followed by mutants RT-15, RT-9, RT-14 and RT-6.

These results are in harmony with those found by Hasan *et al* (2002), who found no cultivar completely resistant to aflatoxin production and invasion with aflatoxigenic fungi, while there were a significant

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

Genotypes	*A. flavus*				*A. parasiticus*				*A. flavus* + *A. parasiticus*					
	% Occurrence (y)		Content of aflatoxin (ppb)		% Occurrence (y)		Content of aflatoxin (ppb)		% occurrence of *A. flavus* (y)		% Occurrence of *A. parasiticus* (y)		Content of aflatoxin (ppb)	
	Seed	Shell	B_1	B_2	Seed	Shell	B_1	B_2	Seed	Shell	Seed	Shell	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

(y) Each value is the mean of three replicates (3 plates / replicate, five seeds or shell pieces per dish). ND (Not detected)

difference in cultivar ability to allow invasion and aflatoxin production. In this regard, Wilson and Flowers (1978) pointed out that, aflatoxin contamination of peanuts is unavoidable. In this study, the extent of aflatoxigenic fungi and aflatoxin contamination varied in harvested pods of peanut entries under natural and artificial inoculation. Furthermore aflatoxigenic fungi colonization and aflatoxin contamination were greater under artificial than natural inoculation.

The variable amount of aflatoxin present in contaminated peanut mutants and their parent variety may be due to the environmental factors, nature of the fungal strains as well as composition of the substratum (Anderson *et al* 1995 and Saleha 1996). 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).

Field evaluation

Yield components, pod grade and kernel quality

The mean values as combined overall the two seasons, of pod yield m-2, pod grade, kernels quality and oil content, of ten mutants, as well as, the parent variety Giza-5 are given in Table (4). The mean values of pod yield m^{-2} in all the mutants were significantly higher compared to the control (Giza-5), except the mutant RT-15 which showed pod yield 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 Giza-5. Also, Hussein *et al.* (1991) selected many mutant lines with higher yielding ability since they proved to be significantly superior to their corresponding parent (Giza-4), with many altered traits.

Concerning to 100-pod weight, the results in (Table 4) indicated that four mutants; RT-7, RT-9, RT-10 and RT-11 were significantly increased, meanwhile the mutants RT-14 and RT-15 had significant decrease in 100-pod weight compared to their parent variety, Giza-5. Kale *et al* (2000) selected seven new peanut mutant lines characterized with large pod size. The results of fancy pods (FP) showed that no significant differences were detected between the mutants or between them and the control variety (Giza-5), except the two mutants RT-8 and RT-11, which showed significant increases in FP%. Branch (2001) released Georgia Valencia peanut mutant line which has a significantly larger pod size with 25% more fancy pods than the Georgia Red (the parent variety). However, the results in Table (4) showed that the shelling percentage in all peanut mutants did not significantly differ from the control (Giza-5).

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

Genotypes	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	479	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
Mean	711	74.31	244	71.66	97.53	62.52	95.86	48.56
LSD 0.05	48	1.31	17.50	NS	NS	1.92	1.57	1.10

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

The data on the kernel quality in Table (4) showed that TSMK% in all mutants did not significantly differ from the original variety (Giza-5). Soriano (1988) reported that only about 88% of the fertilized ovules developed into good seeds in the resistant varieties to *A. flavus* invasion, compared to good seeds in the susceptible lines. In contrast, the large kernels percentage (LK %) of three mutants: RT-11, RT-13 and RT-14 had a significant increase in KL% (74.06, 72.48 and 66.43%, respectively), compared to (62.30%) of the parent variety. Meanwhile, the three mutants: RT-6, RT-9 and RT-15 were significantly decreased in LK% compared to Giza-5. The rest of the mutants were approximately equal to Giza-5 variety in LK%. Pathirana (1991) selected mutants of peanut characterized with large kernel size. The mean values of 100-kernel weight (Table 4) significantly increased in all peanut mutants than Giza-5 variety. The increases ranged between 2.60g for the peanut mutant RT-13 to 8.00g. for the mutant RT-9, while, 100-kernel weight was 92.00g in Giza-5. Kale *et al* (2000) selected many peanut mutant lines characterized by large kernel; also, Sidhu *et al* (1997) and Pathirana (1991) released many peanut mutant lines with high kernel index compared to their parent varieties.

The oil content of all mutants (Table 4) showed significant decrease than their parent. The results indicated that the two mutants RT-6 and RT-9 were the least mutants in oil content. The decrease in oil percentage in all

the mutant genotypes under study ranged between 6.02% (RT-9) to 1.28% (RT-13 and RT-14), compared to the oil percentage in Giza-5. Grami *et al* (1977) reported that increasing oil yield is achieved by increasing kernel yield and /or increasing the percentage of oil content.

Evaluation of peanut genotypes against pod rot diseases complex under field natural infection

Data presented in Table (5) showed the reaction of ten peanut mutants and their parent variety Giza 5 against pod rot diseases complex *i.e.* dry brown lesion, pink discoloration and general breakdown as well as apparently healthy pods 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 disease incidence, followed by dry brown lesion while, the pink discoloration was the least pod rot disease incidence on all studied peanut entries with few exceptions.

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

Genotypes	2004				2005			
	% Dry brown lesion	% Pink discoloration	% General breakdown	% Apparently healthy pods	% Dry brown lesion	% Pink discoloration	% General breakdown	% pparently healthy pods
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	2447	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
SD at 0.05	1.845	1.332	3.087	2.763	1.845	1.260	2.807	2.667

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 descending order. However, Giza 5 was the highest susceptible one. It showed the highest percentages of incidence of all pod rot categories 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 Giza varied in their susceptibility to infection with pod rot disease complex i.e. dry brown lesion, pink discoloration and general breakdown under greenhouse and field conditions. These results are in agreement with Hasan *et al* (2002) and Mahmoud *et al* (2006). In this respect Mahmoud *et al* (2006) found similar results since they recorded that, pod rot diseases were common on all tested cultivars and the highest percentages of the diseases were in Giza 4 and Giza 5.

Occurrence of *A. flavus* and *A. parasiticus* in seed and shell and aflatoxin content in peanut seed under field natural infection

Tables (6 and 7) presented data on *A. flavus* and *A. parasiticus* in seed and shell and aflatoxin content in seed only of peanut genotypes under natural infection of field conditions in seasons 2004 and 2005. Concerning occurrence of both aflatoxigenic fungi, *A. flavus* was more invasive to either pod shells or seeds than *A. parasiticus* and both fungi occurred at high frequency in seeds compared to pod shells of most evaluated mutants and parent variety Giza 5, with few exceptions. However, both aflatoxigenic fungi have occurred in high frequency in pods with dry brown lesion or general breakdown compared with pink discoloration and apparently healthy pods in both seasons 2004 and 2005.

The results also indicated that, mutant RT-10 recorded the least occurrence of aflatoxigenic fungi *A. flavus* and *A. parasiticus* in both seed and shell in all categories of pod rots beside the apparently healthy pods followed by mutants RT-13, RT-12, RT-7 and RT-11, while Giza 5 followed by mutants RT-9, and RT-15 recorded the highest occurrence of aflatoxigenic fungi on either seed or shell, whereas mutants RT-8, RT-6 and RT-14 were intermediate in this respect in season 2004. The same trend of results of aflatoxigenic fungi occurrence was observed in season 2005 with few exceptions. Several workers have screened peanut genotypes for resistance to seed colonization and aflatoxin contamination of aflatoxigenic fungi *A. flavus* and *A. parasiticus* under *in vitro* conditions (Azer *et al* 2002 and Shahin and Azer 2003) and field conditions (Kisyombe *et al* 1985 and Will *et al* 1994).

Regarding aflatoxin contamination, the obtained results in Tables (6 and 7) also indicated that, aflatoxin B_1 was higher than B_2 in all detected

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.

Genotypes	Occurrence of aflatoxigenic fungi of pod rot categories (y)																Content of aflatoxin (ppb)	
	Dry brown lesion				Pink discoloration				General breakdown				Apparently healthy pods					
	A. flavus		*A. parasiticus*		*A. flavus*		*A. parasiticus*		*A. flavus*		*A. parasiticus*		*A. flavus*		*A. parasiticus*			
	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	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

(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

Genotypes	Occurrence of aflatoxigenic fungi of pod rot categories (y)																Content of aflatoxin (ppb)	
	Dry brown lesion				Pink discoloration				General breakdown				Apparently healthy pods					
	A. flavus		*A.parasiticus*		*A. flavus*		*A.parasiticus*		*A. flavus*		*A.parasiticus*		*A. flavus*		*A.parasiticus*			
	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	Seed	Shell	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-	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-	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-	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-	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-	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-	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	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

(y) Each value is the mean of three replicates (3 plates / replicate, five seeds or shell pieces per dish). ND (Not detected)

cases of peanut entries. In this respect in both seasons, mutants RT-7, RT-10 and RT-12 were free from any contamination with aflatoxin while, aflatoxin contamination was the
lowest content 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 Hasan *et al* (2002) and Mahmoud *et al* (2006).

Data also showed that, under field conditions there was no clear correlation between the occurrence of aflatoxigenic fungi in kernels of peanut entries and their aflatoxin contamination, with some exceptions. This is in agreement with Azaizeh *et al* (1989), Will *et al* (1994) and Robin *et al* (1999) who reported no significant correlation between aflatoxin concentration and soil population densities of aflatoxigenic fungi. 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). While peanuts grown under stress conditions will only result in extensive aflatoxigenic mould infection and subsequent aflatoxin contamination of the harvested peanuts (Azaizeh *et al* 1989).

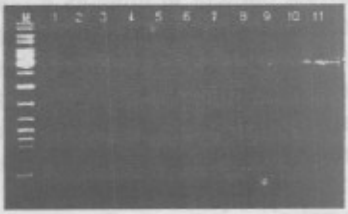
Fingerprinting of peanut mutants and their parent variety, Giza-5

RAPD-PCR was used to evaluate the genetic diversity of the ten peanut mutants from their parent variety (Giza-5) using thirteen arbitrary primers. Of the thirteen 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 (Giza-5). Number of bands ranged from 6 (primer OP-A9) to 14 (primer OP-C7) across all investigated mutants and their parent.

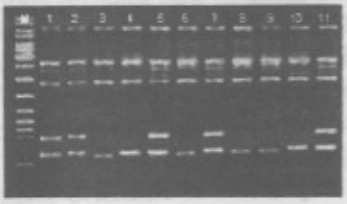
Level of polymorphism varied from one primer to another. Primer OP-A9 showed the highest level of polymorphism (83.3%), while primer OP- V6 showed the lowest level (57.1 %). The resulted amplified fragments are shown in Fig.1, for four primers that presented the highest and the lowest degree of polymorphism.

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 results of genetic similarity are shown in (Table 8). The lowest genetic similarity (0.58 and 0.62) were observed between the parent variety (Giza-5) and both of RT-10 and RT-12, respectively, while the highest genetic

OP-A9



OP-B10



OP-C7

OP-V6

M=100bp Ladder DNA 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 1. Agarose gel (1.2%) in TBE buffer stained with ethidium bromide showing RAPD-PCR polymorphism of DNA for ten peanut mutants and their parent variety (Giza-5) using random primers (OP-A9, OP-B10, OP-C7 and OP-V6).

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

Genotypes	G5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14	RT15
G5	1										
RT6	0.91	1									
RT7	0.67	0.69	1								
RT8	0.71	0.78	0.68	1							
RT9	0.80	0.88	0.72	0.84	1						
RT10	0.58	0.68	0.82	0.71	0.76	1					
RT11	0.80	0.89	0.76	0.79	0.93	0.75	1				
RT12	0.62	0.65	0.88	0.63	0.67	0.90	0.70	1			
RT13	0.74	0.77	0.77	0.78	0.74	0.74	0.75	0.75	1		
RT14	0.71	0.73	0.69	0.79	0.72	0.70	0.67	0.67	0.81	1	
RT15	0.83	0.81	0.71	0.69	0.80	0.66	0.78	0.66	0.79	0.84	1

similarity were scored between RT-11 and RT-9 (0.93), followed by Giza-5 and RT-6 (0.91). Cluster analysis classified the eleven genotypes into two main clusters (Fig. 2). The first main cluster consisted of three peanut mutants (RT-10, RT-7 and RT-12), while the second main cluster divided into two main sub-clusters, the first consisted of three peanut mutants (RT-14, RT-15 and RT-13), whereas, the second 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.

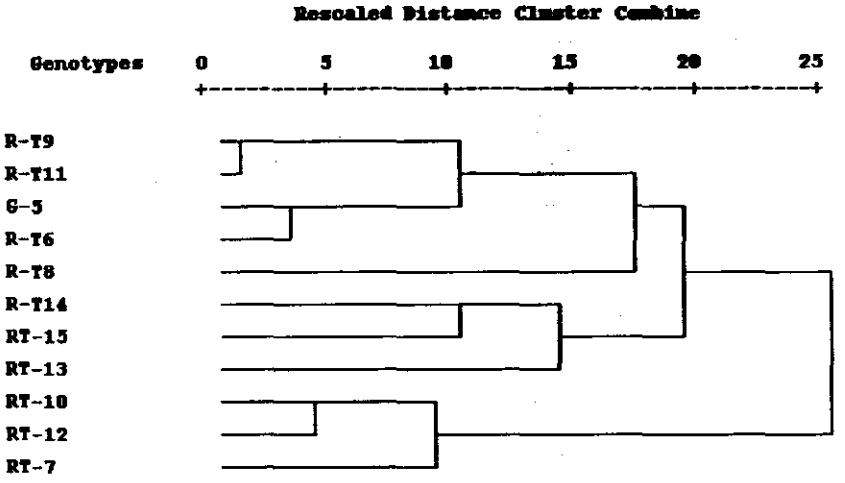
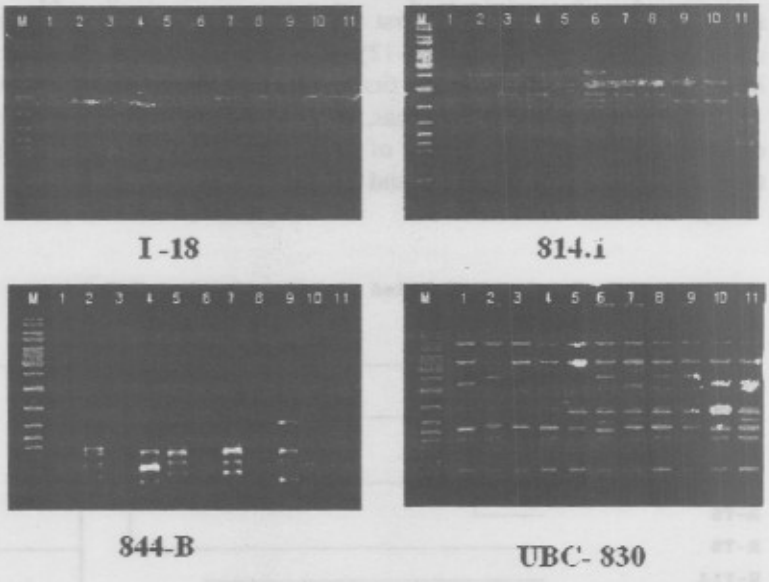


Fig. 2. Dendrogram of the genetic distances among the ten peanut mutants and their parent variety (Giza-5) based on RAPD analysis.

ISSR-PCR was used to evaluate the genetic diversity of the ten peanut mutants from their parent variety (Giza-5) 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 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 (Giza-5). 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 %).



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. Agarose gel (1.2%) in TBE buffer stained with ethidium bromide showing ISSR-PCR polymorphism of DNA for ten peanut mutants and their parent variety (Giza-5) using random primers (I-18, 814.1, 844-B and UBC-830).

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 (0.57 and 0.68) were observed between the mutant RT-15 and both of RT-8 and RT-13, respectively, while the highest genetic similarity index were 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 clusters (Fig.4). RT-15 was located alone in the first main cluster and the second divided into two main sub-clusters, the first consisted of Giza-5 and RT-14, while the second divided into two sub-sub- clusters, one of them consisted of mutant RT-7, RT-10 and RT-12 and the second consisted of the rest of peanut mutants and their parent variety.

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

Genotypes	G5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14	RT15
G5	1										
RT6	0.81	1									
RT7	0.76	0.82	1								
RT8	0.73	0.88	0.80	1							
RT9	0.84	0.92	0.85	0.85	1						
RT10	0.78	0.78	0.93	0.76	0.84	1					
RT11	0.81	0.90	0.88	0.82	0.87	0.81	1				
RT12	0.77	0.83	0.84	0.81	0.81	0.83	0.86	1			
RT13	0.77	0.85	0.83	0.83	0.88	0.80	0.88	0.76	1		
RT14	0.83	0.75	0.72	0.69	0.78	0.71	0.75	0.71	0.79	1	
RT15	0.77	0.72	0.73	0.67	0.72	0.71	0.81	0.77	0.68	0.73	1

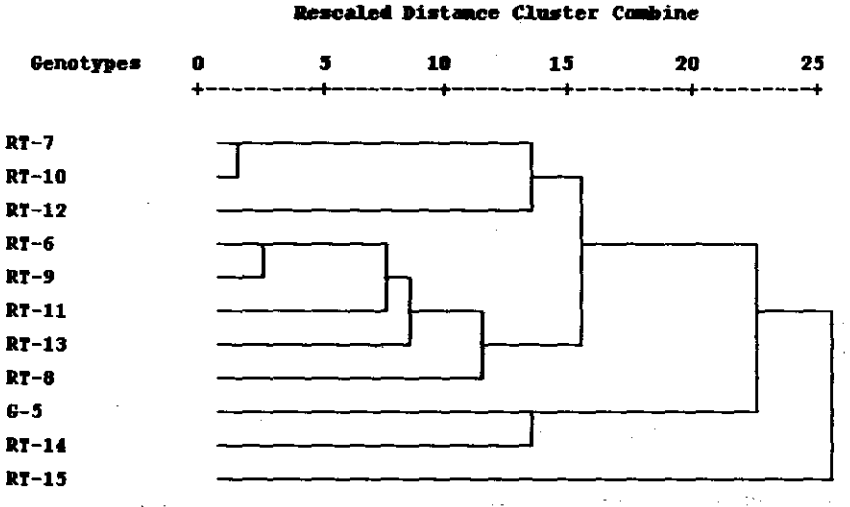


Fig. 4. Dendrogram of the genetic distances among the ten peanut mutants and their parent variety (Giza-5) based on ISSR analysis.

Genotypes distribution on the consensus tree according to the banding patterns of ISSR shuffled than 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 the combination of the two techniques banding patterns to cover as much as possible of the genome, that will increase the validity of the consensus tree. The results the similarity index and consensus tree of the combined data for the ten peanut mutants and their parent variety (Giza-5) as shown in Table (10) and Fig. (5) showed

Table 10 Similarity index for ten peanut mutants and their parent variety Giza-5 as calculated on the base of their banding patterns with *RAPD and ISSR primers.*

Genotypes	G5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14	RT15
G5	1										
RT6	0.88	1									
RT7	0.70	0.73	1								
RT8	0.71	0.82	0.72	1							
RT9	0.81	0.90	0.76	0.84	1						
RT10	0.63	0.71	0.85	0.73	0.79	1					
RT11	0.81	0.89	0.80	0.80	0.91	0.77	1				
RT12	0.67	0.71	0.87	0.69	0.71	0.88	0.75	1			
RT13	0.75	0.80	0.79	0.80	0.79	0.76	0.80	0.76	1		
RT14	0.75	0.74	0.70	0.76	0.74	0.70	0.70	0.68	0.80	1	
RT15	0.81	0.78	0.72	0.68	0.77	0.68	0.79	0.69	0.75	0.80	1

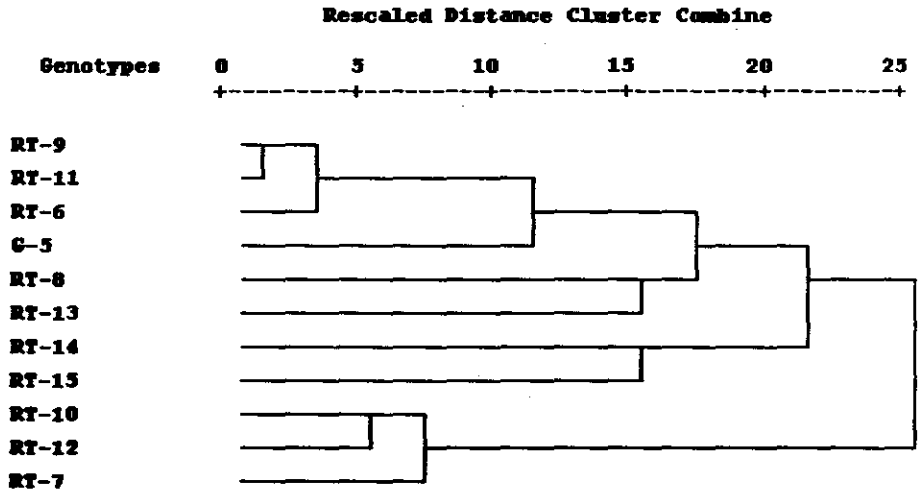


Fig. 5. Dendrogram of the genetic distances among the ten peanut mutants and their parent variety (Giza-5) based on RAPD and ISSR analysis.

that the lowest genetic similarity (0.63 and 0.67) were observed between the parent variety and both of RT-10 and RT-12, respectively, while the highest genetic similarity (0.91 and 0.90) were scored between RT-9 and both of RT-11 and RT-6, respectively. According to 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 consensus tree and the other cluster was divided into two

main subclusters, 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 in one hand alone and on the other hand, mutants RT-9, RT-11 and RT-6 located in the second one. The results indicated that the three most distantly related cultivars were RT-7, RT-10 and RT-12 compared to the parent variety (Giza-5), the similarity index estimates were 0.70, 0.63 and 0.67, respectively. According to RAPD, ISSR and RAPD+ISSR analysis, these three mutants resistant to pod rot diseases always locate in the other main cluster or subcluster in contrast with the susceptible ones, as shown in Figs.(2, 4 and 5). Although the genetic distances between the resistance mutants are very close, there is high variation in their morphological, yield and yield component characters, as shown in Fig. (6) and Table (4).

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.

DNA Molecular markers related to pod rot resistance/susceptibility based on RAPD and ISSR

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 (Giza 5) and the other mutants. However, Giza 5 was highly susceptible against all categories of pod rots diseases and recorded the highest contamination with aflatoxin B_1 and B_2. The presence/absence of a certain DNA fragment in the electrophoregram of a resistant/susceptible variety may be considered as a molecular marker that indicates the resistant/susceptible variety. In this work several molecular markers related to pod rot resistance/susceptibility in peanut mutants and their parent variety, Giza-5 were obtained (Table 11).

Fragments of 200 and 800 bp were present only on the electrophoregram in the three resistance 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 2000bp were present only on the electrophoregram of the three susceptible mutants (RT-6- RT-8 and RT-9) and their parent

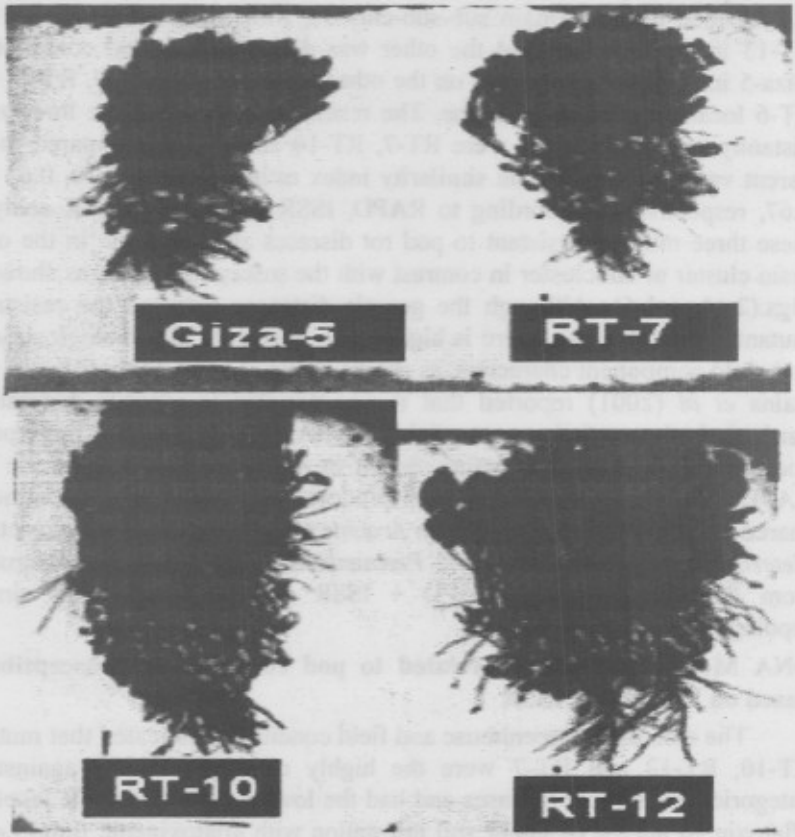


Fig. 6. The morphological variation between the parent variety Giza-5 and mutants; RT-7, RT-10 and RT-12, at Nubaria district, El- Behara governorate, season 2005.

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

variety (Giza-5) and were not observed for other mutants with the same primer; these fragments could be considered as positive markers for susceptibility. 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 1000bp which were present only in the DNA fragments of the three susceptible mutants (RT-6- RT-8 and RT-9) and their parent variety (Giza-5) and were not observed for other mutants with the same primer. A band of 1000 bp was generated on the electrophoregram of all peanut mutants and their parent variety (Giza-5) 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 their parent variety (Giza-5) 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 and to generate molecular genetic markers for pod rot resistance/susceptibility in some peanut mutants and their parent variety. 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 (genome coverage) 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 could be useful to investigate the genetic diversity among the studied peanut genotypes. 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 variety 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. 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, when they analyzed genetic diversity of 48 sweetpotato landraces in China using RAPD (30 primers), ISSR (14 primers) and AFLP (9 primers) markers which generated 227, 249

and 260 polymorphic bands, respectively. Our results revealed that several molecular genetic markers that may be related to pod rot resistance/susceptibility in peanut mutants and Giza-5 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 susceptiblility to TSWV and/or leaf spots in peanut. Guo *et al* (2005) in U.S.A. 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).

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البصمة الوراثية والتوصيف لبعض طفرات الفول السوداني والكشف عن معلمات جزيئية مرتبطة بالمقاومة لأمراض أعفان الثمار و التلوث بالافلاتوكسينات باستخدام ISSR و RAPD

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تم تقييم عشرة طفرات فول سوداني هم RT-6, RT-7, RT-8, RT-9, RT-10, RT-11, RT-12, RT-13, RT-14 and RT-15 بالإضافة إلى الصنف الأب (جيزة 5) لمقاومتهم للمسببات المرضية المسببة لاعفان الثمار ، ولإصابتهم بالفطريات المفرزة للافلاتوكسين بالإضافة للتلوث بالافلاتوكسين، تحت ظروف العدوى والحقل ، في النوبارية – محافظة البحيرة، في الموسمين الصيفيين 2004، 2005 .

أظهرت طفرات 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 العدوى والحقل مقارنة بالصنف الأب (جيزة 5). كانت الطفرات RT-10, RT-12 and RT-7 هي الأكثر مقاومة لأمراض أعفان الثمار و الأقل تلوثا أوالخالية تماما من التلوث بالافلاتوكسينات B1 and B2 تحت ظروف العدوى الصناعية بالفطريات المفرزة منفردة أو مجتمعة أو تحت ظروف العدوى الطبيعية بالحقل.

تحت ظروف الحقل ، تزايد محصول الثمار للمتر المربع معنويا فى الطفرات العشر من الفول السوداني الناتجة من التشعيع بأشعة جاما مقارنة بالصنف الأب (جيزة 5). باستثناء الطفرة RT-15 والتى تعادلت معه. زاد وزن الـــ 100 ثمرة معنويا فى الطفرات RT-7, RT-9, RT-10 and RT-11 بينما تناقصت معنويا فى الطفرات RT-14 and RT-15 مقارنة بالصنف الأب (جيزة 5). زادت نسبة الثمار ذات القطر الاكبر من 14 مم والتى لاتمر من فتحات الغربال ذات الاقطار 36/64 زيادة معنوية فى الطفرتين RT-8 and RT-11 فى حين لم تتغير معنويا نسبة البذور الكلية الناضجة عن الصنف الأب (جيزة 5). وعلى العكس، زادت معنويا نسبة البذور الكبيرة (أكبر من 8 مم) فى الطفرات RT-11, RT-13 and RT-14. نقصت نسبة الزيت معنويا فى الطفرات العشر من الفول السوداني مقارنة بالصنف الأب (جيزة 5).

تم استخلاص الحمض النووي DNA من أوراق الطفرات العشر من الفول السوداني والصنف الأب (جيزة 5)، والتي لم تتعرض للعدوى لإيجاد وإسمات وراثية جزيئية على مستوي الحمض النووي DNA لتمييز المقاومة أو القابلية للإصابة. استخدمت طريقتين للتضاعف العشوائي للحمض النووي هما RAPDوISSR . حيث تم استخدام ثلاثة عشر بادئا عشوائيا ، نجحت عشرة منها في إجراء تضاعف لقطع من الحمض النووي DNA، وأعطت اثنتان منها شظايا أحادية المظهر بينما نجحت الثمانية بادئات الأخرى 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-A9,

(OP-B10, OP-C7, OP-V6) وجود بعض الشظايا من الحمض النووي DNA والتي ارتبطت إما إيجابا أو سلبا مع صفة المقاومة أو الإصابة بالمرض.وبالنسبة لبادئات ISSR ، فقد تم استخدام أثنى عشر بادئا، نجحت ثمانية منها في إجراء تضاعف للقطع من الحمض النووي DNA، وأعطت أربع منها شظايا أحادية المظهر بينما نجحت الأربع بادئات الأخرى في مضاعفة أجزاء متعددة المظهر. وقد أمكن إجراء تعريف وراثي للطفرات المختبرة عن طريق التباينات المظهرية الناتجة من التضاعف العشوائي لأجزاء من الحمض النووي DNA لكل صنف تحت الاختبار باستخدام البادئات (I-18, 814.4, 844-B and UBC-830)، ولم يكتشف وجود أى شظايا من الحمض النووي DNA والتي ارتبطت إما إيجابا أو سلبا مع صفة المقاومة أو الإصابة بالمرض. وقد تم حساب التباعد الوراثي بين الطفرات والصنف الأب والطفرات وبعضها وتم رسم الشجرة التطورية (tree consensus)، والتي أظهرت ان الطفرات المقاومة لامراض أعفان الثمار والخالية من التلوث بالأفلاتوكسين تقع فى نفس المجموعة دائما مع تحليل كل من RAPD و ISSR منفردين ومجتمعين.