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PATHOLOGICAL AND BIOCHEMICAL GENETIC CHARACTERIZATION OF γ- RAY AND EMS-INDUCED PEA MUTANTS RESISTANT TO RUST DISEASE

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ABSTRACT: *Attempts were made to develop rust-resistant mutants of pea through induced mutations. Seed samples of cultivars Little Marvel and Teledo Sugar were irradiated by different gamma-ray doses (50, 100 and 150 Grey). Other samples were chemically mutagenized by three EMS concentrations:(0.25, 0.75 and 1.50%). Deformed and sterile M_1-plants were discarded. Seeds of individual normal M_1-plants were harvested and grown as M_2-families. Resistant and moderately resistant M_2-plants were selected and their progenies were grown and tested against rust disease during M_3 and M_4 generations.*

Some M_2 plants from the progenies of M_1-plants of gamma ray and EMS materials showed moderate symptoms than the control progenies. The percentages of resistant plants in M_2 generation were very low, but they increased gradually by progression of generations; from M_2 (1.1%) to M_4 (11.7-12.0%). The highest percentage of resistant plants was recorded in M_4 from the material of 100 Grey Gamma-rays for cv. Little Marvel (15.3%) and with 50 Grey gamma-rays and 0.75% EMS for cv. Teledo Sugar (13.3% and 14.1%), respectively. Some selected M_4-resistant plants gave high seed yield, some were different in their morphological characters, such as plant height, early and late flowering, number of pods/plant, number of seeds/plant, weight of 100 seed, thickness of stem, pod shape and resistance to rust. Crop parameters of resistant plants in M_4 generation have increased in comparison with the controls.

Higher peroxidase activities as well as lower amino acids content were recorded in the mutants derived after mutagenic treatments with gamma-rays compared to those treated with EMS. All M_4 mutant plants tested varied in their protein patterns. The susceptible plants contained large number of proteins compared with resistant mutants. There were remarkable changes in protein patterns of all tested mutant plants due to infection by Uromyces pisi (Pers) Winter. The induced proteins in the susceptible plants were higher compared with the resistant mutants.

Key words: *Mutants, Uromyces pisi, Pisum sativum, SDS-PAGE, Biochemical assessment.*

INTRODUCTION

Pea (*Pisum sativum* L.) is considered one of the most important legume crops in Egypt for local consumption and exportation. This crop is widely

used as a source of plant protein in human diets due to its high content of protein, ascorbic acid and carbohydrates as well as balanced amino acids composition and good digestibility. In addition, the importance of pea is increasing as an important fodder crop in Egypt (as green fodder, silage, and hay), especially in the newly reclaimed lands. Moreover, including pea in crop rotation improves soil fertility for the succeeding crops. In general, this crop gives high yield and ensures high profits, especially when cultivated for green pods. Therefore, it occupies a prominent position among other legumes in the Egyptian agriculture.

Pea plants are severely attacked by many pathogenic fungi which infect different parts of the plant causing different amounts of losses. Rust disease caused by *Uromyces pisi* (Pers) Winter is the most important disease infecting pea plantations all over Egypt (Khafagi *et al.*,1995 and Abada *et al.*1997). During the past few years, pea rust disease incited by *Uromyces pisi* has been seriously attack pea plantations, especially late in the season. The infection percentages of pea rust disease reached approximately 41-65% during 1997/1998 growing season in the inspected some governorates.

In Egypt, it is difficult to increase the cultivated area of pea on the account of other important crops. Chemical control of pea rust mostly causes environmental pollution and highly affects the growth of the host plants. Moreover, it may induce acquired resistance in the causal pathogens. This may render such chemicals ineffective after a short time of their use. In Egypt as well as in many countries, the trend, in the recent years, is to reduce and/or stop the use of chemical pesticides due to their harmful effects on human and animal health. In addition, several investigators tested several compounds to control this disease, but with low success.

The use of genetically resistant cultivars has been the principal goal of reducing disease and pest losses. Conventional techniques, although useful and still being used, are time consuming and laborious, since they need a series of crosses and backcrosses and selection cycles. The utilization of mutation breeding programs proved, in many cases, to be useful in breeding new lines resistant or tolerant to many fungal diseases (Nazim *et al.*, 1988, Saber, 1993, Abada 1995 and Saber *et al.*, 1998).

Unfortunately, all pea cultivars grown in Egypt are vulnerable to infection with rust. Therefore, increasing pea production may be achieved through increasing the yield per unit area, mainly through developing new high yielding genotypes resistant or tolerant to diseases.

Breeding resistant or tolerant pea cultivars is an important method for plant disease control and crop management. However, conventional breeding methods have been tried, but the results did not contribute much for developing resistant lines. Therefore, it is thought that induced mutations may be a useful tool to enrich the variability with novel mutant genes for resistance or tolerance against the pathogen.

Scope of study:

The aim of the present investigation is to use gamma-rays and EMS as chemical mutagen to induce mutations for resistance or tolerance to rust disease of pea. Selection and characterization of mutant lines derived from some locally adapted varieties is the final goal of the present study. Assessment of resistance on a biochemical basis will be determined.

MATERIALS AND MEHTODS

Survey of infection by rust disease:

Survey of rust disease on pea plantations was carried out in some governorates *i.e.* Behera, Dakahlia, Kafr-El sheikh, Qalubia, Sharkia and Giza during 1997 /1998 growing season.

A Mutation induction program was carried out to improve disease resistance cvs.of Little Marvel and Teledo Sugar pea using Ethyl Methan Sulphonate (EMS) and gamma rays. The experiments were carried out in a farm at Nobariya, Behera governorate during growing seasons lengthened from 1998/99 to 2001/2002. A lot of 850 g seeds representing each cultivar was used for each treatment.

Mutagenic treatments

Dry seeds were exposed to gamma rays at doses of 50, 100 and 150 Grey = (5, 10 and 15 k-rad.) at the Middle Eastern-Radio-Isotope Center for Arab Countries at Dokki, Giza.

Ethyl Methane Sulphonate (EMS) as a potent chemical mutagen, was used in the present study. The following concentrations were applied (0.25, 0.75 and 1.5% at pH=7.0). Treatment conditions are mainly those described by Brunner (1977) based on Manual in Mutation Breeding of the International Atomic Energy Agency in 1977.

Assessment of mutagenic effects

M_1 and further generations

The treated pea seeds were stored in a refrigerator at 10°C for 24 hours and then sown in field plots, each of 1/500 feddan consisted of 3 ridges were sown in hills (20 cm apart) and each treatment was replicated three times.

The seeds of M_1 generation were sown in mid of November of 1998 at the rate of three seeds in each hill with 20 cm apart. The plants were left to natural infection in the field and received all the agricultural practices recommended for pea production. At the end of the 1998 /1999 growing season seeds of the survived plants (M_2-seeds) were collected and stored pea seeds representing the M_2- generation were sown during 1999/2000 growing season. At the end of the season, the seeds of resistant plants were collected separately and were stored for sowing the M_3 and M_4 generations during November of 2000 and 2001, respectively. However, moderately resistant and susceptible plants were scored separately and pooled harvested together, as the interest was focused to select resistant plants.

The mutagenized plants were left to natural infection with rust disease at nubaria locality where favourable environmental conditions are prevalent and suitable for rust infection.

Disease assessment:

Classification of the mutagenized plants to resistant, moderately resistant and susceptible was estimated according to disease index proposed by Hanounik (1986).

Resistant plants (M_4) to rust were checked under artificial inoculation in the greenhouse with urediospores of *Uromyces pisi*.

Impact of the mutagens on some crop parameters

The effect of the mutagens on some crop parameters of the resistant genotypes of M_4 of the two cultivars in comparison with the original types was taken into consideration. The following crop parameters were recorded in M_4 generation, *i.e.* plant height, number of pods/plant, number of seeds/plant and weight of 100 seed.

Biochemical studies for testing resistance

Samples of M_4-plants were biochemically tested to determine the basis of resistance to rust disease. Peroxidase activities were determined following the method of Allan and Hollis (1972), Polyphenol oxidase activities were determined following the method of Maxwell and Batman (1967) Total free amino acids content of the resistant genotypes of M_4 of the two cultivars in comparison with original types was taken into consideration which determined as the percentage content to the dry weight of the seeds according to the method of Rosein (1957). The mutant plants and the controls were grown in pots in the greenhouse under isolated conditions using polyethylene covers. Seedlings 15 days old were artificially inoculated by urediospores of *U. pisi*. Infected leanes were collected after about 45 days from sowing to determine the activities of the enzymes. This was done in the Central Laboratory for Biochemical Analysis, Fac. of Agric. Cairo Univ. Giza.

Electrophoretic detection of protein by sodium dodecyel sulphate poly-acrylamide gel electrophoresis (SDS-PAGE):

Polyacrylamide gel electrophoresis (PAGE) was used to determine the quantitative changes that occur in the soluble proteins of different M_4 mutants of cvs. Little Marvel and Teledo Sugar for resistant and susceptible inoculated and uninoculated plants according to the method of Broglie *et al.* (1986).

Protein extraction:

Leaves of similar age were taken from inoculated and uninoculated plants of M_4 mutants, cvs of Little Marvel and Teledo Sugar. Protein content and staining of protein bands was determined using the method described by Ahmed (2001).

RESULTS

Survey of infection by rust disease

Survey of natural infection by pea rust in the inspected governorates revealed that Behra governorate plantations recorded the highest infection (65 %) followed by those of Kafr EL-Sheikh and Dakahlia governorates (57 and 56%, respectively). Meanwhile, pea plantations of Giza governorate showed the lowest infection (41 %) followed by those of Sharkia and Kalubia governorates (48 and 50 %) respectively .

Response of treated plants to infection

M_2-generation

Table (1) shows the population size of M_2 plants and also the number of selected plants. M_2 seeds of selected plants of pea from each mutagenic treatment were planted and screened for rust resistance. M_2 plants were kept under strict observation until flowering stage in order to observe and record the reaction of the growing plants to rust disease, where, the whole field was severely attacked with the disease. The experiment was kept under the stress of severe infection until ripening. In general, the plants showing delay in infection, less number and smaller pods were considered susceptible (S). On the contrary, plants with slight-infection and normal growth were considered resistant (R). Based on the foregoing classification, the M_2-data are presented in Table (1).

Data presented in Table (1) indicate that the 0.25% EMS did not result in any resistant plants in the cv. Little Marvel. The highest percentage of resistant plants were recorded with 0.75% EMS for cv. Little Marvel being (2.3%).

M_3-generation:

Results shown in Table (1) show that M_3 plants were also classified into resistant (R), tolerant (MR) and susceptible (S) plants in response to rust infection. The reaction of the tested mutants to rust disease was greatly varied due to irradiation and chemical mutagen. Data presented in Table (1) reveal that the highest percentage of resistant plants to rust occurred as a result of the concentration of 1.5% EMS and the dose of 100 Grey gamma rays for cv. Little Marvel being (15.6% and 12.3%), respectively. However, the lowest percentage was recorded for cv. Teledo sugar with 100 and 50 Grey gamma-rays (4.4 and 4.8%), respectively and with 0.75% EMS (5.6%).No resistant plants were obtained with 150 Grey gamma-rays for both cultivars in M3 and with 0.25% EMS for Little Marvel cultivar. In all generation cases the percentage of (MR) plants was higher than the percentage of (S) plants.

M_4-generation

Table (1) and Fig.(1) shows the number of M_4 plants in the different classes of resistance to the pathogen. It is evident that the percentage of resistant M_4 plants (R) is 12.0% in cv. Little Marvel and 11.7% in cv. Teledo Sugar. However, (MR) and (S) plants are the majority.

Table (1): Number and percentage of resistant plants to pea rust disease selected as mutants in M_2 , M_3 and M_4 generations.

Generation	Mutagenic treatment		Cultivars													
			No. of Screened Plants	Little Marvel						No of screened plants	Teledo Sugar					
				(R) *		(MR) **		(S) ***			(R) *		(MR) **		(S) ***	
				No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
M_2 1999/2000		0.25	380	-	-	130	34.2	250	65.8	394	2	0.5	161	40.9	231	58.6
	EMS	0.75	350	8	2.3	116	33.2	226	64.6	383	5	1.3	152	39.7	226	59.0
	%	1.50	360	5	1.4	115	31.9	240	66.7	300	3	1.0	150	50.0	147	49.0
	Gamma	50	390	7	1.8	180	46.2	203	52.1	363	5	1.4	148	40.8	210	57.9
	Rays	100	360	4	1.1	120	33.3	236	65.5	358	6	1.7	120	33.5	232	64.8
	(Grey)	150	350	1	0.3	198	55.6	151	43.1	310	2	0.6	112	31.6	196	63.2
	+ Total		2190	25	1.1	859	39.2	1306	59.6	2108	23	1.1	843	40.0	1242	60.0
	Control		320	0	0.0	0	0.0	320	100	340	0	0.0	0	0.0	340	100
M_3 2000/2001		0.25	-	-	-	-	-	-	-	106	9	8.5	60	56.6	37	34.9
	EMS	0.75	170	18	10.8	100	58.9	52	30.6	195	11	5.6	122	62.6	62	31.8
	%	1.50	64	10	15.6	33	51.7	21	45.7	88	6	6.8	44	50.0	38	43.2
	Gamma	50	120	13	10.8	51	42.5	56	46.7	125	6	4.8	71	56.8	48	47.8
	Rays	100	73	9	12.3	37	50.7	27	37.0	90	4	4.4	46	51.1	40	44.4
	(Grey)	150	50	-	-	32	64.0	18	36.0	30	-	-	17	56.7	13	43.3
	+ Total		477	50	11.1	253	53.0	17.4	36.4	634	36	5.7	360	56.8	238	37.5
M_4 2001/2002		0.25	-	-	-	-	-	-	-	100	9	9.0	60	60.0	31	31.0
	EMS	0.75	193	22	11.4	109	5.65	62	32.1	92	13	14.1	42	45.7	37	40.2
	%	1.50	102	13	7.5	63	61.8	26	25.5	63	7	11.1	36	57.1	20	31.7
	Gamma	50	180	19	10.6	96	53.3	65	36.1	60	8	13.3	30	50.0	22	36.7
	Rays	100	98	15	15.3	45	45.9	38	38.8	43	5	11.6	23	53.6	15	34.9
	(Grey)	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	+ Total		573	69	12.0	313	54.6	191	33.3	358	42	11.7	191	53.4	125	34.9

\+ Mutagenized material (control not included)

\- No resistant plants were obtained

In M_3 and M_4 controls were highly susceptible

* (R) Resistant plants, disease severity less than (20%)

** (M) Moderately resistant, disease severity between (20-50%)

*** (S) Susceptible plants, disease severity over (50%)

A

Fig.(1A): Infected pea plant showed typical symptoms of the rust. (Susceptible plants)

B

C

Fig. (1B&C): Fourth generation of pea plant cv. (Little Marvel) genetically resistant to artificially infection with *U. Pisi* which previously treated with EMS and Gamma-rays, respectively.

Some of the resistant M_4 plants appeared to be more productive than the control. The selected mutants were retested and reselected in M_4 generation.

The data suggest that the lowest EMS concentration (0.25%) was not sufficient to induce mutants for resistance to rust disease. Similarly, highest gamma-ray dose (150 Grey) was too high to get fertile plants.

In total, EMS gave resistant lines more than resistant lines by gamma rays. The data suggest that EMS is more effective than gamma-rays in inducing rust resistant lines.

Some crop parameters of the mutants (R) in the M_4 generation, are presented in (Table 2). Data indicate that (R) lines of both cultivars showed an increase in plant height, number of pods/plant, number of seeds/plant and weight of 100 seed in comparison with control.

Evaluation of Resistant Mutant Plants and their Biochemical Changes:

The mutant plants resistant to rust disease, selected in the M_4-generation, were evaluated agronomically and biochemically compared to the healthy (not infected) and to the susceptible (infected) control plants. The mutant plants selected as resistant (R) in M_4-generation along with the susceptible and healthy ones as controls were also grown under greenhouse conditions. The material was artificially inoculated by fungal spores. Sample of the resistant mutant plants and the controls were used to determine peroxidase, polyphenol oxidase activities of leaves and protein content of seeds.

The biochemical data of the peroxidase and polyphenol oxidase activities were very interesting as they reflect different levels of resistance to the fungus causing rust disease. Taking into consideration the level of peroxidase activity, in susceptible and resistant plants in Table (2), variation in resistance to the fungus was indicated by variation in peroxidase activity.

It is clear from the data in Table (2), that the mutant plants were highly variable in their resistance to *U. pisi*. The same trends was noticed by polyphenol oxidase.

Data presented in Table (2) clearly indicate that infection by rust disease caused pronounced increases in both polyphenol oxidase and peroxidase activities. However, the values obtained for resistant (R) mutant plants were clearly higher compared to susceptible and non-inoculated plants as well as control plants. In addition, there were no clear difference, in amino acids content between EMS and gamma-ray, induced mutants derived from both pea cultivars used in the present study. On the contrary, mutants derived after mutagenic treatments with gamma rays and EMS showed higher amino acids content compared to control. The pronounced increases in enzyme-activities of the (R) mutant plants were good indicators for the changes happening in their genetic background due to mutagenic treatments applied, since the enzymes are direct products of the genes.

However, the mutants will be subjected to further pathogenic tests, and to biochemical and molecular genetic analyses, to re-select the most promising ones to be developed as new varieties.

Changes in protein patterns extracted from mutant plants due to infection by *U. pisi* (with sodium dodecyel sulphate - polyacrylamide gel electrophoresis (SDS-PAGE):

Data presented in Table (3) and Fig. (2) indicate that the pea healthy plants showed considerable variation in their protein patterns. The susceptible plants contained the highest number of proteins as compared with the resistant mutant plants of both cultivars. Infection by *U. pisi* resulted in remarkable changes in protein patterns of all mutant plants. Number of induced proteins in pea plants infected by *U. pisi* were 9 and 8 for the susceptible plants compared with 6 in the resistant mutant plants. Data also indicated that the disappeared proteins in pea plants due to infection by *U. pisi* ,*i.e.* proteins number 10 and 12 for the susceptible plants compared with protein number 7 in the resistant mutant plants of EMS and gamma rays. Other proteins in all mutant plants not changed or slightly increased or decreased due to infection. Generally the changes in protein patterns due to the infection were higher in the susceptible plants compared with the resistant mutant plants.

DISCUSSION

The present investigation was a trial to use mutation breeding as a technique to induce mutant genes for resistance against the fungus *Uromyces pisi* causing rust disease in pea. Both EMS and gamma-rays, as potent mutagens, were used in treating the seeds of two local cultivars, widely grown in Egypt. *i.e.* cvs Little Marvel and Teledo Sugar.

Table (2): Mean yield parameters and biochemical attributes of resistant mutant plants selected from the parent cultivars Little Marvel and Teledo Sugar in M_4 generation.

Cultivar	Mutagen treatment		No. of mut. plants	Plant height (cm)	No. of pods/plant	No. of seeds/plant	Weight of 100 seed (g)	Total free amino acid	Peroxidase activity	Polyphenol oxidase activity
Little Marvel	EMS* %	0.25	-	-	-	-	-	-	-	-
		0.75	22	65.2	17.3	90.7	20.7	1.5	70.3	36.1
		1.50	13	63.4	15.5	93.0	23.9	1.2	72.7	30.9
	Mean			64.3	16.4	91.9	22.3	1.4	71.5	33.5
	Gamma rays * (gray)	50	19	67.7	13.3	91.0	25.7	1.7	79.3	30.3
		100	15	60.9	12.5	80.3	20.3	1.9	74.2	31.6
		150	-	-	-	-	-	-	-	-
	Mean			64.3	12.9	85.7	23.0	1.8	76.0	31.0
	Control** (1) (100 plants)			58.0	10.0	66.2	17.3	0.9	66.6	22.7
	Control *** (2) (100 plants)			36.3	7.3	39.7	15.5	2.3	70.3	26.3
Teledo Sugar	EMS* %	0.25	9	63.7	16.2	98.3	21.7	1.3	75.3	30.2
		0.75	13	60.3	15.3	95.3	21.3	1.2	77.9	36.7
		1.5	7	63.5	12.1	92.7	20.1	0.9	73.1	29.1
	Mean			62.5	14.5	95.4	21.0	1.1	75.4	32.0
	Gamma rays * (gray)	50	8	65.5	14.8	87.3	22.3	1.9	79.0	33.7
		100	5	62.2	13.7	90.5	20.2	2.1	78.3	30.3
		150	-	-	-	-	-	-	-	-
	Mean			63.9	14.3	89.4	21.3	2.0	78.7	32.0
	Control** (1) (100 plants)			60.1	12.4	70.7	18.0	0.7	66.7	23.0
	Control *** (2) (100 plants)			40.3	7.5	40.1	13.3	2.2	70.1	25.9

* = Inoculated plants with *Uromyces pisi*.

** = Control (1) healthy plants (not treated with EMS or gamma rays).

*** = Control (2) susceptible plants (not treated with EMS or gamma rays).

(-) = No resistant plants were obtained

Table (3): Changes in proteins extracted from mutant plants cvs. Little Marvel and Teledo Sugar due to infection by *Uromyces pisi* (with sodium dodecyel) sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

	Mutant plants / cv. Little Marvel									Mutant plants / cv. Teledo Sugar								
	(S) Susceptible			(R) EMS			(R) Gamma-rays			(S) Susceptible			(R) EMS			(R) Gamma-rays		
	*MW of protein (Kd)	Healthy	Infected	*MW of protein (Kd)	Healthy	Infected	*MW of protein (Kd)	Healthy	Infected	*MW of protein (Kd)	Healthy	Infected	*MW of protein (Kd)	Healthy	Infected	*MW of protein (Kd)	Healthy	Infected
1.	103	NC	NC	103	NC	NC	103	NC	NC	102	NC	NC	106	NC	NC	104	NC	NC
2.	96	-	⊕	98	+	θ	102	+	θ	95	-	+	98	+	θ	101	+	θ
3.	71	+	θ	71	+	θ	100	+	θ	71	+	θ	71	+	θ	100	+	θ
4.	73	-	⊕	70	+	θ	83	+	θ	72	-	+	70	+	θ	85	+	θ
5.	68	+	θ	65	-	⊕	80	-	⊕	66	+	θ	68	-	⊕	79	-	⊕
6.	63	-	⊕	63	NC	NC	75	NC	NC	63	-	+	60	+	⊕	74	NC	NC
7.	60	+	θ	62	+	⊕	66	+	⊕	60	+	θ	62	+	⊕	65	+	⊕
8.	53	+	θ	56	NC	NC	63	+	⊕	51	+	θ	57	NC	NC	60	NC	NC
9.	50	-	⊕	55	NC	NC	40	NC	NC	45	-	+	53	NC	NC	39	NC	NC
10.	39	+	θ	50	+	θ	39	-	⊕	38	+	θ	51	+	θ	36	-	⊕
11.	36	-	⊕	43	+	θ	35	+	θ	36	-	+	40	+	θ	33	+	θ
12.	32	+	θ	41	-	⊕	32	-	⊕	31	+	θ	39	-	⊕	30	NC	NC
13.	31	-	⊕	40	NC	NC	30	NC	NC	30	-	+	36	+	θ	29	NC	NC
14.	29	NC	NC	33	-	⊕	35	-	⊕	27	NC	NC	33	-	⊕	25	-	⊕
15.	26	+	θ	30	+	θ	20	+	θ	26	+	θ	30	+	θ	21	+	θ
16.	25	-	⊕	28	NC	NC	18	-	⊕	24	-	+	27	NC	NC	17	-	⊕
17.	22	NC	NC	25	+	θ	17	+	θ	22	+	θ	23	+	θ	15	+	θ
18.	20	-	⊕	23	NC	NC	16	NC	NC	20	-	+	20	+	⊕	14	NC	NC
19.	18	+	θ	19	+	θ	15	NC	NC	17	+	θ	18	+	θ	13	+	θ
20.	15	NC	NC	18	-	⊕	10	-	⊕	14	NC	NC	17	-	⊕	10	-	⊕
21.	13	-	⊕	16	+	θ				12	-	+	13	+	θ			
22.	12	NC	NC	15	NC	NC				11	NC	NC	15	NC	NC			
23.	11	NC	NC							10	NC	NC						
24.	10	+	θ							9	+	θ						

* MW : Molecular weight
+ , - : Indicating proteins present + or absent - in healthy plants.
⊕, θ : Indicating proteins induced ⊕ or disappeared θ in pea plants infected with *U. pisi.*
NC : Not changed

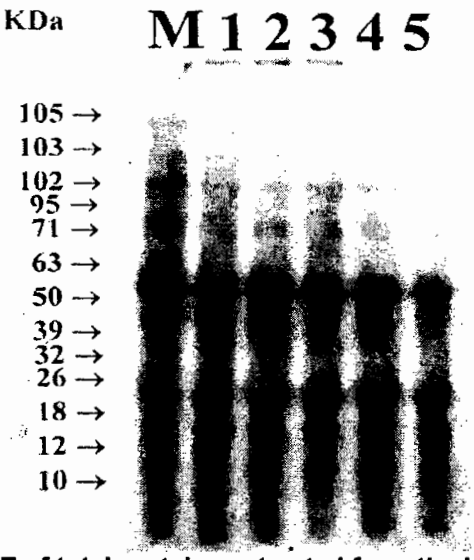

Fig.(2):SDS-PAGE of total proteins extracted from the infected leaves of different mutant plants cvs. Little Marvel and Teledo Sugar.

M = Molecular weight protein markers.
1 = Mutant resistant (EMS) from cv. Little Marvel.
2 = Mutant resistant (gamma-rays) from cv. Little Marvel.
3 = Mutant resistant (EMS) from cv. Teledo Sugar.
4 = Mutant resistant (gamma-rays) from cv. Teledo Sugar.
5 =Susceptible plants.

The results of this study showed that few resistant plants were selected in M_2-generation. However, in M_3 and M_4 generations, a higher number of resistant plants was recorded. The decrease in the ratio of less susceptible mutants in later generations was also recorded by Abada (1995) and Saber *et al.* (1998).

Sowing the seed bulk of each treatment of M_1 resulted in growing plants that could be classified into (R), (MR) and (S) plants in (M_2 progeny). Meanwhile, no (R) plants were grown in the progeny of cultivar, Little Marvel "exposed to 0.25% EMS in M2 generation, and for both cultivars at 150 Grey-gamma rays in M_3 and M_4. This result may suggest that the dose 150 Grey was highly lethal to pea seeds.

Mutagenized population in the M_4 generation recorded an increase, to some extent, in the (R) plants in comparison with those of M_2 and M_3 generations. Moreover, the progeny of all treatments showed noticeable increase in the percentage of (MR) plants than (S) ones in M_2 and M_3 generations compared with those of M_4 generation, where the percentages of (S) plants were higher than those in M_2 and M_3 generations.

The fluctuation in the reaction of each generation to rust infection may be due to the continuous segregation in the later generations. The results, in general, are in agreement with the findings of many investigators (Nazim *et al.*, 1988; Abada 1995 and Saber *et al.* 1998).

The percentages of (R) plants were very low but increased gradually by progression of generations. It may be stated that the low numbers of (R) plants obtained in the different generations are due to the high population density of aggressive inoculum and favourable environmental conditions prevailing in Nobariya region.

The results showed a positive correlation between resistance and yield parameters. Treatments which decreased the susceptibility, resulted in plants with higher seed yield than those of control. In addition, low differences were found in the vegetative growth of the mutagenized plants in most cases. Enhancing effect was recorded by Abada (1995) and Saber *et al.* (1998).

Seeds of plants marked as resistant mutants were collected separately and sown in the next season to follow their reactions to rust disease. Most of the selected plants yielded homogenous populations of resistant plants, mostly with a better vegetative growth than the control. To confirm, that novel genes for resistance could be induced by the present mutagenic treatments, samples of the mutant plants were subjected to biochemical investigations to assess the basis of resistance.

The results of the present investigation, together with those of Abada (1995) and Saber *et al.* (1998) substantiate the importance of mutation breeding as a tool in breeding legumes for disease resistance. It is well established that activities of the pathogen and the response of the host plant to infection establish the basis for the outcome of the struggle between both organisms. The interaction is usually accompanied by some alterations in the plant biochemical pathways and constituents. This has been the area of interest of much research activities aiming to establish possible relation of susceptibility or resistance to certain groups of plant constituents, (Sunder *et al.*, 1998). During this investigation, the activities of polyphenol oxidase, peroxidase and total protein content were detected in the tissues of healthy and infected pea plants. The findings obtained indicate that there are biochemical changes in the studied plants. However, the increment of the chemical components, was always higher in the tissues of resistant plants in comparison with the susceptible ones and control. In addition, the highest values of each component were recorded in the tissues of the M_4 plants. It was evident from the results that infection with pea rust causes pronounced increase of polyphenol oxidase, peroxidase activities and protein content in the inoculated mutant and control plants, compared with non-inoculated plants. This increase was more pronounced in tissues of (R) plants especially in M_4 generation, which reflects the fact that a change has happened in the genetic background of the mutated plants. On the other

hand, polyphenol oxidase and peroxidase activities were greater in the compatible host-parasite combinations. With respect to polyphenol oxidase activities, the enzyme have an influence on the virulence of some pathogens as well as upon the host-parasite coexistence. Also peroxidase is known to catalyze the redox reaction between H_2O_2 as an electron acceptor and many kinds of substrates, it also showed an oxidative activity beside the peroxidative activity, i.e. the oxidation of different substrates by atmospheric oxygen without exogenous peroxidase (Sunder *et al.* 1998).

Chemical analysis of the infected and healthy pea plants showed variation in amino acids content in infected plants compared with healthy ones. The increase in total amino acids may be due to host pathogen interaction. Amino acids would have accumulated in the infected leaves due to the blockage of protein synthesis or due to proteolysis caused by the pathogen. (Ahmed, 2001).

The healthy tested pea plants showed considerable variation in their protein profiles. Infection by *U. pisi* resulted in changes and induction of new proteins. The induced proteins in the susceptible plants were 9 and 8, respectively, compared with 6 for resistant mutants. These results indicate that pathogenic related proteins (PR proteins) varied according to the genotypes as mentioned by Ahmed, (2001). On the other hand, Hlinkova and Sykora (1996) registered eight new proteins for sensitive reactions during their studies on the changes of protein patterns induced by barley powdery mildew infection.

Other proteins in tested pea mutant plants were disappeared, increased or decreased due to infection by *U. pisi*. It may be concluded that PR-proteins reflected a particular type of stress which induced changes in plant metabolism. The results were in harmony with those reported by Ahmed (2001).

REFERENCES

Abada, K. A. (1995). Induced mutations by gamma rays for rust resistance in faba-beans. Bull. Fac. Agric. Univ. Cairo, 46: 299-310.

Abada, K. A.; M. M. Saber and M. A. Mostafa (1997). Control of pea rust disease under Egyptian conditions. 8th Congress of the Egyptian Phytopathol. Soc., Cairo, 199-210.

Ahmed (Saieda) S. A. (2001). Further studies on soybean anthracnose disease in Egypt. Ph.D. Thesis, Fac. of Agric., Cairo Univ.

Allan, A. I. and J. P. Hollis (1972). Sulfide inhibition of oxidase in rice roots. Phytopathology, 62:634-639.

Broglie, K. E.; J. J. Gaynor and R. M. Grogli (1986). Ethylene-regulated gene expression: Molecular cloning of the encoding on endochitinase from *Phaseolus vulgaris*. Proc. Nat. Acad. Sci., 83:6820-6824.

Brunner H. (1977). Mutagenicity of ethyl methane sulfonate and sodium azide in grain legumes. Mutation Breeding Newsletter. IAEA, Vienna, 9:8.

Hanounik, S. B. (1986). Screening techniques for disease resistance in faba-bean. International Center for Agricultural Research in Dry Areas. (ICARDA). Aleppo. Syria.

Hlinkova, E. and M. Sykora (1996). Changes in extracellular protein patterns induced by powdery mildew (*Erysiphe graminis* f.sp. *hordei)* infection. Proc. of the 9th European and Mediterranean Cereal Rusts and Powdery Mildew Conf. 2-6 September, Lunteren, the Netherlands.

International Atomic Energy Agency (IAEA) (1977). Manual in mutation breeding.

Khafagi, Y. S.; A. M. Abo El-Ella; Ahmed, Fatma S.; and El-Gizy, Samira, M. (1995). Pea rust in Egypt. Menofiya J. Agric. Res., 20 (6): 2155-2170.

Maxwell, D. P. and D. E. Batman (1967). Changes in the activities of some oxidases in extracts of Rhizoctonia- infected been hypocotyls in relation to lesion maturation. Phytopathology. 57:132-136.

Nazim M.; A. El-Hosary and M. S. Rady (1988). Induced mutations for disease resistance to leaf spot fungi in soybean. International Atomic Energy Agency, Vienna, 383-397.

Roseon, H. (1957). A modified ninhydrine colorimetric analysis for amino acids. Arch. Biochlem. Biophysiol., 67:10-51.

Saber, M. M. (1993). Pathological and genetical studies on peas powdery mildew disease Ph. D. Thesis., Fac. of Agric., Cairo Univ.

Saber M. M.; Hussein Mona H. and S. H. Ahmed (1998). Pathological and Biochemical Assessment of Gamma-Ray induced Mutants for Resistance to Yellow Rust in Wheat. Arab J. Biotech. 1:59-668

Sunder A. R.; R. Viswanathan; J. D. Mohanra and P. Padmanaban (1998). Role of oxidative enzyme in sugar cane and *Colletotrichum falcatum* interaction. Acta Phytopathologica et Entomologica-Hungarica, 33: 3-40, 297-304.

دراسات باثولوجية وبيوكيميائية لتحديد درجة مقاومة الطوافر المستحدثة بأشعة جاما والمطفر الكيميائي EMS لنبات البسلة لمقاومة مرض الصدأ

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الملخص العربي

تعتبر البسلة أحد المحاصيل البقولية الهامة ليس فقط على المستوى المحلي بل أيضا على المستوى العالمي. تتعرض نباتات البسلة خلال موسم النمو للإصابة بالعديد من الأمراض التي تؤثر على إنتاجية المحصول وجودته، ومن أهم هذه الأمراض مرض الصدأ الذي ينتشر في العديد من مناطق زراعة البسلة في مصر. وقد أجرى هذا البحث بهدف مقاومة هذا المرض بعيداً عن استخدام المبيدات وذلك باستحداث طفرات مقاومة للمرض، حيث تم تعريض اثنين من أصناف البسلة التجارية لأشعة جاما بتركيز ٥٠، ١٠٠ ، ١٥٠جراى وكذلك للـ (EMS) بتركيزات مختلفة هي ٠,٢٥ ، ٠,٧٥ ، ١,٥٠ %، وأجريت هذه التجارب لمدة أربعة أجيال طافرة حيث أدت المعاملة بالمطفرات إلى الحصول على نباتات مقاومة، ومتوسطة المقاومة، وقابلة للإصابة بمرض الصدأ وذلك في الأجيال الثاني والثالث والرابع، وكانت أعداد هذه النباتات متباينا خلال الأجيال الثلاثة، كانت أعداد النباتات المقاومة قليلة جداً في الجيل الثاني، وبدأت تتزايد في الأجيال المتعاقبة حيث بلغت النسبة المئوية للنباتات المقاومة (١٢%) في الجيل الرابع. كانت أعلى نسبة نباتات مقاومة متحصل عليها في الجيل الرابع عند تركيز ٠,٧٥ % لـ EMS ، ١٥٠ جراى لأشعة جاما وذلك للصنف ليتل مارفل، في حين كانت أعلى نسبة نباتات مقاومة للصنف تيليدو شوجر عند المعاملة بواسطة أشعة جاما عند جرعة قدرها ٥٠ جراى وكذلك لـ EMS عند تركيز ٠,٧٥%. لم يتحصل على أي نباتات مقاومة من الصنف ليتل مارفل عند تركيز قدره ٠,٢٥%EMS في كل الأجيال المتعاقبة، وكذلك لم يتحصل على أي نباتات مقاومة عند جرعة قدرها ١٥٠ جراى لأشعة جاما للصنفين وذلك في كل من الجيلين الثالث والرابع. وأظهرت نباتات الجيل الرابع المقاومة زيادة في طول النباتات وعدد قرون وبذور لكل نبات ووزن الـ ١٠٠ بذرة مقارنة بالنباتات القابلة للإصابة غير المعاملة.

دلـــت الدراســـات البيوكيميائية على المنتخبات الجديدة للطوافر أن النباتات القابلة للإصابة كـــان محـــتواها مـــن الأحمـــاض الأمينية أعلى من النباتات المقاومة، وأن معدل نشاط أنزيم البيروكســـيديز والبولـــي فينول اوكسيديز كانت عالية في الطوافر المقاومة عن النباتات القابلة للإصـــابة وكانـــت هـــناك علاقة موجبة بين معدل نشاط الأنزيم والمقاومة لكل المعاملات.وقد أظهـــرت نباتات الجيل الرابع اختلافات في عدد البروتينات المستحدثة وقد أدت الإصابة بالفطر إلى استحداث عدد كبير من البروتينات في جميع المنتخبات الجديدة للنباتات بالإضافة إلى زيادة أو نقـــص فـــي بعض البروتينات الأخرى وكان عدد البروتينات التي استحدثت في النبات القابل للإصابة كانت كبيرة مقارنة بعدد البروتينات التي استحدثت في الطوافر المقاومة.