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MOLECULAR GENETICS CHARACTERIZATION OF SOME GAMMA RAYS PEANUT MUTANTS

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ABSTRACT

Local peanut cultivars; Giza 4 and Giza 5 were exposed to different doses (50, 100, 150, 200, 250 and 300 Gy) of Co^{60} gamma rays source. All irradiated materials were cultivated to give the M_1 and M_2 generations. Then, high yielding mutants were selected from the M_2 generation and cultivated separately to give the M_3 generation. Some yielding component traits were measured for the three generations (M_1, M_2 and M_3) and M_3 families. The M_3 selected mutants (highest yielding mutants and lowest yielding mutants) were artificially infected by *Aspergillus flavus* L spores (Aflatoxins - B1 and B2 groups - producer strain). Aflatoxin concentrations were estimated and the analysis showed that the aflatoxin concentrations in seeds of the M_3 selected mutants from Giza 4 cultivar were higher than those in seeds of the M_3 mutants selected from Giza 5 cultivar. However, the concentration of aflatoxin was increased when the yield (weight of seeds per plant trait) increased. RAPD-PCR analysis using primers A19, A20, B01, B08, C13 and C18 were successfully differentiated these mutants from their controls and showed high polymorphism among the M_3 families.

Key-words: peanut, *Arachis Hypogaea* L., RAPD-PCR, yield component traits, mutants, gamma rays, irradiation, fungs, *Aspergillus flavus* L., aflatoxins.

INTRODUCTION

Peanut (Arachis hypogaea L.) is one of most oil crops all over the world which cultivated on a large scale. The seeds contain more than 40% oil and about 25-30% protein. The world's largest producers of peanut are India (nearly 35% of world production), China and USA (Weiss, 2000).

In Egypt, Peanut is grown mainly for direct consumption and export but the local cultivars low production comparing with the foreign cultivars and sensitive to infection by fungus such as Aspergillus flavus L. which produce aflatoxins in seeds, these aflatoxins are very harmful for human and animal. So the ultimate aim of peanut breeding programs is to develop cultivars with high yielding potential and lower sensitivity to infection by fungus.

Aflatoxin contamination in peanut is due to Aspergillus flavus L. infection. Aflatoxins are an acutely toxic, carcinogenic and immuno suppressive class of mycotoxins. Aflatoxin B1 is the most potent and carcinogenic naturally substance, it causes liver damage to most domestic and experimental animals and humans (Diener et al., 1987). Aspergillus flavus invade the lowers, travel down the pegs and become established in the developing seeds (Styer et al., 1983).

Difficulties of traditional breeding programs in peanut led to use mutation induction as alternative technique. More than 265 grain legume cultivars developed using induced mutations in 32 countries, 44 peanut cultivars were developed (Bhatia et al., 2001).

Improvement of peanut through induced gamma irradiation mutants has been investigated (Kale et al., 1997 and Qiu et al., 1997). Many researcher successes to obtain useful mutations, Venkatachalam et al. (1999) induced seven types of chlorophyll mutants (albino, xantha, chlorina, viridis, alboviridis, xanthoviridis and maculate) using gamma rays, ethyl methanesulfonate and sodium azide. Branch (2002) induced larg-seeded 'Georgia Browne' mutant breeding lines using gamma irradiation. Gowda et al. (2002) induced late leaf spot resistant mutant form Spanish genotype using ethyl methanesulfonate.

Molecular markers, in general have proven to be very useful for crop improvements and studies of crop evolution in many species (Mohan et al., 1997). In peanut, including randomly amplified polymorphic DNA (RAPD) have been used of introgression from wild

crosses (Garcia el al., 1995) and for measuring genetic variations in wild species (Stalker et al., 1994).

The main objectives of the present study are selecting new mutants that have high yielding production and studying them for infection by Aspergillus flavus L. and aflatoxins content. Then, use molecular technique to study the polymorphism among these mutants.

MATERIALS AND METHODS

M_1 generation

Dry seeds of two Egyptian peanut cultivars (Giza 4 and Giza 5) obtained from Oil Crop Research Department, Field Crop Research Institute (ECRD), Agriculture Research Center (ARC) were irradiated with gamma ray doses (50, 100, 150, 200, 250 and 300 Gy) obtained from Co^{60} source at NRC, EAEA Inshas, Egypt. Treated seeds with untreated seeds (control) were sown (500 seeds for each) immediately after irradiation in a complete randomized block design with three replicates to give the M_1 generation. Random samples of 30 individual plants (10 plants for each replicate) were used to measure the following traits: plant height (cm), number of branches/plant, number of pods/plant, weight of pods/plant (g), number of seeds/plant, weight of seeds/plant (g), shelling (%), weight of 100-seeds (g), protein content percentage (%) and oil content percentage (%).

M_2 generation

Ten random plants were taken from the M_1 generation from each plot (replicate). The seeds of these 30 plants for each treatment were bulked and sown in a complete randomized block design with three replicates to give the M_2 generation. These random samples of 30 individual plants (10 plants for each replicate) were used to measure the same previous traits in the M_1 generation.

M_3 generation

Ten random plants were taken from the M_2 generation from each plot (replicate). Then seeds of these 30 plants for each treatment were bulked and sown in a complete randomized block design with three replicates to give the M_3 generation. These random samples of 30 individual plants (ten plants for each replicate) were used to measure the same mentioned traits.

M3 selected mutants

Three segregates of each cultivar were selected from the M_2 generation plants based on the highest yielding component traits (1, 2 and 3 for Giza4 cultivar and 4, 5 and 6 for Giza5) to give the M_3 highest selected mutant plants. In addition, three segregates of each cultivar were selected based on the lowest yielding component traits (1ˇ, 2ˇ and 3ˇ for Giza4 cultivar and 4ˇ, 5ˇ and 6ˇ for Giza5) to give the M_3 lowest selected mutant plants. Random samples of 30 individual plants for each high and low mutant (ten plants for each replicate) were used to measure the same mentioned traits.

The data of M_1, M_2 and M_3 generations and M_3 selected mutant plants were statistically analyzed according to Gomes and Gomez (1984). Duncan's test was used to verify the validity of the differences between means. Estimation of seed protein and oil content for M1, M2 and M3 generations was performed by Instalab 600 Near InfraRed Product Analyzer.

Artificial infection and estimating of aflatoxin concentration

The seeds of selected mutant plants of the two cultivars were exposed to *Aspergillus flavus* L. suspension (6 spore/ml), then incubated in Potato Dextrose Agar media (PDA) in 26˚ C for two weeks. Aflatoxins (B1 and B2 groups) producer strain of *Aspergillus falvus* Link, anamorph was obtained from Egyptian Microbial Collection, MIRCN, Cairo, Egypt. B1 aflatoxin concentration was estimated by Aflatoxin AOAC fluorometer procedure (Truckess et al., 1991)

DNA extraction and RAPD-PCR analysis

Ten seeds from each highest and lowest M3 mutated plants obtained from Giza 4, Giza 5 cultivars and the seeds of their controls were germinated for two weeks. Total genomic DNA was extracted according to the method of Bushra et al. (1999). PCR reactions were conducted using six arbitrary ten mer primers with the following sequences: A19: 5ˇCAAACGTCGG3ˇ, A20: 5ˇGTTGCGATCC3ˊ, B01: 5ˊ GTTTCGCTCC3ˊ, B08: 5ˊ GTCCACACGG 3ˊ , C13: 5ˊ AAGCCTCGTC3ˊ and C18 5ˊ TGAGTGGGTG 3ˊ . PCR technique was performed in 30µl volumes tubes according to Williams et al. (1990) which containing the following: 3.0µl DNTPs (2.5 mM), 3.0µl MgCl2 (25 mM), 3.0µl Buffer (10 x), 2.0µl Primer (10 pmol), 0.2µl

Taq DNA polymerase, 2.0µl template DNA (25 ng) and 16.8 µl H2O (d.w). The amplification was carried out in a DNA thermocycler (MWG-BIOTECH Primuse) programmed as follows:

(94°C/4min)1 cycle, (94°C/30sec, 35°C/1min, 72°C/2min)40 cycles, (70°C/5min)1 cycle

Agarose gel (1.5%) electrophoresis was used for separating the PCR products of DNA fragments. Gels were photographed using a digital camera and scanned with Bio-Rad video densitometer Model 620, at a wave length of 577. Software data analyses for Bio-Rad Model 620 USA densitometer and computer programs were used.

RESULTS AND DISCUSSION

Depending on the highest weight of seeds per plant trait, three mutants were selected from each peanut cultivar (Giza 4 and Giza 5) from the M_2 generation plants. These mutants were grown to give the M_3 generation lines, mutant 1, mutant 2 and mutant 3 from Giza 4 and mutant 4, mutant 5 and mutant 6 from Giza 5. Analysis of variance for means of yielding components traits was used to test the significant changes among each three mutants and their controls. L.S.D test was used to verify the test of significance for trait means.

Means of yielding components traits for the selected M_3 mutants from Giza 4 cultivar are presented in Table (1). No significant changes were observed for means of plant height and number of branches traits but significant changes were obtained for means of number of pods / plant, weight of pods / plant, number of seeds / plant, weight of seed / plant, shelling percentage, 100-seeds weight, oil content % and protein content % traits.

For mutant 1, significant increase for means was observed between mutant 1 and its control for number of pods / plant, weight of pods / plant, weight of seeds / plant and 100-seeds weight traits with less oil content %.

For mutant 2, significant increase for means was observed between mutant 2 and its control for number of pods / plant, weight of pods / plant, number of seeds / plant, weight of seeds / plant, 100-seeds weight and protein content % traits with less oil content %.

For mutant 3, significant increase for means was observed between mutant 3 and its control for number of pods / plant, weight of pods / plant, number of seeds / plant, weight of seeds / plant, shelling percentage, 100-seeds weight traits with less oil content %.

Table (1): Means of studied yield component traits for the selected mutants (1, 2 and 3) form M_3 generation of Giza 4.

Mutants	Plant height (cm)	No. of Branches/ plant	No. of pods/ plant	Weight of pods/ plant	No. of seeds/ plant	Weight of seeds/ plant	Shelling %	Weight of 100-seeds (g)	Protein content %	Oil content %
Control	46.96	3.44	42.78 c	65.35 c	48.38 c	30.48 b	46.91 b	61.59 c	28.22 b	50.51 a
(1)	59.11	3.72	54.05 b	104.83 b	70.61 bc	53.93 a	51.72 b	76.50 ab	27.76 b	39.52 b
(2)	60.00	3.56	68.94 a	129.47 a	82.83 ab	67.95 a	53.29 ab	80.97 a	29.11 a	39.86 b
(3)	58.00	3.49	63.07 ab	112.51 ab	94.51 a	67.05 a	59.95 a	71.05 b	28.25 b	40.27 b
$L.S.D_{0.05}$	N.S	N.S	**	**	*	**	*	**	*	**

(N.S): not significant (*): significant at 0.05 level of probability (**): significant at 0.01 level of probability

Table (2): Means of studied yield component traits for the selected mutant (4, 5 and 6) form M_3 generation of Giza 5.

Mutants	Plant height (cm)	No. of Branches/ plant	No. of pods/ plant	Weight of pods/ plant	No. of seeds/ plant	Weight of seeds/ plant	Shelling %	Weight of 100-seeds (g)	Protein content %	Oil content %
Control	32.69 c	3.20	34.21 b	54.25 c	44.64	33.94 b	61.08	75.22 b	26.57	37.63
(4)	46.94 a	3.50	51.17 a	112.56 a	69.34	63.32 a	56.47	90.95 a	28.28	41.43
(5)	41.96 ab	3.39	35.62 b	71.55 bc	47.88	42.22 ab	56.89	88.84 a	27.08	37.35
(6)	40.07 b	3.34	44.45 ab	93.34 ab	60.79	56.93 a	60.63	94.57 a	27.20	36.83
$L.S.D_{0.05}$	**	N.S	*	*	N.S	*	N.S	*	N.S	N.S

(N.S): not significant (*): significant at 0.05 level of probability (**): significant at 0.01 level of probability

These results were in partial agreement with the results of Sorour (1989) who found no significant variations in means for radiation doses on peanut Giza4 cutivar for plant height, number of branches/plant, number of pods/plant, weight of pods/plant, weight of seeds/plant and weight of 100-seeds in M2 and M3 generations, while he found significant variations in means for radiation doses on the same cultivar for the same traits in M1 generation.

Means of yielding components traits of M3 mutants selected from Giza5 cultivar are presented in Table (2). No significant changes were observed for means of number of branches / plant, number of seeds / plant, shelling percentage, protein content % and oil content % traits but significant changes were observed for means of plant height, number of pods / plant, weight of pods / plant, weight of seeds / plant and weight of 100-seed traits.

For mutant 4, significant increase for means was observed between mutant 4 and its control for plant height, number of pods / plant, weight of pods / plant, weight of seeds / plant and 100-seeds weight traits.

For mutant 5, significant increase for means was observed between mutant 5 and its control for plant height and 100-seeds weight traits.

For mutant 6, significant increase for means was observed between mutant 6 and its control for plant height, weight of pods / plant, weight of seeds / plant, and 100-seeds weight traits.

Under the effect of gamma rays, Giza 5 had the highest values for plant height. There were similar results as obtained by El-shazly et al. (2005). Mean values of oil and protein % of irradiated Giza 4 cultivar surpassed those of Giza 5 cultivars. Similar results were obtained by Kassem and Esawy (2003).

Evaluation of selected mutants for resistance to *Aspergillus flavus* L.

Aflatoxin concentrations in seeds of M_3 selected mutants (based on highest weight of seeds) from Giza 4 cultivar (1, 2 and 3) were higher than the concentrations in seeds of M_3 selected mutants from Giza 5 cultivar (4, 5 and 6). While the Aflatoxin concentrations in seeds of M_3 selected mutants (based on lowest weight of seeds) from Giza4 cultivar (1`, 2` and 3`) and Giza5 (4`, 5` and 6`) were approximately similar. However, in the two cultivars, the

concentrations of aflatoxin increased when the yield was increased as shown in Table (3).

Table (3): Aflatoxin concentrations for the highest yielding (1, 2, 3, 4, 5 and 6) and lowest yielding (1', 2', 3', 4', 5' and 6') plants in M_3 generation of Giza 4 and Giza 5 selected mutants.

Giza 4	afla/gram (µg)	Giza 5	afla/gram (µg)
Control	42.76	Control	18
1	151.11	4	18.75
2	80.19	5	33.23
3	170.21	6	27.24
1'	31.11	4'	41.58
2'	52.71	5'	26.99
3'	188.46	6'	39.06

Aflatoxin concentrations in seeds for the M3 selected mutants from Giza 5 cultivar were 18.00 µg/g for control, 39.5 µg/g for mutant 4, 22.00 µg/g for mutant 5 and 36.00 µg/g for mutant 6. It is clear that there were changes of the mutants which were more sensitive to Aspergillus flavus infection than their controls. Results were obtained by Azer et al (2002) who induced some breeding lines from peanut (Giza 5 cultivar) using gamma rays shwoed more resistant to Aspergillus flavus infection than their controls.

DNA variations induced by gamma rays for peanut cultivars

DNA variations which caused by gamma rays for the peanut cultivars based on RAPD polymorphism for the two cultivars are shown in Table (4).

In Giza 4, the number of total bands decreased in most plants compared with the control, especially in the highest yielding plants in mutants 1 and 3. The lowest yielding plant in mutant 2´ had the highest number of new bands over the control, while the highest yielding plant in mutant 3 showed the highest number of absent bands with regard to the control.

In Giza 5, the number of total bands decreased in most plants compared with the control, especially in the lowest yielding plants in mutant 5´ and 6´. The highest yielding plants in mutant 5 and 6 showed the highest number of new bands over the control, while lowest

yielding plant in mutant 5´ exhibited the highest number of absent bands with regard to the control.

Percentages of new bands over the control in the two cultivars nearly equal to each other (14.28 % in Giza 4 and 14.81 % in Giza 5) while, the percentages of absent bands with regard to the control were closer to each other in the two cultivars (44.72 % in Giza 4 and 36.80 % in Giza 5).

Table (4): DNA fragment variations based on RAPD polymorphism using six primers which caused by gamma rays for the two peanut cultivars (Giza 4 and Giza 5).

Cultivars	Mutants	No. of total bands	No. of new bands over the control	No. of absent bands with regard to the control
Giza 4	C	30	-	-
	1	**16**	3	17
	2	20	2	12
	3	**17**	5	**18**
	1'	22	3	11
	2'	31	7	6
	3'	25	3	8
	Total	161	23	72
	Polymorphism %	-	14.28 %	44.72 %
Giza 5	C	28	-	-
	4	24	4	8
	5	25	**6**	9
	6	23	**6**	11
	4'	22	2	8
	5'	**20**	4	**12**
	6'	**20**	2	10
	Total	162	24	58
	Polymorphism %	-	14.81 %	35.80 %

RAPD-PCR for Giza 4 selected mutants

The results of RAPD analysis using A19, A20, B01, B08, C13 and C18 primers with Giza 4 are illustrated in Figure (1). All primers gave polymorphisms between the highest and lowest plants (based on weight of seeds / plant) for M_3 selected mutants and varied from their control as shown in Table (5). Primer A19 produced a positive unique DNA fragment for mutant 1, 1` (highest and lowest) with a fragment size of 100 bp and another one for the lowest plant in mutant 2` with a fragment size of 250 bp. Primer B01 produced two positive unique

DNA fragments for the lowest plant in mutant 2` with fragments sizes of 300 and 500 bp. Dendrogram tree for Giza 4 and its mutants are shown in Figure (3). The lowest plants for the three mutants were distant from their control.

Table (5): Polymorphism generated by primers A19, A20, B01, B08, C13 and C18 for peanunt cultivar Giza 4 (control) compared with the highest yielding (1,2 and 3) and lowest yielding (1', 2' and 3') mutants.

Primers	M.S (bp)	Giza4	Highest mutant			Lowest mutant		
		Control	1	2	3	1'	2'	3'
	950	0	1	1	1	1	0	1
	800	1	1	1	1	1	1	1
	700	1	0	0	0	1	1	1
A19	500	1	0	1	0	1	1	1
	250	0	0	0	0	0	1	0
	150	0	0	1	1	0	1	0
	100	0	1	0	0	1	0	0
	900	1	0	0	0	0	0	1
	650	1	1	1	1	1	1	1
	500	1	1	1	1	1	1	1
A20	250	1	0	0	0	1	0	0
	200	1	0	0	1	0	1	1
	50	1	0	0	0	0	0	0
	550	1	0	0	0	1	0	1
	500	0	0	0	0	0	1	0
B01	350	1	0	1	0	1	1	1
	300	0	0	0	0	0	1	0
	170	1	1	0	1	0	1	0
	700	0	0	0	1	1	1	0
	500	1	0	0	1	1	1	1
	400	1	1	1	0	0	1	1
B08	300	1	1	1	0	1	1	0
	200	1	0	1	0	1	1	0
	100	1	0	1	1	1	1	1
	75	0	1	0	1	1	1	1
	50	1	1	1	1	1	1	1
	1500	1	1	1	1	1	1	1
	900	1	0	1	0	0	0	0
	600	1	0	1	0	1	1	0
C13	400	1	0	1	0	1	1	1
	250	1	0	0	0	0	1	1
	150	1	1	1	1	1	1	1
	50	1	1	1	1	1	1	1
	900	1	1	0	0	1	1	1
	650	1	0	0	0	0	1	1
C18	400	1	1	1	0	0	1	1
	300	1	1	1	0	0	1	1
	200	0	0	0	1	1	1	1
	100	1	0	0	1	0	0	0

RAPD bands presence : (1) RAPD bands absence: (0)

RAPD-PCR for Giza 5 selected mutants

The results of RAPD analysis using A19, A20, B08, 8C13 and C18 primers with Giza 5 are illustrated in Figure (2). All primers gave polymorphisms between the highest and lowest plants (based on weight of seed / plant) for M_3 selected mutants and varied from their control as shown in Table (6). Primer A19 produced a negative unique DNA fragment for the highest plant in mutant 6 with fragment size of 450 bp. Primer A20 produced a negative unique DNA fragment for the lowest plant in mutant 5 with a fragment size of 500 bp and another one for the lowest plant in mutant 6 with a fragment size 700bp. Primer B08 produced three negative unique DNA fragments; one for the highest plant in mutant 4 with a fragment size of 70 bp, one for the highest plant in mutant 6 with a fragment size of 650 bp and the last one for the lowest plant in mutant 5` with a fragment size of 350 bp. Primer C13 produced a negative DNA fragment for the lowest plant in mutant 6` with a fragment size of 1300bp.

Dendrogram tree for Giza 5 and its mutants are shown in Figure (4). Mutant 4, 4` (lowest and highest) and its control were related to each other, while the highest plants in mutant 5 and 6 were distant from the lowest plants in mutant 5` and 6`.

RAPD-PCR is a useful technique in band characterization for detection gamma ray effect on DNA level in peanut and the assessment of genetic diversity among groundnut germplasms. Dwivedi et al. (2001) found about 18.74 % of polymorphism among selected peanut accessions using RAPD-PCR technique. Massawe et al. (2003) found high level of polymorphism among bambara peanut landraces using RAPD-PCR analysis.

Gamma irradiation was successful in creating variability within peanut plants for inducing and developing variable and desirable advanced mutant breeding lines within peanut cultivars.

Table (6): Polymorphism generated by Primers A19, A20, B01, B08, C13 and C18 for peanut cultivar Giza 5 (control) compared with the highest yielding (4,5 and 6) and lowest yielding (4', 5' and 6') mutants.

Primers	M.W (bp)	Giza5						
		Highest mutant				Lowest mutant		
		Control	4	5	6	4'	5'	6'
A19	1500	0	0	1	1	1	0	0
	1200	1	0	1	1	0	0	1
	900	1	1	1	1	1	1	1
	850	1	0	0	1	1	0	0
	650	1	0	0	0	0	1	0
	500	1	1	1	0	1	0	1
	450	1	1	1	0	1	1	1
	400	0	0	1	1	1	0	0
	350	1	1	0	0	0	0	0
A20	850	1	1	0	0	1	0	0
	700	1	1	1	1	1	1	0
	500	1	1	1	1	1	0	1
	300	1	0	0	1	0	0	0
	200	1	0	1	1	1	0	1
B08	800	1	1	1	0	0	0	1
	650	1	1	1	0	1	1	1
	500	1	1	1	0	1	0	0
	350	1	1	1	1	1	0	1
	200	1	1	0	1	0	1	1
	100	1	1	1	1	1	1	1
	80	1	1	1	1	1	1	1
	70	1	0	1	1	1	1	1
	50	0	1	1	1	0	0	1
C13	1700	0	0	0	0	0	1	0
	1300	1	1	1	1	1	1	0
	700	1	1	1	0	0	1	0
	500	1	1	1	1	1	1	1
	400	1	0	0	1	0	0	0
	300	1	0	1	1	1	0	0
	150	1	1	1	1	1	1	1
C18	1000	0	1	1	1	0	1	1
	750	0	1	1	1	0	1	0
	500	0	1	1	1	0	1	0
	300	1	1	0	0	0	1	1
	100	1	1	0	0	1	1	1

RAPD bands presence : (1) RAPD bands absence: (0)

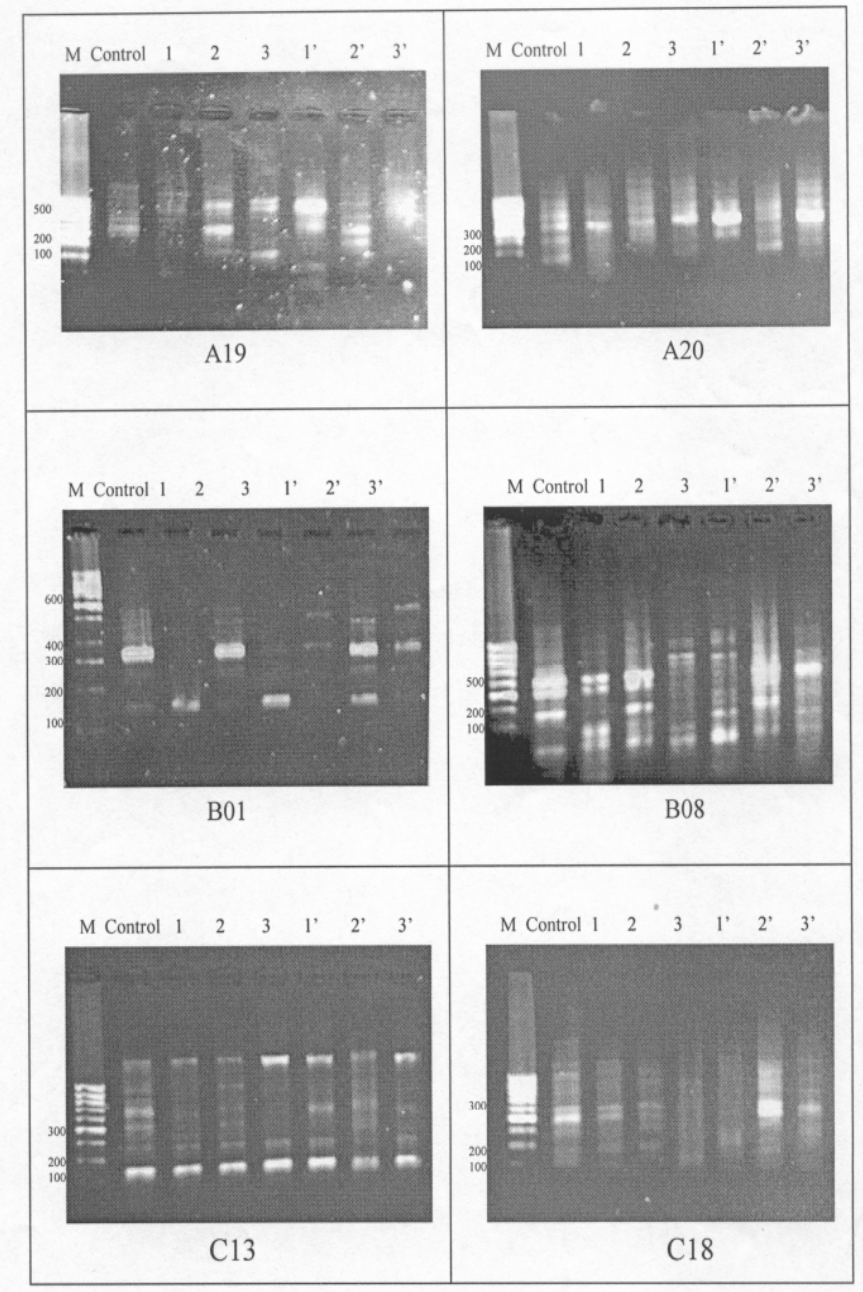

Figure (1): DNA polymorphism generated by A19, A20, B01, B08, C13 and C18 primers for Giza 4 (control) which compared with its highest yield (1,2 and 3) and lowest yield (1', 2' and 3') mutants.

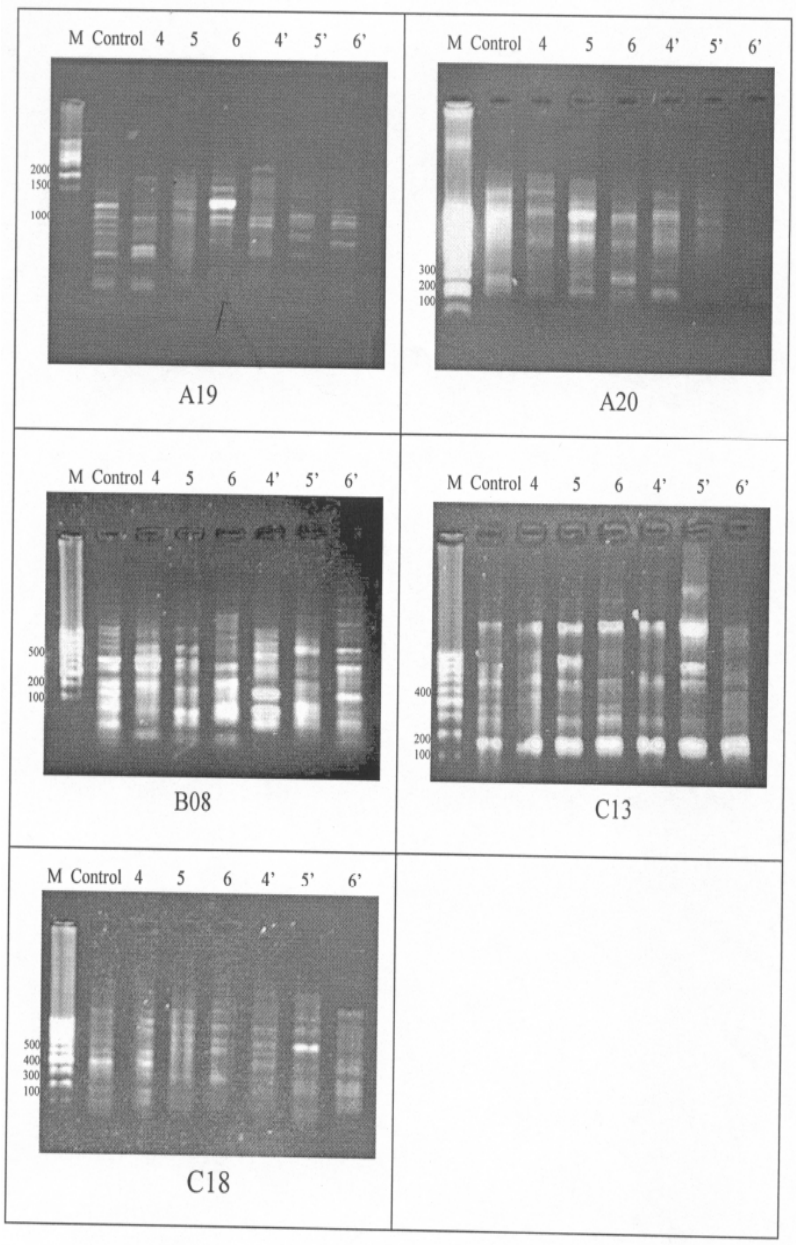

Figure (1): DNA polymorphism generated by A19, A20, B01, C13 and C18 primers for Giza 5 (control) which compared with its highest yield (1,2 and 3) and lowest yield (1', 2' and 3') mutants.

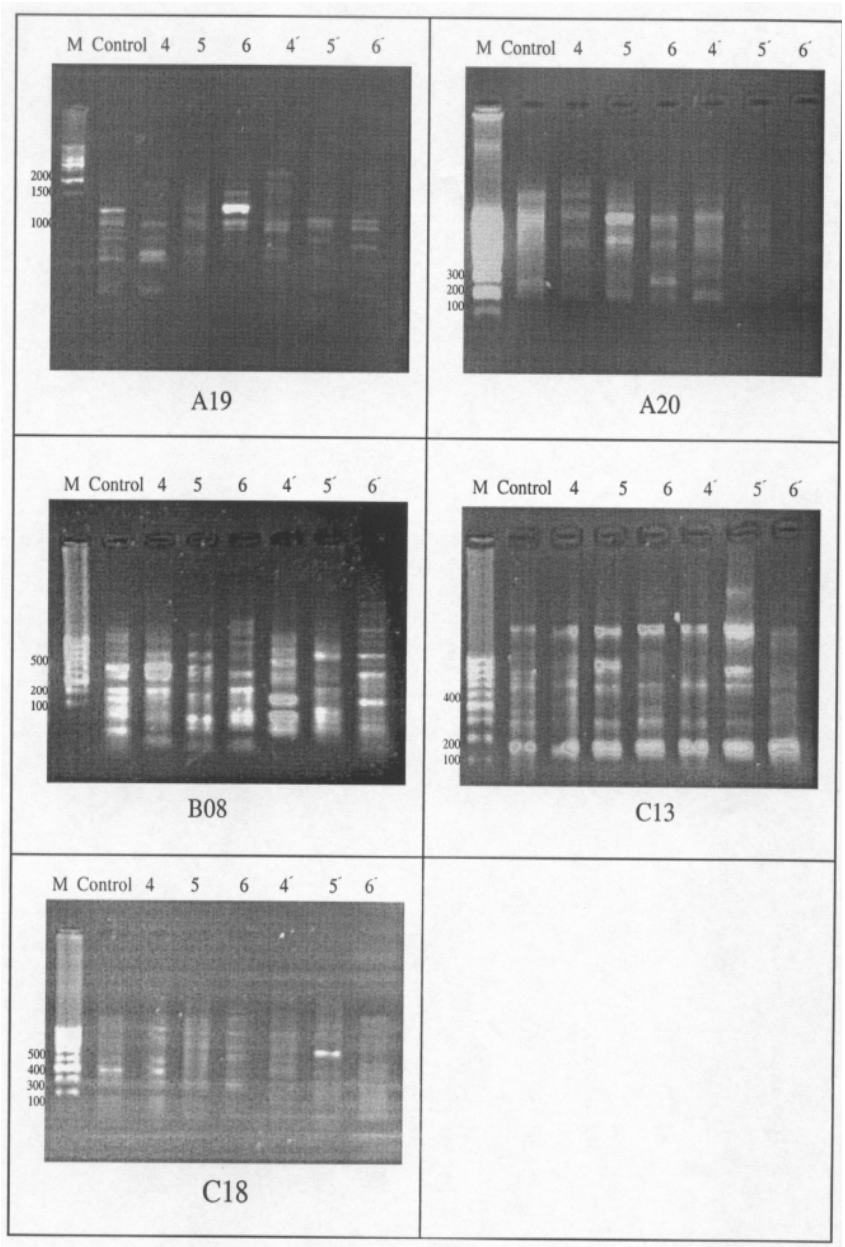

Figure (2): DNA polymorphism generated by A19, A20, B01, C13 and C18 primers with Giza 5 (control). The columns represent the highest yield (4, 5 and 6) and the lowest yield (4´, 5´ and 6´) mutants.

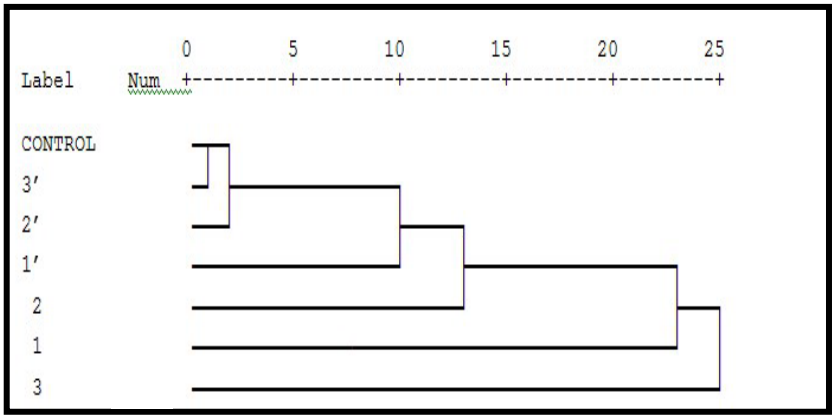

Figure (3): Dendrogram tree based on similarity indices for Giza 4 (control) compared with its highest yield (1, 2 and 3) and lowest yield (1', 2' and 3') mutants.

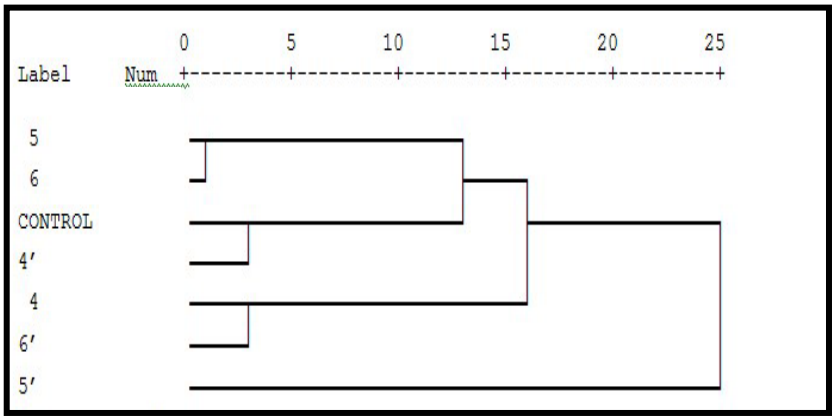

Figure (4): Dendrogram tree based on similarity indices for Giza 5 (control) compared with its highest yield (4, 5 and 6) and lowest yield (4', 5' and 6') mutants.

REFERENCES

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

Bhatia, C. R., M. Maluszunski, K. Nichtherein and L. Vanzanten (2001). Grain legume cultivars derived from induced mutations, and mutations affecting nodulation. Mutation Breeding Review, 13: 44.

Branch, W. D. (2002). Variability among advanced gamma-irradiation induced large-seeded mutant breeding lines in the 'Georgia Browne' peanut cultivar. Plant Breeding, 121(3): 275-277.

Bushra, C., A. Yasmeen, T. Husnain and S. Riazuddin (1999).Mini-scale Genomic DNA Extraction from Cotton. Plant Molecular Biology Reporter, 17: 1-7.

Diener, U. L., R. J. Cole, T. H. Sanders, G. A. Payne, L. S. Lee and M. A. Klich (1987). Epidemiology of Aflatoxin formation by Aspergillus flavus. Annu, Rev. Phytopathol, 14: 249-270.

Dwivedi, S. L., S. Gurtu, S. Chandra, W. Yuejin and S. N. Nigam (2001). Assessment of genetic diversity among selected groundnut germplasm. I: RAPD analysis. Plant Breeding, 120: 345-349.

El-shazly, M. S., A. A. Aly, M. A. El-Ashry, S. El. M. M. Ammar and M. S. H. Salamam (2005). Induction of genetic variability in peanut using gamma rays and sodium azide. Journal of plant Breed 9(1): 171-183 special issue.

Garcia, G. M., H. T. Stalker and G. Kochert (1995). Introgression analysis of an interspecific hybrid population in peanuts (Arachis hypogaea L.) using RFLP and RAPD markers. Genome, 38: 166-176.

Gomes, K. A. and A. A. Gomez (1984). Statistical procedure for agricultural research, 2nd edition (Wily Interscience Publication) John Wily and Sons Inc. New York.

Gowda, M. V. C., B. N. Motagi, R. Sheshagiri, G. K. Naidu and M. N. Rajendraprasad (2002). Mutant 28-2: a bold-seeded disease and pest resistant groundnut genotype for Karnataka, India. International Arachis Newsletter, 22: 32-34.

Kale, D. M., C. Mouli, G. S. S. Murty and M. V. P. Rao (1997). Development of a new groundnut variety "TG-26" by using

induced mutants in cross breeding. Mutation Breeding Newsletter, 43: 25.

Kassem, M. and W. A. Esawy (2003). Changes in growth and yield characters and in genetic variation of Peanut (Arachis hypogaea L.) plants due to gamma ray irradiation. Arab Journal of Nuclear Science and Applications 36(2): 311-324.

Massawe, F. J., J. A. Roberts, S. N. Azam-Ali and M. R. Davey (2003). Genetic diversity in bambara groundnut (Vigna subterranean (L.) Verdc) landraces assessed by Random Amplified polymorphic DNA (RAPD) markers. Genetic Resources and Crop Evolution, 50(7): 737-741.

Mohan, M.; S. Nair, A. Bhagwat, T. Gl. Krshna, M. Yano, C. R. Bhatia and T. Saski (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed., 3: 67-103.

Qiu, Q., Z. Li, F. Shen, C. Wang and H. Miao (1997). Peanut breeding through mutation techniques in China. Mutation Breeding Newsletter, 43: 6.

Venkatachalam, P., N. Geetha and N. Jayabalan (1999). Frequency and spectrum of chlorophyll mutations in groundnut (Arachis hypogaea L.). Journal of Botany, 28 (1): 17-25.

Sorour, W. A. I. (1989). Genetic studies in peanut (Arachis hypogaea L.) developing genetically improved mutants through mutation induction. Unpublished Ph. D. Thesis, Faculty of Agriculture, Cairo University, Giza, Egypt.

Stalker, H. T., T. D. Phillips, J. P. Murphy and T. M. Jones (1994). Variation of isozyme patterns among Arachis species. Theor. Appl. Genet., 87: 746-755.

Styer, C. H., R. J. Cloe and R. A. Hill (1983). Inoculation and infection of peanut flowers by Aspergillus flavus. Proceedings of the American Peanut Research and Education Society, 15: 91.

Truckess, M. W., M. E. Stack, S. Nesheim, S. W. Page, R. H. Albert, T. J. Hansen and K. F. Donahue (1991). Immunoaffinity column coupled with solution fluorometry or liquid chromatography post column derivatization for determination of aflatoxins in corn, peanuts and peanut butter. Journal of the Association of the Official Analytical Chemistry, 74(1): 81-88.

Weiss, E. A. (2000). Oilseed crops (second edition), chapter three, Blackwell Science, (In edt.) pp. 53-92.

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

التصنيف الوراثى الجزيئى لبعض طفرات أشعة جاما فى الفول السودانى

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عرضت الأصناف المحلية جيزة 4 وجيزة 5 من الفول السودانى لجرعات مختلفة (100و 150و 200و 250و 300 جراى) لمصدر أشعة جاما. تم زراعة البذور المشعة لتعطى الجيل الطافر الأول ثم لتعطى الجيل الطافر الثانى. أنتخبت الطفرات العالية فى صفات المحصول من الجيل الطافر الثانى وزرعت لتعطى الجيل الطافر الثالث. تم قياس صفات مكونات المحصول فى الثلاث أجيال. عرضت بذور النباتات المنتخبة (الطفرات ذات المحصول العالى والطفرات ذات المحصول المنخفض) لعدوى صناعية بجراثيم فطر الأسبرجلس فلافوس (سلالة ب1 وب2) وتم قياس تركيز التوكسينات فى بذور الطفرات المنتخبة فى الجيل الثالث لكلا الصنفين من الفول السودانى لتظهر أن الصنف جيزة 4 يحتوى على تركيز أعلى من الصنف جيزة 5. وأزدادت تركيزات التوكسينات بزيادة المحصول (وزن الحبوب لكل نبات).

بأستخدام تفاعل التضخم العشوائى لجزيئات DNA بواسطة بصمات جزيئية وهى: A19, A20, B01, B08, C13, C18 كانت تميز بنجاح بين أختلاف الطفرات والكنترول وأظهرت وجود أختلافات بين الصنفين.