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Biochemical Markers Associated with Disease Resistance to Damping-Off and Root-Rot Diseases of Peanut Mutants and their Productivity

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Ten mutants of peanut and their parental variety (Giza-5) were screened against damping off and root-rot diseases. Under greenhouse conditions (soil infested with *Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina* and *Fusarium solani* each alone or in mixture), the parental variety Giza-5 was the most susceptible to these fungi and produced the lowest percentage of healthy survived plants whereas the mutants RT-10, RT-11 and RT-7 were the most resistant mutants against both diseases and gave the highest percentage of healthy plants. Similar trend was observed under field conditions as the mutants RT-10, RT-11, RT-7 and RT-12 were the most resistant mutants whereas RT-6, RT-7 and RT-8 were the superior mutants for plant growth, yield and yield components comparing with the parent variety Giza-5 in two successive seasons. Activities of oxidative enzymes, the total and free phenols as well as total and reducing sugars were mostly higher in healthy plants than in infected ones and in the resistant mutants than the susceptible ones. Meanwhile the total free amino acid was lower in healthy and infected roots of resistant mutants in comparison with susceptible ones.

Studying the SDS-protein banding patterns of the ten peanut mutants leaves (as a result of gamma irradiation) and their parental variety grown under normal (non-stressed) and disease stress conditions was found to be useful in selection for resistance against damping-off and root-rot diseases. The obtained results revealed no resemblance between any mutant and its parental variety and a unique fingerprint characterized each.

Key words: Amino acids, damping off, disease resistant, mutants, oxidative enzyme activities, peanut, phenolic contents, protein electrophoresis, sugar contents and root-rot.

Peanut (*Arachis hypogaea* L.) is one of the most important leguminous and oil crops in Egypt and in many parts of the world. Damping-off and root-rot diseases (*Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina* and *Fusarium* spp.) are among the most destructive diseases, which attack peanut plants (Abdel-Momen and Starr, 1998) causing quantitative and qualitative losses of yield. Reaction of peanut varieties and strains as well as, their yield components were affected differently by damping-off and root- rot infections (Hilal *et al.*, 1994). Amounts of peanut losses could be minimized by using resistant varieties

(Hilal *et al.*, 1994) or through induced gamma-irradiation mutants (Venkatachalam *et al.*1999; Azer *et al.*, 2002). Gamma irradiation has been used for improving plant characters and increasing genetic variability in peanut species (Sorour *et al.*,1999; Azer *et al.*, 2002 and Azzam and El-Sawy, 2005).

The post infection resistance (active resistance) might be correlated with specific biochemical changes in phenols, sugars, amino acids, phytoalexin accumulation, lignifications and activation of oxidative enzymes in host plant (Metraux and Raskin, 1992). Also, the accumulation of host synthesized new polypeptides is associated with the disease resistance. (Broglie *et al.*, 1986).The new protein contents depended on host genotype and virulence genes of the pathogens (Hlinkova and Sykora,1996).

Biochemical genetic markers offer specific advantage in assessment of genetic diversity and trait-specific crop improvement. Such markers can facilitate appropriate choice of parents for crosses to mapping/tagging of gene blocks associated to economically important traits and in turn permits marker-assisted selection (MAS) in backcross, pedigree and population improvement programs (Mohan *et al.*, 1997). Azzam and El-Sawy (2005) studied the protein banding patterns of seed storage proteins in peanut and indicated that differences caused by gamma ray irradiation were due to real genetic structure changes. They added that, the densitometric analysis of the SDS-protein banding patterns was found to be useful in identifying the induction of variations in the irradiated populations. The similarity index indicated that Giza 6 and R 92 are more sensitive genotypes to irradiation with gamma rays than Giza 4. It is important to determine the suitable dose, which induces the needed variation to start a breeding program that depends on inducing mutations.

The present work aimed to evaluate the behaviour of some peanut mutants under artificial and natural infection for damping-off and root-rot pathogens and their yield components, and to determinate specific biochemical changes in resistant and susceptible mutants comparison with the parent variety (Giza-5) and to find out biochemical genetic markers for resistance.

Materials and Methods

Greenhouse evaluation:

Ten entries of peanut (Table 1 a & b) selected from previously mutant generations (Azer *et al.*, 2002) in addition to the parent variety (Giza-5) were evaluated for their reaction against *Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina* and *Fusarium solani* in the M5 generation during season 2003. Glass bottles containing autoclaved sorghum-coarse sand- water (2:1:2 v/v/v) medium were inoculated by a given fungus using agar discs taken from the periphery of its 5 days old colony, incubated at 26°C for two weeks. The inocula were used either separately or in mixture at the rate of 2% w/w, to infest sterilized potted soil, mixed thoroughly with the soil, then watered and lift for one week before sowing. Apparently healthy seeds of peanut entries were surface disinfested by sodium hypochlorite solution (3%) for 2 min then sown at the rate of 5seeds/pot

(30 cm diam.). Three pots were used for each particular treatment. Pots containing sterile non-infested soil were used as control. Percentages of pre- and post-emergence damping-off were calculated after 15 and 60 days from sowing, respectively while percentages of plants having root- rot symptoms and survived healthy plants were estimated after uprooting (120 days from sowing). Disease estimation in each stage was calculated based on number of seeds that were sown per each pot.

Biochemical changes associated with the resistant and susceptible peanut entries:

Root samples were taken from healthy and infected peanut plants (30 days old) that grown in soil infested with the mixture of the tested fungi to investigate the following biochemical changes. The root samples were extracted according to Goldschmidt *et al.* (1968), then activities of the oxidative enzymes, *i.e.* peroxidase (PO); polyphenoloxidase (PPO) and catalase (CAT) were determined as described by Allam and Hollis (1972); Matta and Dimond (1963) and Maxwell and Bateman (1967) and assayed using a spectrophotometer at 425, 495 and 240nm., respectively. The reaction substrate of each oxidative enzyme was pyrogallol, catechol and H_2O_2 for determining activity of peroxidase; polyphenoloxidase and catalase, respectively. Another root samples were extracted in soxhlet units using 75% ethanol for 10-12 hrs then used to determine phenolic compounds; sugars content and total free amino acids as described by A.O.A.C. (1980); A.O.A.C. (1975) and Moore and Stein (1954), respectively and calculated as milligrams equivalent of catechol, glucose and argenin /1 g fresh weight of peanut roots, respectively.

Sodium Dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

For direct visual protein comparisons, proteins extracted from leaves of tested peanut entries (30 days old) grown under fungal stress conditions (mixed infested soil) and in pathogen free potted soil (control) then size fractionated based on the molecular weight by SDS-PAGE performed as described by Laemmli (1970). Vertical slab gels (0.75 mm-thick) were cast and electrophoresed using the Bio Rad Mini-Protean II system. Gels were stained with commassie brilliant blue R-250 solution, photographed and scored using gel documentation system manufactured by Alpha Ease FC (Alphaimager 2200), U.S.A. The similarity matrices were done using Gel works 1D advanced software UVP-England Program. The relationships among the mutants and their parent variety as revealed by dendrograms were done using SPSS windows (Version 10) program.

Field evaluation:

Field experiments performed in a randomized complete block design with three replicates were conducted during 2004 and 2005 seasons at Nubariya district, El-Behera governorate. The field soil was sandy loam, heavily infested with the damping-off and root-rot causal pathogens. Seeds of each of the previously mentioned peanut entries were sown at the first week of May of each season, in rows spaced 40-cm., with 20-cm between plants. Overhead sprinkler system (Central pivot system) was used for irrigation and the recommended fertilizer levels and agronomical practices were used as usual in the reclaimed sandy soils. Disease assessment was estimated as mentioned before in greenhouse evaluation.

Plant growth, yield and yield components:

To estimate the yield potential of peanut entry, the pod yield of $21m^2$ (2.1m x 10m) of each replicate was calculated. Harvest time was determined 120 days after sowing. The plants were dug by hand inverted and dried in the field for a week then pods were harvested by hand. At harvest, fifteen air-dried plants from the inner rows from each replication were selected to determine the dry weight of plant growth above the ground aerial (gram/plant^{-1}), number of pods/plant^{-1}, weight of pods/plant^{-1}, 100-Kernel weight (grams).

Statistical analysis:

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

Results and Discussion

1. Reaction of peanut entries against damping-off and root-rot pathogens of peanut under greenhouse conditions:

The obtained results (Tables 1a & 1b) show that, the tested ten peanut entries reacted differently against tested soil borne pathogens either singly or in mixture.

Regardless pathogens, the parent variety Giza-5 showed the highest percentages of pre-emergence, post- emergence; root rotted plants and lowest percentage of healthy standing plants while, the entry RT-10 shows the highest significant decrease in the first three criteria followed by entries RT-11, RT-7, RT-12 and RT-13 comparing with parent variety. Moreover, all tested entries recorded significant increase in percentages of healthy standing plants but entries RT-10 and RT-11 were the best while the entry RT-15 gave the lowest increase comparing with the parent Giza-5 variety. The obtained results are in agreement with Hilal *et al.*, (1994) and El-Deeb and Ibrahim (1998). The observed variations among the peanut entries against infection with the tested pathogens might be due the differences in their genetic make up and/or to variations in genetic pool of the peanut mutants that resulted from gamma ray irradiation (Venkatachalam *et al.*, 1999).

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Table 1a. Percentages of pre- and post-emergence damped-off seedlings of some peanut entries due to artificial infested soil with pathogens each alone or in mixture at seedling stage under greenhouse conditions

Damping-off	Peanut entry	Tested pathogen and disease incidence (%)					Mean
		S. rolfsii	*R. solani*	*F. solani*	*M. phaseolina*	Mixed	
Pre-emergence damping-off	Giza-5	40.0	33.3	26.7	20.0	46.7	33.3
	RT-15	26.7	26.7	26.7	20.0	46.7	29.4
	RT-14	33.3	20.0	20.0	20.0	40.0	26.7
	RT-9	33.3	26.7	20.0	13.3	26.7	24.0
	RT-8	33.3	26.7	20.0	13.3	33.3	25.3
	RT-6	33.3	33.3	13.3	13.3	33.3	25.3
	RT-13	20.0	20.0	20.0	13.3	33.3	21.3
	RT-12	26.7	13.3	13.3	13.3	20.0	17.3
	RT-7	20.0	20.0	13.3	6.7	20.0	16.0
	RT-11	20.0	13.3	13.3	6.7	13.3	13.3
	RT-10	13.3	6.7	6.7	0.0	20.0	9.3
Post-emergence damping-off	Giza-5	26.7	26.7	13.3	13.3	26.7	21.3
	RT-15	13.3	20.0	13.3	6.7	33.3	17.3
	RT-14	20.0	26.7	6.7	13.3	26.7	18.7
	RT-9	13.3	20.0	13.3	6.7	26.7	16.0
	RT-8	20.0	13.3	6.7	6.7	26.7	14.7
	RT-6	13.3	20.0	13.3	6.7	20.0	14.7
	RT-13	13.3	13.3	13.3	6.7	20.0	13.3
	RT-12	13.3	13.3	6.7	0.0	13.3	9.3
	RT-7	13.3	6.7	0.0	6.7	13.3	8.0
	RT-11	6.7	13.3	6.7	0.0	13.3	8.0
	RT-10	13.3	6.7	0.0	0.0	6.7	5.3

L.S.D. at 5% for:	Pre-emergence damping-off	Post-emergence damping-off
Mutants (M)	8.58	7.71
Pathogenic fungi (F)	5.78	5.20
M x F	19.18	17.25

To facilitate comparison between reactions of the tested peanut entries against damping-off and root-rot pathogens, three categories of different varietals reactions were suggested based on the obtained results of % of survived healthy plants in Table (1-b) and could be classified as follows:

Reaction	Range of % survived healthy plants
Resistant (R)	Up to 72.0%
Moderate (M)	72.0%< 44.0 %
Susceptible (S)	< 44.0 %

Table 1b. Reaction of some peanut entries against artificial infested soil with pathogens each alone or in mixture at mature stage under greenhouse conditions

Disease incidence (%)	Peanut entry	Tested pathogens					Mean
		S. rolfsii	*R. solani*	*F. solani*	*M. phaseolina*	Mixed	
Root-rotted plants	Giza-5	13.3	20.0	13.3	6.7	20.0	14.7
	RT-15	13.3	13.3	6.7	6.7	13.3	10.7
	RT-14	0.0	6.7	13.3	13.3	20.0	10.7
	RT-9	13.3	6.7	6.7	6.7	20.0	10.7
	RT-8	6.7	13.3	6.7	6.7	13.3	9.3
	RT-6	6.7	6.7	6.7	0.0	13.3	6.7
	RT-13	6.7	6.7	0.0	0.0	13.3	5.3
	RT-12	6.7	0.0	6.7	6.7	13.3	6.7
	RT-7	6.7	6.7	0.0	0.0	6.7	4.0
	RT-11	6.7	0.0	0.0	0.0	0.0	1.3
	RT-10	0.0	0.0	0.0	0.0	6.7	1.3
Survived healthy plants	Giza-5	20.0	20.0	46.7	60.0	6.7	30.7
	RT-15	46.7	40.0	53.3	66.7	6.7	42.7
	RT-14	46.7	46.7	60.0	53.3	13.3	44.0
	RT-9	40.0	46 7	60.0	73.3	26.7	49.3
	RT-8	40.0	46.7	66.7	73.3	26.7	50.7
	RT-6	46.7	40.0	66.7	80.0	33.3	53.3
	RT-13	60.0	60.0	66.7	80.0	33.3	60.0
	RT-12	53.3	73.3	73.3	80.0	53.3	66.7
	RT-7	60.0	66.7	86.7	86.7	60.0	72.0
	RT-11	66.7	73.3	80.0	93.3	73.3	77.3
	RT-10	73.3	86.7	93.3	100.0	66.7	84.0

L.S.D. at 5% for:	Root-rotted plants	Survived healthy plants
Mutants (M)	6.91	9.45
Pathogenic fungi (F)	4.51	6.37
M x F	14.96	21.12

Based on previously classification, it could be concluded from the obtained results, that the peanut entries RT-10, RT-11 and RT-7 could be classified as resistant entries (R) which produced the highest % survived healthy plants (72.0–84.0 %) while the parental variety Giza-5, RT-15 and RT-14 were as susceptible entries (S) which produced the lowest % survived healthy plants (30.7-44.0 %), however, the other tested peanut entries could be classified as moderate entries (M) which resulted % survived healthy plants ranged from 49.3 to 66.7%.

2. Biochemical changes in healthy and infected peanut plants:

2.1. Oxidative enzymes:

Data in Table (2) show that the healthy and infected plants of resistant entries RT-10 and RT-11 showed the highest activity of peroxidase (PO) enzyme followed

by RT-12, RT-7 and RT-13 comparing with those of the susceptible variety Giza-5. Also, healthy and infected plants of resistant entries RT-11, RT-7 and RT-10 showed the highest activity of polyphenoloxidase (PPO) enzyme comparing with those of the susceptible entries RT-15 and the parent variety Giza-5. On the other hand, the entries RT-7, RT-10 and RT-13 recorded the highest level of catalase activity while the lowest catalase activity was detected in the entry RT -14. In general, activity of the oxidative enzymes was higher in tissues of healthy plants than in infected plants and in resistant mutants more than susceptible ones. In this respect Jagdish and Tyagi (1993) showed a greater increase in peroxidase activity in leaves of resistant mungbean cultivars following inoculation with *M. phaseolina* than the susceptible ones. Pathak *et al.* (1998) reported that the activity of polyphenoloxidase was highest in immune sunflower cultivar and lowest in the highly susceptible one. Enhanced oxidative enzymes activity in disease development has been correlated with the expression of resistance in different host- pathogen system (Cadena-Gomez and Nicholson, 1987). Avdiushko *et al.* (1993) mentioned that, many plants enzymes are involved in defence reaction against plant pathogen. These include oxidative enzymes such as peroxidase and polyphenoloxidase which catalase the formation of lignin and other oxidative phenols that contribute to formation of defence barrier for reinforcing the cell structure. In fact, the oxidative enzymes play an important role in plant diseases resistance (Mahmoud *et al.*, 2006).

Table 2. Activities of some oxidative enzymes expressed as optical density/minute/g. fresh weight in roots of healthy and infected peanut mutants (entries) plants under greenhouse conditions

Peanut entry	Reaction*	Peroxidase (PO) at 425 nm		Polyphenoloxidase (PPO) at 495 nm		Catalase (CAT) at 240 nm	
		Healthy plants	Infected plants	Healthy plants	Infected plants	Healthy plants	Infected plants
Giza-5	S	0.25	0.32	0.35	0.30	0.89	1.02
RT 6	M	0.30	0.22	0.63	0.48	0.69	0.80
RT 7	R	0.88	0.78	1.34	0.74	1.37	1.25
RT 8	M	042	0.50	0.98	0.89	0.87	1.19
RT 9	M	0.49	0.72	0.68	0.74	0.54	0.63
RT 10	R	1.33	0.94	1.24	0.88	1.12	1.76
RT 11	R	1.28	0.79	1.49	1.07	0.74	0.98
RT 12	M	0.94	0.76	0.61	0.57	0.93	1.18
RT 13	M	0.82	0.56	0.73	0.68	1.37	1.54
RT 14	S	0.76	0.58	0.64	0.66	0.47	0.59
RT 15	S	0.60	0.47	0.39	0.44	0.57	0.75

* (S)= susceptible entry, (M)= moderate entry and (R)= resistant entry.

2.2. Phenolics, sugars and total free amino acid contents:

Data in Tables (3 a & b) indicate that the healthy and infected plants of resistant peanut entries showed, with few exceptions, higher amounts of phenols (total and free) and sugars (total and reducing) and lower amount of total free amino acid in comparison with susceptible ones.

Comparing with the susceptible parent variety Giza-5, the resistant mutants RT-10, RT-7 and RT-11 showed the highest free and total phenols followed by entries RT-8, RT-6, and RT-14 while, mutant RT-10 shows the highest amount of conjugated phenols followed by RT-12 and RT-15. In all tested peanut entries including parent variety Giza-5, the infected plants contained higher amounts of total, free and conjugated phenols than the healthy ones with few exceptions. Such behaviour of phenolic compounds was detected also in the healthy and infected plants of the resistant and susceptible cultivars of sesame (El-Fiki *et al.*, 2004). In fact, phenolics might play an important role in plant defence. Phenols are essential for the biosynthesis of lignin, which consider an important structural component of plant cell walls and most notably phytoalexins (Fajardo *et al.*, 1994 and Mahmoud *et al.*, 2006).

The same data show also that the amounts of sugars (total, reducing and non-reducing) were higher in healthy plants of mutants No.10 and 8 and infected plants of mutants RT. 15, RT-10, RT-9 and RT-8, whereas were lower in healthy plants of mutants RT-6 and in infected plants of mutants RT-11 and RT-7. The obtained results were in harmony with those of El-Fiki *et al.*, 2004. Abdel-Kader, 1983 suggested that, the level of total soluble carbohydrates might be a critical factor in determines resistance.

Table 3a. Determination of phenolic compound content in healthy and infected roots of peanut mutants (entries) plants (mg/ g fresh weight)

Peanut entry	Reaction*	Healthy plants			Infected plants		
		Total phenols	Free phenols	Conjugated phenols	Total phenols	Free phenols	Conjugated phenols
Giza-5	S	0.12	0.09	0.03	0.74	0.47	0.27
RT 6	M	2.00	1.94	0.06	3.91	3.42	0.49
RT 7	R	2.34	2.23	0.11	2.50	2.29	0.21
RT 8	M	2.15	1.99	0.15	1.58	1.12	0.46
RT 9	M	0.91	0.80	0.11	1.62	1.54	0.08
RT 10	R	3.07	2.26	0.81	3.66	3.35	0.32
RT 11	R	2.29	2.02	0.27	2.55	2.22	0.33
RT 12	M	1.58	1.16	0.42	1.46	1.15	0.31
RT 13	M	1.44	1.20	0.24	2.41	2.15	0.26
RT 14	S	1.94	1.56	0.28	2.30	1.99	0.31
RT 15	S	1.17	0.78	0.39	2.74	2.33	0.41

* (S) = susceptible entry, (M) = moderate entry and (R) = resistant entry

Table 3b. Determination of sugar content and free amino acids in healthy and infected roots of peanut mutants (entries) plants

Peanut entry	Reaction	Sugars content (mg/g fresh weight)						Total free amino acids	
		Healthy plants			Infected plants				
		Total sugars	Reducing sugars	Non-reducing sugars	Total sugars	Reducing sugars	Non-reducing sugars	Healthy plants	Infected plants
Giza-5	S *	2.82	2.04	0.78	1.58	1.32	0.26	1.63	1.76
RT 6	M	0.67	0.48	0.19	2.06	1.58	0.50	0.85	1.20
RT 7	R	2.25	1.80	0.46	1.23	1.10	0.22	0.51	0.11
RT 8	M	4.70	3.41	1.29	2.80	1.43	1.36	0.39	0.44
RT 9	M	2.57	1.58	0.98	3.30	1.87	1.43	0.56	0.71
RT 10	R	5.99	4.31	1.70	3.62	2.21	1.41	0.51	0.23
RT 11	R	1.58	1.24	0.35	1.07	0.86	0.21	0.61	0.56
RT 12	M	2.19	1.93	0.25	1.61	1.41	0.20	0.41	0.51
RT 13	M	2.15	2.00	0.15	1.70	1.68	0.02	0.54	0.61
RT 14	S	2.18	2.01	0.16	1.87	1.36	0.51	0.77	0.40
RT 15	S	1.32	0.93	0.39	4.92	3.17	1.75	0.99	0.36

* (S)= susceptible entry, (M)= moderate entry and (R)= resistant entry.

The total free amino acids, in general, was higher in infected plants than in healthy ones, and in the susceptible entries than the resistant ones. The healthy and infected plants of susceptible parent variety Giza-5, entries RT-15, RT-14 and RT-6 recorded the highest amount of total free amino acids, while the lowest amounts were recorded in those of peanut entries RT-8, RT-12, RT-10, RT-7 and RT-11 (with few exceptions). These results are in agreement with Gangopadhyay and Wyllie (1970) who noted that, healthy and infected roots of susceptible soybean cultivar contained relatively high concentrations of free amino acids as compared to those in the resistant one. The free amino acids content appeared closely related with the symptom severity and the increase in amino acids content might be due to the decomposition of host protein or the reduction of protein synthesis due to fungal growth. Hegazy (1987) stated that, the free amino acids were higher in the infected plants compared to controls and this may be indicating the inhibition of some enzymes involved in protein synthesis and other complex compounds or an increase in the level of some proteolytic enzymes.

3. Protein patterns:

Protein markers, including electrophoresis protein and isozymes were among the first group of molecular markers exploited for genetic diversity assessment. The electrophoretic banding patterns of proteins extracted from leaves of non-stressed (control) and stressed plants (grown in soil infested with mixture of the tested fungi) of different evaluated peanut entries are shown in Figs. (1) and (2), respectively, and their densitometric analysis are illustrated in Tables (4) and (5), respectively, where the presence and absence of bands were assessed with (1) and (0), respectively.

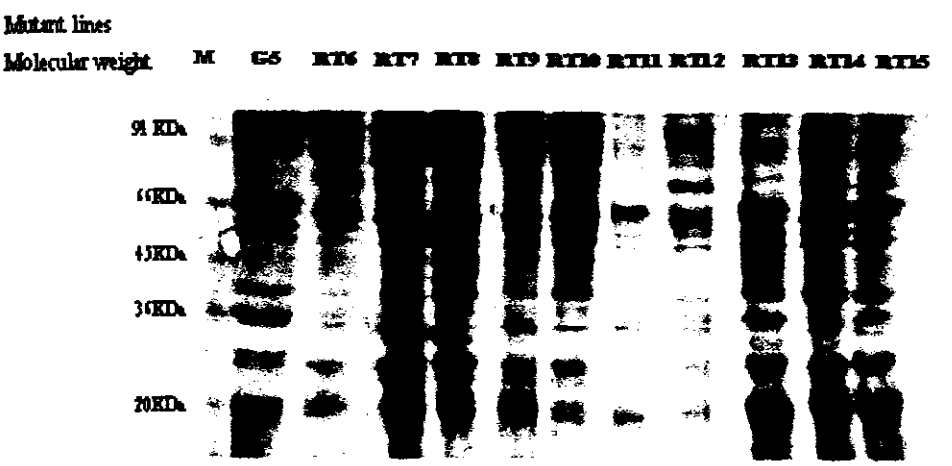

Fig. 1. SDS-protein banding patterns for ten peanut mutants and their parental variety Giza-5 (G5) grown under non-stressed normal condition.

Fig. 2. SDS-protein banding patterns for ten peanut mutants and their parental variety Giza-5 (G5) grown under fungal stress condition.

The results of SDS-PAGE revealed a total number of 36 bands (with molecular weights (MW) ranging from about 12.45 to 96.36 KDa and RF values ranging from 0.989 to 0.042) were detected in control plants grown under normal conditions whereas a total number of 25 bands (with molecular weights (MW) ranging from about 14.58 to 89.83 KDa with RF ranging from 0.951 to 0.070) were found in those grown under disease stress conditions, which were not necessarily present in all mutants and Giza-5 (parental variety). The SDS-protein banding patterns of the ten peanut mutants and their parental variety under both non-stressed and stressed conditions was found to be useful in identifying the variation induced between them and also between them and their parental variety as a result of irradiation with different gamma ray doses and selection for resistance based on the protein level. The total number of bands differed for all mutants under both conditions except RT-8 (13 bands) and the parental variety (11 bands).

Data showed four common bands (monomorphic), while the remaining bands were polymorphic with polymorphism under the stress conditions, while under the control conditions all bands were polymorphic with polymorphism and there were no common bands. There is no resemblance between any mutant and its parental variety and a unique fingerprint characterized each. The variations among these ten peanut mutants and their parental variety might be due the differences in their genetic make up and\or to variations in genetic pool that resulted from irradiated with gamma rays doses. In general, that the four protein bands, *i.e.* number 5, 12, 25, and 30 were detected in the non-stressed plants of the resistant peanut entries RT-10 and RT-11 and lacking from same plants of the most susceptible parent variety Giza-5 while the opposite situation was found regarding bands number 8, 24 and 31. Regarding with plants grown under disease stress conditions, the bands number 3, 15, 20 and 22 were detected while bands number 21 and 23 were lacked from plants of the two resistant peanut entries meanwhile the opposite situations were found in the parent susceptible variety Giza-5. In general, some of the new bands might be considered as positive marker for resistance against damping-off and root-rot diseases and the higher productivity in peanut, while other bands might be considered positive markers for susceptibility against damping-off and root-rot diseases and the lower productivity in peanut.

The occurrence of new bands and absence of others, represented by different genotypes, under fungal stress conditions would indicate either enhancement or repression of gene expression in these plants. This may alter the produced proteins in response to pathogen stress either on the transcriptional or post transcriptional levels of gene expression. In fact, medium doses of gamma radiation can often lead to some mutations, free radicals are generated from sparsely ionizing radiation and they attack DNA randomly. They can also cleave one DNA strand at a time of irradiation, and generate single strand breaks (S.S.Bs), alternate the cellular DNA and generate loss of bases.

Table 4. Densitometric analysis for SDS leaves storage protein (water soluble fraction) of ten peanut mutants grown under normal (non-stressed) condition

Band No.	RF	MW	Peanut entries (mutants and their parental variety)										
			Giza-5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14	RT15
1	0.04	96.36	1	1	1	1	0	1	1	1	1	1	0
2	0.08	88.53	0	0	0	0	0	0	0	0	1	1	0
3	0.01	85.57	1	0	1	1	0	1	0	1	0	0	0
4	0.11	83.39	0	1	0	0	0	0	1	0	0	0	1
5	0.13	79.00	0	1	1	1	0	1	1	1	1	1	0
6	0.19	70.39	1	1	1	0	0	1	1	1	1	1	0
7	0.21	66.63	0	0	0	1	0	0	0	0	0	0	0
8	0.25	61.83	1	0	0	0	0	0	0	0	0	0	1
9	0.27	59.71	0	1	0	1	0	0	0	0	0	0	0
10	0.28	57.38	1	0	1	0	1	1	1	1	0	1	1
11	0.30	55.68	0	1	0	0	0	0	0	0	1	0	0
12	0.32	53.24	0	0	0	1	0	1	1	1	0	0	0
13	0.34	51.67	1	1	1	1	0	0	0	0	0	1	1
14	0.35	50.65	0	1	0	0	1	0	0	0	1	0	0
15	0.37	48.19	0	0	1	0	0	1	1	1	0	0	0
16	0.38	47.00	1	0	0	1	1	0	0	0	1	1	1
17	0.41	43.40	0	0	1	0	0	0	1	1	1	1	0
18	0.43	41.70	0	0	0	0	0	1	0	0	0	0	0
19	0.46	39.08	0	0	0	0	0	0	0	0	0	0	1
20	0.50	36.27	0	1	0	0	0	0	0	0	0	0	0
21	0.52	34.16	1	0	1	1	1	1	1	1	1	1	1
22	0.54	32.83	0	0	0	0	0	0	0	0	1	0	0
23	0.56	31.46	0	0	0	1	0	1	0	1	0	0	0
24	0.58	30.16	1	0	0	0	0	0	0	0	0	0	1
25	0.60	28.69	0	1	1	1	1	1	1	1	1	1	0
26	0.64	26.62	0	0	1	0	0	0	0	1	1	0	0
27	0.66	25.84	0	0	0	0	1	0	0	0	0	0	0
28	0.69	24.34	1	0	0	0	0	0	0	0	0	0	1
29	0.71	23.50	0	1	1	1	1	1	1	1	1	1	0
30	0.74	21.17	0	0	0	0	0	0	1	1	0	0	0
31	0.81	18.87	1	1	0	0	1	0	0	1	1	0	0
32	0.84	17.25	0	0	1	1	1	1	1	1	1	1	1
33	0.92	15.23	0	0	0	0	0	0	0	0	1	0	0
34	0.47	14.14	0	0	1	0	0	0	0	0	0	0	0
35	0.90	13.50	0	1	0	0	0	0	0	0	0	0	1
36	0.99	12.45	0	0	0	0	0	0	0	0	1	0	0
Total bands			11	13	14	13	9	13	13	16	17	12	11

Table 5. Densitometric analysis for SDS leaves storage protein (water soluble fraction) of ten peanut mutants under disease stressed condition

Band No.	RF	MW	Peanut Entries (Mutants and their parental variety)										
			Giza-5	RT6	RT7	RT8	RT9	RT10	RT11	RT12	RT13	RT14	RT15
1	0.07	89.8	0	1	1	1	1	1	1	1	0	1	1
2	0.11	82.30	0	0	1	1	0	0	1	0	0	0	0
3	0.14	77.63	0	0	0	0	0	1	1	0	0	0	0
4	0.18	72.42	0	0	0	1	0	0	0	1	1	1	1
5	0.20	69.65	1	1	1	0	0	1	1	0	0	0	0
6	0.24	65.43	0	0	0	0	0	0	0	0	1	1	1
7	0.24	63.02	1	1	1	1	1	1	1	1	0	0	0
8	0.29	57.02	0	0	0	0	0	0	0	1	0	0	0
9	0.30	56.31	1	1	1	1	1	1	0	0	1	1	1
10	0.32	53.78	0	0	0	0	0	0	1	1	0	0	0
11	0.40	45.33	1	1	1	1	1	1	1	1	1	1	1
12	0.47	39.83	1	0	0	1	1	1	1	1	1	1	1
13	0.53	34.85	1	1	1	1	1	1	1	1	1	1	1
14	0.55	33.99	0	0	0	0	0	0	1	0	0	0	0
15	0.62	29.62	0	0	0	0	0	1	1	0	0	0	0
16	0.67	26.36	0	0	0	0	1	0	0	0	0	0	0
17	0.69	25.18	1	1	1	1	1	1	1	1	1	1	1
18	0.73	23.35	0	0	0	0	0	0	0	1	0	0	0
19	0.74	22.31	1	1	1	1	1	1	1	0	0	1	1
20	0.79	20.52	0	0	0	0	0	1	1	1	1	0	0
21	0.83	19.04	1	1	1	1	1	0	0	0	1	1	1
22	0.85	18.11	0	0	1	1	0	1	1	1	0	0	0
23	0.86	17.11	1	1	0	0	0	0	0	0	0	1	1
24	0.90	16.59	1	1	1	1	1	1	1	1	1	1	1
25	0.95	14.58	0	1	1	0	0	0	1	0	0	1	0
Total bands			11	12	13	13	11	14	17	13	10	13	12

Loss of bases would cause considerable gene damage during subsequent DNA replication processes. Also, there is a flow of information from the genes of an organism into the construction of specific proteins, generally referred to as gene expression; it is the spectrum of proteins produced that provides the connection between genotype and phenotype. Many environmental factors are now known to greatly influence the extent to which specific genes are activated to produce proteins that are protective and which enable organisms to survive (Burdon, 1999). Radiation may cause genetic damage to DNA by creating unusual bonds between DNA bases. If radiation-induces un-repaired genetic damage, mutation or cell death may result. Ionizing radiation may also cause chromosomal aberrations (Russell, 1994).

Effect of gamma ray doses on the genetic relationships between evaluated peanut entries (mutants and the parental variety) under non-stressed (control) and disease stressed conditions are shown in Figs. 3 & 4, respectively. Under the control conditions (Fig. 3), the peanut entries were separated into two clusters; cluster 1

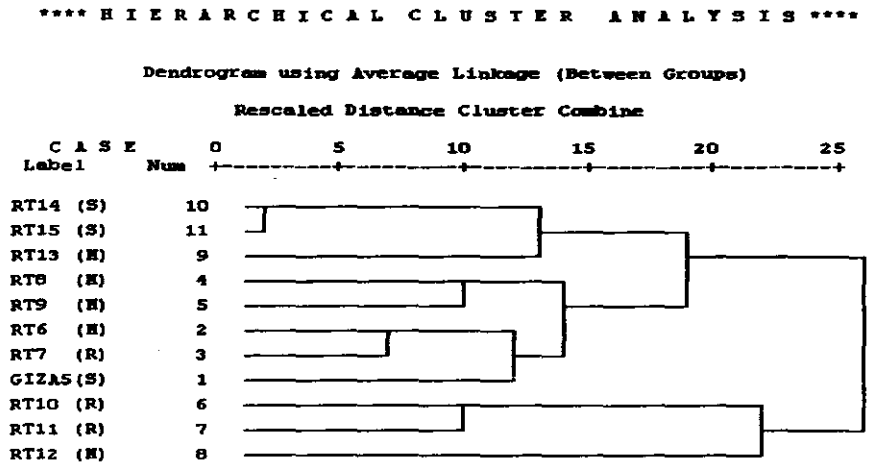

Fig. 3. A dendrogram showing the genetic distance among the ten peanut mutants and their parental variety (Giza-5) under control condition using SDS- protein data. Whereas, (S)= susceptible entry, (M)= moderate entry and (R)= resistant entry.

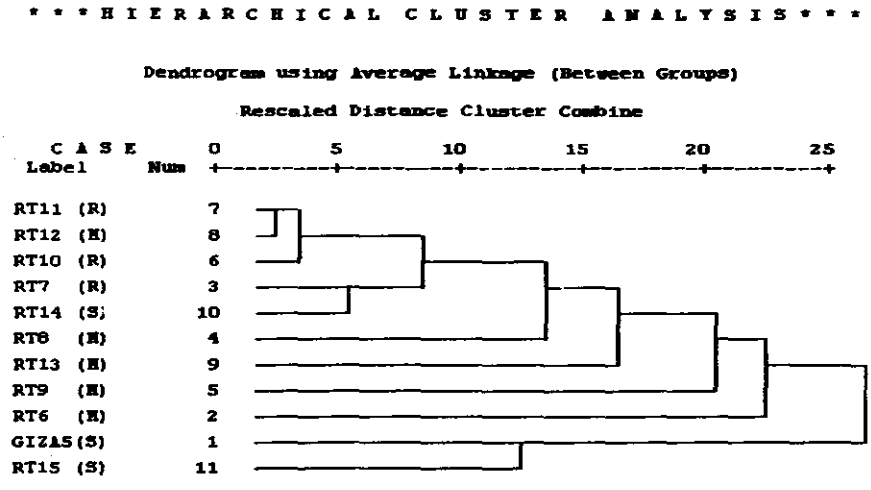

Fig. 4. A dendrogram showing the genetic distance among the ten peanut mutants and their parental variety (Giza-5) under fungal stress condition using SDS- protein data. Whereas, (S)= susceptible entry. (M)= moderate entry and (R)= resistant entry.

included almost all peanut mutants and Giza-5, except RT-10, RT-11 and RT-12, which are appeared in cluster 2 and are the most resistant mutants against damping-off and root-rot diseases. Cluster 1 included two subclusters, the first subcluster included RT-13, RT-14 and RT-15 which conceders as susceptible mutants, while the second subcluster included Giza-5, RT-7, RT-6, RT-9 and RT-8 that separated also in two clusters.

However, under disease stress (Fig. 4), the studied peanut entries were separated into two clusters; cluster 1 included all peanut mutants, except RT-15, which are appeared in cluster 2 with the parental variety; Giza-5 and are the most susceptible ones against damping-off and root-rot diseases. Cluster 1 included two sub clusters, the second sub cluster included RT-6, while the first one includes the rest that were separated in 5 sub clusters, the last one include RT-10, RT-11 and RT-12, the most resistant mutants.

The development of disease resistance was found to be correlated with the accumulation of host synthesized new polypeptides (Broglie *et al.*, 1986). Hlinkova and Sykora (1996) recorded that the new protein contents depended on host genotype and virulence genes of the pathogens. Proteins with peroxidase activity differed at the level of susceptibility host-pathogen interaction. Radwan (2000) detected new proteins in the mutagenized resistant and immune plants of barely to powdery mildew (new genotypes) from the two tested cultivars in comparison with the susceptible mutant ones and the control (parents). The changes of proteins depended on the host genotype and sensitivity to infection.

4. Field evaluation:

Data in Table (6) showed that the aerial dry weight/plant and number of pods/plant in mutants RT -14 and RT-15 were not differed significantly when comparing with the parent variety (Giza-5). The other mutants, however, showed significant increase in the aerial dry weight/plant (between 126.45-213.2%) and number of pods/plant (between 113.96-156.7%) in comparison with the parent variety (Giza-5). The same trend was noticed concerning weight of pods/plant (between 60.33-81.85g) and weight of 100-kernels (between 94.59-100.0g) comparing with 43.91 and 92.0g/plant, respectively in the parent variety (Giza-5),

As for aerial dry weight/plant, the mutant RT- 8 exhibited the highest significant increase (213.2%) followed by RT-7 (203.0%) and RT-6 (200.93%) while, RT-14 produces the lowest significant increase (115.6%) comparing with Giza-5 variety. Regarding with number of pods/plant, the mutant RT-7 produces the highest increase (156.7%) followed by RT-6 (143.26%) while the lowest increase was produced by mutant RT-12 (113.96%) comparing with Giza-5 variety. These results indicated a positive correlation between the aerial dry weight and the pod yield per plant. Bhatia *et al.* (1991) reported that the selection for higher yield mutants in peanut was associated with an increase in aerial dry matter and pods and grain size. Also, Ball (1981) reported that 100 genetically divers genotypes of peanut displayed significantly positive correlation of fruit weight with total biomass and harvest index.

Table 6. Mean values of some agronomical traits and pod yield of some peanut mutants (entries) resistant to damping-off and root rot causal pathogens under naturally infested field conditions during 2004 and 2005 seasons

Peanut entry	Aerial dry weight		Pods/plant		Pods weight (g/plant)	100-Kernel weight (g.)
	g/plant	(%) of parental var.	Number	(%) of parental var.		
Giza-5	44.69	100	24.57	100	43.91	92.00
RT 6	89.80	200.93	35.20	143.26	75.50	96.03
RT 7	90.72	203.00	38.50	156.70	81.85	98.00
RT 8	95.28	213.20	33.93	130.10	81.57	94.61
RT 9	75.24	168.36	31.62	128.69	68.71	100.00
RT 10	56.51	126.45	28.63	116.52	60.33	95.85
RT 11	73.80	165.14	30.88	125.68	63.37	95.22
RT 12	62.72	140.35	28.00	113.96	61.30	94.59
RT 13	70.49	157.73	32.02	130.32	62.53	94.67
RT 14	51.66	115.60	24.28	98.82	51.92	98.00
RT 15	45.84	102.57	22.98	93.53	42.71	95.47
L.S.D. at 5%	6.28		4.17		8.01	1.57

Comparing with the parent variety (Giza-5), the mutant RT-7 produces the highest increase in weight of pods/plant (81.85g) followed by RT-8 (81.57g) and RT-6 (75.5g) whereas RT-10 produces the lowest significant increase 60.33g). It is clear also that all tested peanut mutants exhibited significant increase in their 100-Kernel weight comparing with variety Giza-5. In this regard, the mutant RT-9 produces the highest weight (100.0g) followed by RT-7 and RT-14 (98.0g) while, RT-12 produces the lowest significant increase comparing with Giza-5 (92.0g). In fact, number of pods could be used as an index of productivity of the peanut crop (Soriano, 1988). Kale *et al.* (2000) selected many peanut mutants characterized with large kernels (100-Kernels wt.). Also, Sidhu *et al.* (1997) released many peanut mutants with high kernels index (100-Kernels weight) compared to their parent varieties. They selected superior two mutants showed an increase in pod yield over respective parents and were superior in number of pods per plant and 100-kernels weight.

5. Reaction of certain peanut mutants against infection with damping-off and root-rot diseases under field conditions:

Data presented in Table (7) show that all tested peanut mutants were more resistant against damping-off and root-rot diseases at seedling and maturity stages, in seasons 2004 and 2005, respectively, under field conditions comparing with their parental variety Giza-5.

Table 7. Reaction of some peanut mutants (entries) against incidence of damping-off, root-rot and healthy plants under naturally infested field conditions

Peanut entry	2004				2005			
	At seedling stage (%)		At maturity stage (%)		At seedling stage (%)		At maturity stage (%)	
	Pre	Post	Root-rotted	Healthy	Pre	Post	Root-rotted	Healthy
Giza-5	24.2	16.7	19.1	40.0	18.2	17.0	16.0	48.8
RT 6	13.2	8.3	10.7	67.8	5.9	11.0	9.0	74.1
RT 7	8.3	7.7	4.1	79.9	4.6	8.8	8.3	78.3
RT 8	10.7	7.8	5.7	75.8	4.7	6.8	7.5	81.0
RT 9	14.8	8.9	8.1	68.2	6.2	9.5	13.0	71.3
RT 10	4.6	6.4	2.1	86.9	5.4	3.3	5.5	85.8
RT 11	12.9	5.1	4.8	77.2	6.2	4.3	6.1	83.4
RT 12	5.9	10.5	7.4	76.2	4.8	6.9	6.3	82.0
RT 13	9.9	9.8	13.4	66.9	12.2	5.5	9.5	72.8
RT 14	14.6	14.3	11.9	59.2	11.9	7.4	14.8	65.9
RT 15	13.8	15.1	13.1	58.0	12.7	10.7	13.7	56.9
L.S.D. at 5%	6.76	4.77	3.09	7.62	4.19	4.48	5.58	8.22

All mutants exhibited significant decrease in percentages of dead seedlings [pre emergence (between 4.6-14.8%) and post emergence (between 5.1-15.1%) in season 2004 and (4.6-12.7% and 3.3-11% of pre and post emergence, respectively) in season 2005]. As for root rotted plants (2.1-13.4% in season 2004 and 5.5-13.7% in season 2005) and produced significant increase in percentages of healthy survived plants (58.0-86.9% in season 2004 and 56.9-85.8% in season 2005), in both seasons, comparing with the parent variety which recorded 24.2, 16.7, 19.1and 40.0 in the first season and 18.2, 17.0, 16.0 and 48.8% in the second season for the four disease parameters, respectively.

The reaction of these mutants against damping-off (seedling stage) and root rot (mature stage) was obviously varied. Mutants RT-10, RT-7 and RT-12 were the highly resistant mutants at seedling stage in seasons 2004 and RT-10, RT-11, RT-8 and RT-12 in seasons 2005 followed by mutants RT-13, RT-8 and RT-11 as well as RT-7, RT-9, and RT-6 in two successive seasons 2004 and 2005, respectively, whereas the parent variety Giza-5 was the highly susceptible one at this stage.

On the other hand, at maturity stage, mutants RT-10, RT-7 and RT-8 were the highly resistant at maturity stage in season 2004 and mutants RT-10, R-11, RT-12 and RT-8 in season 2005, where gave the lowest and highest percentages of root-rotted and healthy plants, respectively. However, the parent variety Giza-5 was the highly susceptible one at this stage, followed by mutants RT-14 and RT-15 which gave highest and lowest percentages of root-rotted and healthy plants, respectively, in two successive seasons 2004 and 2005.

The results might be attributed to variations in root exudates of tested peanut mutants. In fact, root exudates may change environmental circumstances at court of infection which led eventually to susceptibility or resistant reaction as well as inhibited or enhanced the pathogens to infected peanut roots. In this respect Abd-El-Moneem *et al.* (2003) found that, the growth of *F. oxysporum* and *S. rolfsii* in vitro was inhibited by the root exudates of cv. Local 235 but was enhanced by the root exudates of cv. Giza-5. The root exudates of cv. Giza-5 had higher sugar and amino acid contents than that of cv. Local 235. The resistance and susceptibility to damping-off and root-rot infection might be chemical in nature. The present results indicated that the amounts of free phenols, total phenols, reducing and total sugars, in general were obviously higher in mutants that were classified resistant than those classified as susceptible.

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المعلمات الكيموحيوية المرتبطة بالمقاومة لأمراض موت البادرات وعفن الجذور لطفرات الفول السوداني وإنتاجيتها

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*** المركز القومي لبحوث وتكنولوجيا الإشعاع، القاهرة.

تباينت الطفرات العشر من الفول السوداني الناتجة من التشعيع بأشعة جاما تحت ظروف الصوبة، أثناء مراحل النمو المختلفة في مقاومتها للمسببات المرضية المختبرة و مخلوطها المسببة لأمراض موت البادرات و أعفان الجذور مقارنة بالصنف الأبوي جيزة 5 و كانت الطفرات RT 10 تلاها RT 11 , RT 7 الأقل إصابة بأمراض موت البادرات ما قبل وبعد الإنبات وعفن الجذور والأكثر نسبة للنباتات السليمة المتبقية. كذلك كانت RT 10 تلاها RT 11 , RT 12 , RT 7 هم الأكثر مقاومة تحت ظروف الحقل. ومن ناحية أخرى كانت الطفرات RT 6 ,RT 7 و RT 8 هي الأكثر تميزا في المحصول ومكوناته بالمقارنة بالصنف الابوى جيزة 5 في موسمين متعاقبين.

أرتفع النشاط الانزيمى للإنزيمات المؤكسدة والفينولات الحرة والكلية والسكريات المختزلة والكلية في أنسجة جذور النباتات السليمة عن أنسجة النباتات المصابة لمعظم طفرات الفول السوداني المختبرة وفي الطفرات المقاومة عن القابلة للإصابة. بينما انخفضت الأحماض الامينية الحرة في النباتات المصابة والسليمة للأصناف المقاومة عن القابلة للإصابة

أظهرت نماذج حزم البروتين المفردة كهربائيا المستخلص من أوراق عشر طفرات من الفول السوداني والصنف الابوى (جيزة 5) تحت كلا من ظروف التربة المعدية (إجهاد) وغير المعدية بمخلوط من المسببات المرضية، تباينا في عدد حزم البروتينات المتكونة تحت ظروف عدم العدوى (كنترول) وتحت ظروف العدوى (ظروف التربة المعدية بمخلوط من المسببات المرضية) وقد تم التعبير عن بعض الحزم فقط تحت ظروف العدوى وبخاصة في الطفرات المقاومة والمتوسطة المقاومة RT 7, RT 8, RT 10, RT 11 و RT 12.
أظهر دليل التشابه للبروتينات المفردة كهربائيا وصلة القرابة بين العشر طفرات من الفول السوداني العلاقة الوراثية بينهم وبين الصنف الأبوي (جيزة 5) وأن أعلى دليل تشابه وجد بين RT 10، RT 11 وRT 12.