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BIOCHEMICAL GENETIC MARKERS FOR LEVELS OF RESISTANCE TO COWPEA APHID BORNE MOSAIC POTYVIRUS IN SESAME IRRADIATED WITH GAMMA RAYS

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ABSTRACT

This is the first record of Cowpea Aphid Borne Mosaic Potyvirus (CABMV) on sesame in Egypt. CABMV was originally isolated from naturally infected sesame plants growing in Giza Res. Station, which showing mosaic, stunt, necrosis, and deformation symptoms. After biological purification, the identity of the virus isolate was confirmed by Dot- blot immunoassay (DBIA) using specific antiserum against CABMV. The virus isolate was used to inoculate sesame plants in M_2 and M_3 generations. Three sesame cultivars: Giza 32, Toshky 1 and Shandaweel 3, were exposed to 0, 50, 100, 150, 200, 250 and 300 Gy of gamma rays at a dose rate of 0.8 Gy min^{-1}. They were grown at Giza Research Station, ARC in three successive generations. In M_2 generation, significant differences were found between the three sesame cultivars over the gamma ray doses and inoculation by CABMV for plant height, first capsule height, number of branches, seed yield per plant and oil content. Almost all the interactions were significant. It seems that 150 Gy treatment increased most of studied characters in M_2 generation of sesame. The interaction of cultivars x dose treatment was significant for all studied characters indicating various responses of studied cultivars to applied gamma ray doses. In M_3 generation after selection for the relatively resistant plants to CABMV using 10% selection intensity, the statistical analysis showed that the differences between the cultivars were significant for plant height, first capsule height and seed yield. All irradiation doses increased seed yield/plant comparing with control. It means that selection for resistance to CABMV in M_2 generation had positive effects on the resistance for this virus in M_3, beside its positive effect on the yield and its components and oil content. Reduction was observed in almost all studied characters and genotypes as affected by the virus infection. The effect of CABMV on three enzyme activities; Peroxidase (POD), Polyphenyloxidase (PPO) and Catalase (CAT) of 21 sesame genotypes in M_2 and M_3 derived from irradiation by gamma ray doses was studied. Analysis of variance for enzyme activities showed significant differences for all studied enzymes, infection statuses, genotypes and all their interactions. The activities of all studied enzymes were increased in almost all genotypes developed via irradiation compared with the un-irradiated ones under both control and infection conditions except (CAT) in Toshky 1 genotypes under the two conditions. On the other hand, POD, PPO and CAT were increased under infection conditions comparing with the uninfected one and vice versa for PPO in the Toshky 1 genotype developed via irradiation with 250 Gy dose. In the same time, this genotype was the most resistant one which showed the mild symptom in the field. The electrophoretic banding patterns of proteins (SDS-PAGE) extracted from the leaves of three sesame cultivars and their genotypes (that developed via irradiation and selection) under CABMV stress and unstressed conditions revealed a

different number of bands according to the genotype, the irradiation dose and the infection status, with different molecular weights (MW). Data showed four, one and four common bands (monomorphic), in Giza 32, Toshky 1 and Shandaweel 3, respectively. The SDS-protein banding pattern was found to be useful in identifying the induction of variations in the genotypes as a result of irradiating three sesame cultivars with different gamma ray doses based on the protein level. We can conclude that, proteins with molecular weight 82.0 and 38.0 KDa which were found only under infected conditions were found as proteins markers associated with mild to CBAMV symptoms as in Toshky 1 genotype developed via irradiation with 250 Gy.

Key words: *Sesame, Sesamum indicum L., Gamma ray, Mutation, Virus, Cowpea Aphid Borne Mosaic Potyvirus (CABMV), Enzyme activity, SDS-PAGE.*

INTRODUCTION

Sesame (*Sesamum indicum* L) is an important oil crop. It is a rich source of oil and protein. This crop is cultivated in different parts of the world. Cowpea Aphid Borne Mosaic Virus (CABMV) which is considered a member of Potyvirus virus was recorded in Africa (Kenya, Uganda and Nigeria), Europe (Italy, and Rumania), and Asia (India, Iran, Japan, and China) (Brunt *et al* 1996). Also, this virus (CABMV) was first recorded on sesame plants in USA (Pappu *et al* 1997). Bashir *et al* (2002) reported that Cowpea aphid-borne mosaic potyvirus (CABMV) is a cosmopolitan, economically significant seed-borne virus of cowpea. It can cause a yield loss of 13 - 87% under field conditions depending upon crop susceptibility, virus strain and the environmental conditions. CABMV has spread world-wide through the exchange of virus-infected germplasm material. The virus-infected seed provides the initial inoculum and aphids are responsible for the secondary spread of the disease under field conditions. The virus symptoms vary with the cowpea genotype and virus strain. Excellent sources of resistance are available for the breeding of resistant cultivars. Resistance in cowpea is conferred by either a dominant or a recessive gene. Enzyme-linked immunosorbent assay (ELISA) is the most appropriate method for the detection of the virus in the seed or plant tissue for seed certification programmes.

The goals of plant breeding are to contribute to a qualitative and quantitative improvement in crop production. Breeding for resistance to diseases has played a significant role to avoid crop losses directly. Among plant pathogens, viruses are known to cause significant losses to most of the major crops around the world. Therefore, extensive work has been done on breeding for resistance to plant viruses (Russell 1978 and Fraser 1992). The breeding of resistant varieties of plants, if available, is an effective strategy for minimizing the losses caused by viral diseases. This is largely due to intrinsic properties of the plant viruses which do not permit their control by simple physical and chemical methods. The major advantage of breeding for

resistance to viruses is that once a resistant cultivar is developed no specific action is required by farmers to achieve control.

The natural genetic variability may be exhausted and/or the conventional breeding methods became insufficient to realize the needed improvement. Therefore, since 1950's more researchers turned towards the mutagenic treatments to enhance useful genetic variation. Several physical and chemical mutagenic agents: X-rays, neutrons, ionizing radiation (gamma and beta rays), UV, ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), ethylene imine (EI), diethyl sulphonate (DES) and colchicines were applied for inducing mutations in different oil seed crops (Shrief 1998).

In general, mutation breeding of oil crops covers wide fields, e.g., modified plant architecture, higher yields, biotic resistance, abiotic tolerance, earliness and the quality as well as the quantity of produced oil. Induced mutations produced directly or indirectly 110 cultivars in the main oil crops species by 1990 (Ashri 1994).

Ragab (1978) investigated the effects of acute gamma ray doses on the magnitude of variability in the economic characters of the two sesame cultivars: Giza 23 and Giza 24 and the possibility of isolation of some mutants with superior economic characteristics. Encouraging and wide ranges of variability were detected for yield and yield components by Shrief (1983) who selected and evaluated a number of superior mutants under Egyptian conditions.

Sesame appears to be an ideal material for mutation breeding, using both naturally occurring and induced mutants (Rajan 1981). This approach may lead not only to induce changes of characters controlled by single genes but also to improve polygenic traits. Sesame seeds are quite resistant to gamma rays and EMS, therefore higher dose levels should be utilized (Ashri 1982). Mutation breeding has been employed successfully in many countries to combine important desired traits of yield and adaptability (Lee and Choi 1985).

Kang *et al* (1994) selected some mutant lines characterized by desirable traits (e.g. semi-dwarf, quadric-carpels, erect branched, determinate, diseases tolerance and higher yielding ability).

Breeding for diseases resistance is very important for sesame crop. It is also very important to breed against some abiotic stress conditions (Pathirana 1994 and Potan *et al* 1994).

The enzymatic pools and their metabolic pathways are the most important factors affecting pathogenicity especially with viruses. Increase in oxidative enzyme activities can be applied as a tool for virus detection in plants (Hammeschmidtt *et al* 1982). Also, Mahmoud (2004) reported that the oxidative enzyme is one of the most investigated enzymes mainly due to its involvement in so many molecular, physiological and morphological

events in the plant life cycle. Peroxidase and polyphenyl oxidase activity frequency increased in plants infected by pathogens, and the level of its activity is often closely correlated with disease resistance. In particular, oxidative enzyme activity has been reported to be a biochemical marker for resistance and to be associated with systemic resistance. Mahmoud (2004), stated that the correlation between induced resistance and some biochemical changes in plant tissues like increased activity of enzymes and appearance of new polypeptides protein had become a model in the study of plant disease resistance; these biochemical changes became a marker to induce resistance.

The objectives of this investigation were to induce *Cowpea Aphid Borne Mosaic Virus* resistant mutants in sesame by a mutation breeding program, to characterize these mutants for their yield and enzymatic activity under CABMV infection and to identify some molecular markers on the protein level for resistance to CABMV.

MATERIALS AND METHODS

Irradiation and field experiments

Dry seeds (10% moisture content) of three sesame cultivars: Giza 32, Toshky 1 and Shandaweel 3 (obtained from the Oil Crops Research Department, Field Crops Research Institute, ARC) were exposed to 0, 50, 100, 150, 200, 250 and 300 Gy of gamma rays at a dose rate of 0.8 Gy min^{-1} at the National Center for Radiation Research and Technology, Atomic Energy Authority. Irradiated seed lots and non-irradiated controls were grown (immediately after irradiation) on 19th of May, 2003 at Giza Research Station, ARC to give M_1 generation. At harvest, seeds of each genotype (21genotypes; 3 cultivars x 7 gamma ray doses) were bulked separately.

In M_2 generation, seeds from each irradiated M_1 treatment (18 genotypes) as well as controls were grown on 17thof May, 2004 to obtain M_2 plants. At harvest, seeds of 10% of the plants in each stratum which was the most resistant to CABMV has been saved to produce the next generation (M_3).

In M_3 generation, seeds of the selected plants from each irradiation treatment (18 genotypes) as well as controls were grown on 30th of May, 2005 to obtain M_3-plants.

Virus isolation

Samples from naturally infected sesame plants exhibiting Cowpea Aphid Borne Mosaic Potyvirus symptoms consisted of mosaic, stunt, necrosis, and deformation were collected from the Agricultural Research Center Experimental Station in 2003 season. The local lesion technique (Kuhn 1964) was used for biological purification of the virus using *Chenopodium amaranticolar* Costs & Reyn as a local lesion host and the

samples were tested for the presence of CABMV using Dot- blot immunoassay (DBIA) on Nitrocellulose membrane (NCM), 0.45 µm pore size (Smith and Banttari 1987) the IgG was kindly provided by Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark.

Identification of isolated virus

Host range and symptomatology

Tweleve species cultivars belonging to four families were mechanically inoculated by CABMV; ten seedlings of each host plants were inoculated and examined daily for symptoms development. An equal number of healthy seedlings of the same species and age were left without inoculation to serve as a control.

Dot-blot immunoassay (DBIA)

The technique of DBIA described by Smith and Banttari (1987) was adopted. Leaves were homogenized 1:2 (W/V) in coating buffer pH 9.6 passed through a double layer of cheesecloth, then 1µl of each sample was spotted onto NCM. The NCM was washed 3 times with phosphate buffer saline-Tween at 5 min intervals, the NCM blocked with 2% Bovine serum Albumin in PBST and incubated overnight at 4 °C with primary antibody (1/1000) in PBS then, washed 3 times in PBST. The membrane was incubated with secondary antibodies (anti-anti-rabbit) dilution 1/7000 in conjugate buffer for one hour at laboratory temperature. The NCM was washed. The substrate solution nitro blue tetrazolium 5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) was added as one tablet in 10 ml H_2O. Development of a purple color on the blot indicates positive reaction; a negative reaction developed no color on the blot.

Degrees of virus infection

Sesame leaves Infected with CABMV were extracted with 0.01 M phosphate buffer of pH 7 using carborundum powder (400mesh). Twenty one genotypes were inoculated (field grown) in M_2 and M_3 generations, as the method used by Aboul-Ata *et al* (1996). Degree of infection severity was recorded after 2-3 weeks. Three degrees of severity were used, i.e. 1= mild (symptoms are mild and can be recognized well), 2= moderate (symptoms are more than mild and less than severe) and 3= severe (symptoms are severe). The virus infection data were conjugated with protein patterns analysis to identify some markers for different resistance levels.

Experiments designs and statistical analysis

In M_2 and M_3 generations, a split-split plot design with four replications was used for each generation. The two infection statuses were devoted to main plots, three cultivars to subplots and seven irradiated doses to sub-sub plots. The irradiated and non-irradiated seeds were sown in plots; each plot consisted of ten rows, 4 meters long, 60 cm apart and 10 cm between hills. Seeds of 40 plants of every treatment in M_2 (10% selection intensity) were bulked and sown to arise M_3 generation. Random samples of 20 individual plants for both un-infected (healthy) and infected with CABMV per treatment were used to measure studied characters: plant height (cm), first capsule height (cm), number of branches, number of capsules, capsule length (cm), seed yield/plant (g.) in M_2 and M_3 generations. Percentage of oil content of sesame seeds was determined according to the AOAC (1990).

The statistical analysis of the two generations; M_2 and M_3 was performed using MstatC statistics program and means were compared by using L.S.D. at 0.05 level of probability.

Enzyme activities

The leaf tissue 3:1 (buffer volume : fresh weight) was homogenized in a mortar with 100 mM phosphate buffer of pH 7.5 containing pyrogalol, catycol, and hydrogen peroxide (H_2O_2), respectively to determine the enzymes activity of peroxidase, polyphenol oxidase and catalase. The homogenate was centrifuged in Sigma 2-16 K at 10000 rpm for 30 min and the supernatant was kept and stored in separated aliquots at – 80 °C prior to determine peroxidase, polyphenol oxidase and catalase activity. Determination of peroxidase was assayed in leaf extracts by photochemical method as described by Amako *et al* (1994), Polyphenol oxidase (Coseteng and Lee 1987) and catalase (Daojun *et al* 1997).

The statistical analysis of the two generations; M_2 and M_3 (combined) was performed using MstatC statistics program and means were compared by using L.S.D. at 0.05 level of probability.

Protein patterns

Sodium dodesyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for direct visual protein comparisons in M_3 sesame leaves between all used cultivars and gamma ray doses under infection and healthy conditions. Proteins were size fractionated based on the molecular weight by SDS-PAGE performed as described by Laemmli (1970). Gels were stained with commassie brilliant blue R-250 solution, photographed and scored using gel documentation system manufactured by Alpha Ease FC (Alphimager 2200), U.S.A.

RESULTS AND DISCUSSION

Virus isolation

Naturally infected sesame plants showing symptoms were used as source of virus. The isolated virus was transmitted mechanically to sesame plants which exhibited the same symptoms (Fig. 1). The isolate was previously purified by single local lesion technique on *Chenopodium amaranticolar* Costs & Reyn leaves (Fig. 2).

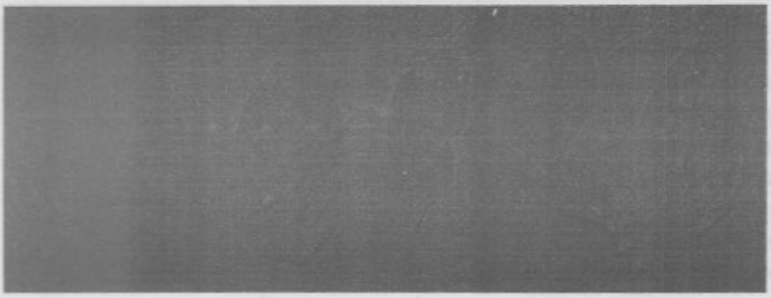


Fig. 1. Mosaic, stunt, necrosis, and deformation symptoms in inoculated sesame leaves by CABMV under greenhouse conditions.

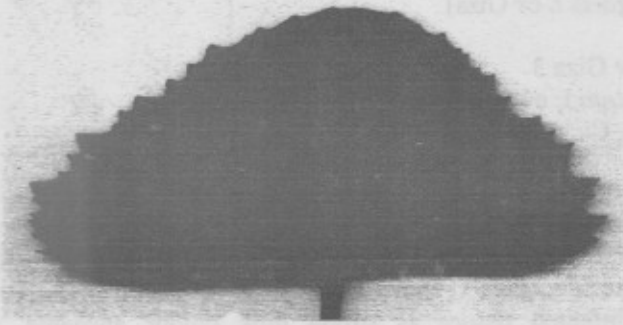


Fig. 2. Local lesion on *Chenopodium amaranticolar* Costs & Reyn leaves was inoculated with CABMV under greenhouse condition.

Identification of virus

Host range and symptomatology

CABMV potyvirus was isolated from naturally infected sesame and symptoms on some plants species were recorded, as shown in Table (1). CABMV induced yellowing on *Vigna unguiculata* L cv cream 7 and *Phaseolus vulgaris* L. cv Giza1, whereas induced local lesion on inoculated leaves of *Chenopodium amaranticolar* Costs& Reyn and *Chenopodium*

quinoa Wild. Similar results were obtained by El-Sharkawy (2005). Also CABMV induced mosaic symptoms on *Petunia hybrida* Vilm and *Glycine max* L. cv. Giza 21. This agrees with Chaicharoen *et al* (2003). On the other hand plants species not susceptible to CABMV infection *Nicotiona glutinosa* L. *Nicotiona tabacum* L. White Burely, *Nicotiona rustica*, *Capsicum annuum* cv. California wonder, *Cucumis sativus* L. (Balady), and *Vicia faba* L cv Giza 3, as shown in Table (1).

Table 1. Symptoms on different plant species inoculated with CABMV.

Test plant	The main symptoms
Chenopodiaceae:	
Chenopodium amaranticolar Costs& Reyn	LL
Chenopodium quinoa Wild	LL
Cucurbitaceae:	
Cucumis sativus L. (Balady)	-
Solanaceae:	
Nicotiona glutinosa L.	-
Nicotiona tabacum L.white Burely	-
Nicotiona rustica	-
Capsicum annuum cv. California wonder	-
Petunia hybrida Vilm	M
Leguminosae:	
Phaseolus vulgaris L cv Giza1	Y
fabaceae	
Vicia faba L cv Giza 3	-
Vigna unguiculata L cv cream 7	Y
Glycine max L. Cv. Giza 21	M

LL = local lesion Y = yellow M = mosaic - = no symptoms

CABMV detection by DBIA

Identification of CABMV was confirmed serologically using DBIA technique. The results (Fig. 3) showed that positive reaction was obtained with CABMV-infected tissues as strong pink colour appeared, when CABMV antiserum (primary antibodies) was used at 1/1000 titration, while negative reaction was obtained with the sample of healthy plants.

This is the first time that CABMV was isolated from sesame plant. DBIA is routinely used for detection of plant viruses and diagnosis of infection. On the other hand DBIA technique was used to confirm the identity of the isolated virus. The advantages of DBIA test for detection of small amounts of antigen was recorded by Dijkstra and De-Jager (1998). Several methods have been used for detection of CABMV using

host range, symptom, and serological technique. The use of dot immunobinding assay (DIBA) in the detection of CABMV was reported by Bhat *et al* (1999), Chaicharoen *et al* (2003), and El-Sharkawy (2005).

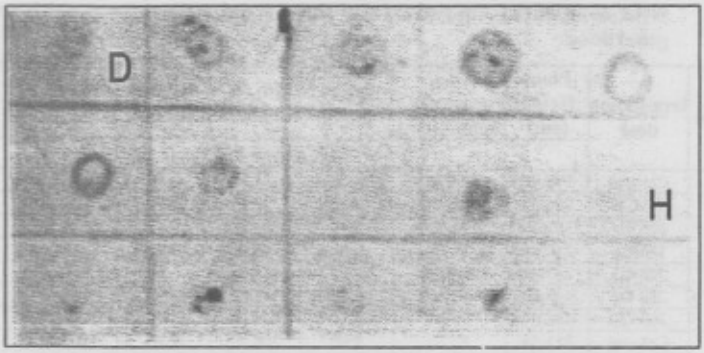


Fig. 3. Immunological detection of CABMV in tissue blot of sesame leaves where primary antibodies were used. Pink color indicates positive reaction (D), while negative reaction appears as white color (H).

Effect of Gamma ray irradiation treatments on sesame characters in M_2 generation

The mean values of studied characters for three sesame cultivars in M_2 generation after irradiation with gamma ray doses are presented in Table (2).

In M_2 generation, significant differences were found between the three sesame cultivars over the gamma ray doses and treatment with CABMV for plant height, the highest genotype was Giza 32, followed by Toshky 1 and Shandaweel 3 (196.89, 174,34 and 158.68 cm, respectively), (Table 2). The statistical analysis indicated significant differences among irradiation doses on plant height; all used doses decreased plant height comparing with the control. The shortest plant height was observed with the highest gamma ray dose (300 Gy.). Inoculation with CABMV caused decreases in plant height over all other factors (140.30 cm) comparing with the control (212.98 cm). Ragab and Kassem (2001) indicated significant sizable reduction in plant height for Taka 1, Taka 2 and Taka 3 cultivars as compared to local variety Giza 32 by 8.90 %, 10.95 % and 19.67 %, respectively over locations. He also obtained shorter plants than the commercial variety Giza 32 *via* induced mutantions in sesame (Ragab, 1996).

Table 2. **Effect of gamma ray doses on mean values of morphological, yield and yield component characters for three irradiated sesame cultivars in M_2 generation.**

Cultivars	Irradiation dose	Plant Height (cm)	First capsule height (cm)	No. of branches	No. of capsules	Capsule length	Seed yield/plant (g.)	Oil %
		Un-infected plants						
Giza 32	Control	273.75	82.75	2.25	221.25	3.35	16.00	54.00
	50 Gy	246.50	104.50	3.00	210.00	3.60	15.50	61.00
	100 Gy	252.50	97.25	3.00	194.50	3.53	14.00	60.00
	150 Gy	240.00	91.25	3.50	208.75	3.93	25.50	58.00
	200 Gy	253.25	103.25	2.00	225.75	3.18	17.50	56.00
	250 Gy	243.00	77.25	2.50	195.25	3.50	14.50	55.00
	300 Gy	232.50	95.00	2.25	197.50	3.95	14.25	61.00
Toshky 1	Control	211.25	37.50	0.00	208.25	4.13	21.50	52.00
	50 Gy	196.75	47.25	0.50	203.50	3.98	16.75	64.00
	100 Gy	192.00	35.00	0.75	203.25	4.23	17.25	65.00
	150 Gy	202.00	36.25	2.00	215.50	4.28	28.00	58.00
	200 Gy	189.00	38.00	0.75	196.75	3.73	16.50	54.00
	250 Gy	186.00	22.00	0.25	211.50	4.18	17.25	53.00
	300 Gy	181.25	39.25	1.00	218.00	3.58	16.50	57.03
Shandaweel 3	Control	226.25	34.00	0.00	91.75	4.00	25.50	57.00
	50 Gy	192.25	41.25	0.50	87.25	4.70	17.75	60.00
	100 Gy	192.00	45.00	1.00	90.25	4.35	17.75	66.00
	150 Gy	193.75	32.75	1.50	207.75	4.38	22.00	59.00
	200 Gy	187.50	44.25	0.50	92.50	4.75	20.50	58.00
	250 Gy	193.50	38.25	1.25	94.50	4.18	19.00	56.00
	300 Gy	187.50	46.00	1.00	84.50	4.33	16.75	55.00
		Infected plants (under stress)						
Giza 32	Control	132.50	49.75	0.58	93.50	3.20	13.78	52.00
	50 Gy	152.50	62.50	0.82	100.50	3.45	15.72	58.00
	100 Gy	140.00	50.00	0.63	97.75	3.54	16.78	55.00
	150 Gy	153.75	66.25	0.98	94.50	3.57	17.34	54.00
	200 Gy	147.50	52.50	0.88	104.25	3.51	12.82	54.00
	250 Gy	146.25	53.75	0.95	92.25	3.56	13.95	53.00
	300 Gy	142.50	48.75	1.19	101.50	3.89	14.69	58.00
Toshky 1	Control	131.25	63.75	1.18	77.75	3.63	15.64	50.00
	50 Gy	160.00	72.50	0.63	75.25	3.54	15.54	60.00
	100 Gy	156.25	60.00	0.13	74.50	4.25	13.24	63.00
	150 Gy	151.25	48.75	2.15	174.00	4.45	13.98	53.00
	200 Gy	177.50	66.25	0.68	89.00	5.07	14.00	53.00
	250 Gy	156.25	71.25	0.78	82.25	4.36	16.22	56.00
	300 Gy	150.00	45.00	0.48	87.25	4.93	15.79	56.00
Shandaweel 3	Control	130.00	38.75	0.73	109.50	4.80	14.73	55.00
	50 Gy	120.00	37.50	1.05	95.75	4.72	16.82	57.00
	100 Gy	112.50	40.00	1.01	99.25	4.13	15.85	58.25
	150 Gy	118.75	35.00	2.90	151.50	4.07	15.83	57.00
	200 Gy	118.75	35.00	1.91	95.00	2.82	15.59	56.00
	250 Gy	133.75	50.00	2.19	91.75	3.11	15.49	54.25
	300 Gy	115.00	53.75	2.55	80.75	3.30	11.85	55.50
$LSD_{0.05}$								
Cultivars(C)		3.35	3.82	0.25	8.24	0.28	0.81	0.11
Irradiation doses (R)		5.45	5.37	0.40	9.43	NS	1.12	0.25
Infection (I)		9.40	NS	NS	9.84	NS	0.34	0.13
Cultivars x irradiation		9.44	9.31	0.70	16.35	0.40	1.94	0.44
Cultivars x infection		4.73	4.27	0.35	11.65	0.39	1.14	0.16
Irradiation x infection		7.71	7.61	NS	NS	NS	1.59	0.36
C x R x I		13.35	13.17	NS	23.12	0.57	2.75	0.62

All the interactions were significant. The same trend was observed with the first capsule height, except for the treatment with the CABMV, while this stress didn't affect the height of the first capsule.

Number of branches differed significantly according to the used cultivar; Giza 32 had the highest mean of branches, followed by shandaweel 3 and Toshky 1; 1.75, 1.29 and 0.80, respectively. The irradiation doses significantly increased no. of branches over the cultivars and CABMV treatment. The highest no. of branches was found in the genotype developed *via* irradiation with 150 Gy. (2.17), while no. of branches for the non-irradiated control was 0.79 over all used cultivars. In this respect, Ragab and Kassem (2001) obtained significant differences for number of branches between the new cultivars (produced from mutation breeding by gamma ray irradiation) and the local variety Giza 32, since Taka 1 and Taka 2 displayed higher number of branches than Giza 32 by 22.83% and 35.62%, respectively.

The interactions between cultivars x irradiation and cultivar x infection were significant. The differences between cultivars, irradiation doses and infection statuses were significant for no. of capsules per plant. Giza 32 showed the highest no. of capsules/plant (152.66) followed by Toshky 1(151.20) and Shandaweel 3 (105.14). The 150 Gy. gave the highest no. of capsule (175.33) over all cultivars and infection statuses. In this respect, Ragab and Kassem (2001) reported that Taka 1, Taka 2 and Taka 3, significantly surpassed the local variety Giza 32 for number of capsules/plant by 59.93%, 51.55% and 71.99%, respectively. The CABMV affected the no. of capsules/plant; it was reduced to 98.46 in infected plants comparing with un-infected ones over all cultivars and irradiation doses. All the interactions between factors were significant, except for the interaction between irradiation doses and infection statuses.

The differences in capsule length between cultivars were significant. The highest capsule lengths were observed in Toshky 1 and Shandaweel 3, (4.16cm) and (4.11cm), respectively, while Giza 32 had the shortest capsule length (3.55 cm). Irradiation doses, infection statuses and the interaction between them had no significant effect on capsule length in sesame in M_2 generation.

Seed yield per plant and oil content differed significantly between all cultivars, irradiation doses, infection statuses and all the interactions between all factors. All irradiation doses decreased seed yield, except 150 Gy. gamma ray dose, which increased seed yield per plant by about 13% compared with the un-irradiated control. Shandaweel 3 had the highest oil content (57.4%), while Toshky 1 had (56.7%) and Giza 32 had (56.4%). Gamma ray doses had positive effect on oil percentage; the highest oil content was obtained from the genotypes developed *via* irradiation with 100 Gy gamma ray dose (61.21%), while the control had 53.33 % oil content.

Azzam (1993) obtained similar results after treating sunflower with 100, 200 and 300 Gy gamma ray doses in sunflower. On the other hand, the infection of CABMV decreased oil content from 58.0% to 55.6% for un-infected plants and infected ones, respectively. Seed yield for the new varieties; Taka 1, Taka 2 and Taka 3 were higher as compared to the general mean of the local variety Giza 32 in 1998 season (Ragab and Kassem 2001).

It seems that 150 Gy treatment increased the most of studied characters in M_2 generation in sesame. The increments in mean number of branches as a result of applying 150 Gy gamma ray dose increased no. of capsules/plant, capsule length and seed yield/plant which finally improved seed yield.

The interaction of cultivars x irradiation doses was significant for all studied characters, indicating various responses of the studied cultivars to the applied gamma ray doses. Similar results were stated by Datta and Biswas (1987) in India, who improved sesame *via* mutation breeding when they subjected sesame to gamma ray irradiation (10KR to 50 KR).

Yield and its components in M_3 generation results from selected plants for CABMV tolerance in M_2 generation

In M_3 generation, after selection in M_2 generation for the tolerant plants to CABMV using 10% selection intensity, results in Table (3) show that plant height was significantly increased by all gamma ray doses (except 100 Gy.). On the other hand, irradiation significantly decreased the first capsule height, which resulted in a significant beneficial effect of irradiation treatments on fruiting zone length.

The statistical analysis showed that the differences between cultivars were significant for plant height and first capsule height. Giza 32 was the tallest genotype and had the highest first capsule height (194.52 and 71.57 cm, respectively), while Shandaweel 3 was the shortest one and had the shortest height of the first capsule height (155.78 and 41.18, respectively). These results were similar with these obtained by Ragab and Kassem (1995), Ragab (1996), Ragab *et al* (2000) and Ragab and Kassem (2001), who mentioned that the length of fruiting zone of Taka 3 significantly increased than that of variety Giza 32 by 9.01%. But the length of the fruiting zone of Taka 2 cultivar was significantly decreased than that of Giza 32 by 10.87%. Fruiting zone length is associated with either plant height or the first capsule height, where the taller stem might reject increased length of fruiting zone which has a direct impact on seed yield.

The mean no. of branches indicated that the effect of infection with CABMV after selection for tolerant plants to this virus, reflected in higher number of branches under this stress than under the un-infected conditions,

Table 3. Effect of gamma ray doses on mean values of morphological, yield and yield component characters for three irradiated sesame cultivars in M_3 generation.

Cultivars	Irradiation dose	Plant height (cm)	First capsule height (cm)	No. of branches	No. of capsules	Capsule length (cm)	Seed yield/plant (g.)	Oil %
		Un-infected plants						
Giza 32	Control	252.50	110.50	1.25	101.75	3.10	16.50	56.00
	50 Gy	232.50	85.00	0.75	96.50	3.48	17.00	62.00
	100 Gy	253.25	100.75	1.25	105.00	3.63	18.25	59.00
	150 Gy	253.50	113.75	1.50	206.75	3.45	29.00	59.50
	200 Gy	259.50	115.75	1.50	108.75	3.68	17.00	57.50
	250 Gy	236.00	75.25	1.50	108.50	3.65	17.50	57.50
	300 Gy	246.75	83.50	1.00	97.25	3.53	16.50	61.75
Toshky 1	Control	182.00	39.50	0.75	218.25	3.93	24.75	50.50
	50 Gy	179.25	41.25	1.00	180.75	3.93	23.00	57.50
	100 Gy	178.25	36.50	1.25	188.75	3.75	23.50	62.00
	150 Gy	193.75	41.00	3.00	301.00	3.85	30.25	55.50
	200 Gy	197.00	39.50	1.00	213.75	3.85	25.50	53.50
	250 Gy	183.75	43.00	1.00	201.00	4.03	23.75	53.50
	300 Gy	189.50	35.25	1.25	190.25	3.75	24.00	55.25
Shandaweel 3	Control	181.50	34.25	0.50	231.25	3.83	23.75	55.50
	50 Gy	188.25	40.00	1.00	189.75	4.08	22.25	58.00
	100 Gy	178.25	12.50	1.00	206.25	4.35	22.50	64.00
	150 Gy	193.75	40.00	2.25	325.50	4.35	32.50	57.25
	200 Gy	197.00	48.00	1.25	216.25	4.18	24.25	57.50
	250 Gy	183.75	45.25	1.00	198.75	4.28	25.75	53.50
	300 Gy	189.50	44.00	0.75	199.50	3.83	24.50	53.50
		Infected plants (under stress)						
Giza 32	Control	122.50	78.75	2.46	92.83	3.04	13.90	55.50
	50 Gy	135.00	38.75	2.75	98.63	3.48	13.80	57.50
	100 Gy	135.00	47.50	2.08	123.38	3.26	15.01	61.50
	150 Gy	122.50	40.00	2.29	152.34	3.36	16.03	56.50
	200 Gy	200.00	35.00	2.21	96.33	3.60	18.33	55.50
	250 Gy	138.00	37.50	3.21	119.50	3.35	19.26	57.50
	300 Gy	144.25	40.00	1.29	123.50	3.40	18.01	57.50
Toshky 1	Control	131.25	45.00	1.67	44.17	2.68	19.18	56.50
	50 Gy	127.50	53.75	2.83	97.54	3.11	19.84	60.50
	100 Gy	153.75	52.50	1.58	120.33	3.48	20.67	65.50
	150 Gy	138.25	68.75	1.29	73.00	3.45	19.90	53.50
	200 Gy	157.50	50.00	2.79	108.08	3.26	20.55	55.50
	250 Gy	167.00	68.75	1.83	109.83	3.15	21.55	56.50
	300 Gy	142.50	70.00	2.96	279.67	3.48	20.83	57.50
Shandaweel 3	Control	136.25	55.00	1.88	65.88	2.89	20.70	58.00
	50 Gy	118.75	48.75	1.59	127.83	3.31	27.20	55.50
	100 Gy	125.00	41.25	1.75	98.67	3.41	21.10	56.50
	150 Gy	122.50	50.00	1.63	72.54	3.01	23.22	57.50
	200 Gy	.10	40.00	1.00	105.50	3.67	35.10	54.50
	250 Gy	100	50.00	1.00	106.00	3.50	13.14	57.50
	300 Gy	122.50	35.00	2.75	70.38	3.46	26.59	57.50
LSD 0.05								
Cultivars(C)		4.14	4.88	NS	12.91	0.14	1.47	0.24
Irradiation doses (R)		6.99	5.45	NS	34.15	0.15	2.06	0.51
Infection (I)		2.25	5.10	0.32	16.78	0.17	NS	NS
Cultivars x irradiation		12.11	9.44	NS	NS	NS	NS	0.88
Cultivars x infection		5.86	6.90	NS	18.26	0.20	2.08	0.43
Irradiation x infection		9.89	7.71	0.73	48.34	NS	2.91	0.72
C x R x I		17.13	13.35	NS	NS	0.37	NS	1.25

(it was 2.08 and 1.23, respectively). Although, there wasn't a significant difference for number of branches due to gamma ray doses. Ragab (2001) obtained significant differences for number of branches between the new cultivars compared with the local variety Giza 32, since Taka 1 and Taka 2 displayed higher number of branches than Giza 32 by 22.83% and 35.62%, respectively.

Concerning no. of capsules per plant, the differences between cultivars, irradiation doses and infection statuses were significant. All gamma ray doses increased number of capsules per plant. 150 Gy. gave the highest number of capsules per plant (188.52). Also, Ragab and Kassem (2001) found that Taka 1, Taka 2 and Taka 3, significantly surpassed the local variety Giza 32 for number of capsules/plant. The infection with CABMV decreased significantly number of capsules per plant. It was 185.02 and 107.67, respectively for control conditions and infected ones. Toshky 1 cultivar gave the highest number per plant (213.39). On the other hand, the mean values of capsule length significantly differed between cultivars, irradiation doses and infection statuses. All irradiation doses increased capsule length. Shandaweel 3 had the highest capsule length (3.70 cm).

Seed yield differed significantly according to the genotype. All irradiation doses increased seed yield/plant as compared with the control. Also 150 Gy dose treatment gave the highest seed/plant (24.77 g.) across all cultivars and infection statuses. The effect of treatment with the virus was not negative, because the difference between control and infected plants was in-significant due to selection for the most tolerant plants in M_2. This means that the selection for tolerance to CABMV had a positive effect on the tolerance to this virus, beside its positive effect on the yield and its components and oil content, while the differences in oil content between infected and non infected plants were not significant. Also, all irradiation doses increased oil content. The highest oil content was observed in Giza 32 genotype. Both seed yield and total oil yield were reduced significantly as a result of virus infection but oil percentages have not been affected as a result of virus infection.

The interactions between cultivars and infection statuses and between irradiation doses and infection statuses were significant for all studied traits, except for no. of branches of the first order interaction and for capsule length for the second order one.

Sesame was improved *via* mutation breeding by using gamma ray irradiation (10KR to 50 KR) in India, (Datta and Biswas 1987).

Reduction according to infection with CABMV:

Data in Table (4) showed that, there was a reduction in almost all studied characters and cultivars as affected by the virus infection, except for

Table 4. Reduction % in infected sesame plants compared with un-infected plants in M_2 and M_3 generations.

Genotype	Generation	Plant height (cm)	First capsule height (cm)	No. of branches	No. of capsules	Capsule length (cm)	Seed yield/ plant (g.)	Oil %
Giza 32	M_2	41.72	41.89	67.78	52.91	1.67	10.12	5.25
Toshky 1		20.28	-67.34	-16.00	54.69	-7.46	21.93	2.95
Shandaweel 3		38.17	-3.01	-113.25	3.34	12.30	23.77	3.78
Giza 32	M_3	42.96	53.61	-86.40	2.18	4.82	13.22	2.85
Toshky 1		21.92	-48.11	-62.12	44.26	16.54	18.46	-4.57
Shandaweel 3		36.35	-21.21	-49.55	58.73	19.61	4.60	0.56

the first capsule height and no. of branches in Toshky 1 and Shandaweel 3 cultivars and for capsule length in Toshky 1 cultivar, these decreases were 67.34, 3.01, 16.00, 113.25 and 7.46, respectively. The plant height decreased in all cultivars and generations because of the infection, while the first capsule height was decreased in Giza 32, which was reflected in increasing the seed weight per plant, and vice versa, for Shandaweel 3 and Toshky 1. Main and Gurtz (1989), reported that the viruses were responsible for 20% of all losses in North Carolina during 1988 and Fernandez - Suarez and Lastres -Gonzalez (1983) mentioned that *Cowpea Mosic Potyvirus* (CPMV) caused yield reductions of 64-75% in four soybean varieties in inoculation trials in 1979-1981. The most affected yield components were number of pods/plant and seed yield/plant.

The effect of CABMV virus infection on oil percentage was studied. The results in Table (3) showed that a reduction occurred in both generations and all cultivars, except Toshky 1 in M_3, while there was an increase in oil percentage. In our study oil percentage was much less than that obtained by Annonymous (1996) and Roshdy (1999). This decrease may be attributed to differences in varieties, treatments and the growth conditions. On the other hand, Zein and Shafie (2005) reported that both seed yield and total oil yield were reduced significantly as a result of virus infection but oil percentage have not been affected as a result of virus infection.

Enzymes activity

Data in Table (5) illustrated the effect of CABMV on three enzyme activities; Peroxidase (POD), Polyphenyloxidase (PPO) and Catalase (CAT) of 21 sesame genotypes combined across M_2 and M_3 generations derived from irradiation of three sesame cultivars with different gamma ray doses. The differences between cultivars, irradiation doses and the infection statuses were significant. Also all their interactions, (cultivars x irradiation doses, cultivars x infection statuses, irradiation doses x infection statuses and cultivars x irradiation doses x infection statuses) were significant. The activity of POD, PPO and CAT enzymes were increased in almost all genotypes developed *via* irradiation compared with the un-irradiated ones under both infected and healthy status except (CAT) in Toshky 1 genotypes under the two conditions. Esanu and Dumitrescu (1971) reported that the appearance of an additional peroxidase enzyme, or increases in the amounts of existing enzymes had been reported for various hosts and viruses including combinations where necrosis was not a feature of the disease, and others where it was the main symptom. On the other hand, POD, PPO and CAT were increased under infection conditions compared with the uninfected one and vice versa for PPO in Toshky 1 genotype developed *via* irradiation with 250 Gy dose (the increase of PPO activity in infected plants relative to uninfected one was -225.0). In the same time, this genotype was the most resistant one which gave the mild symptoms in the field.

The present result indicated that the activities of the oxidative enzymes; peroxidase, polyphenol oxidase and catalase were obviously higher in leaves of treated plants (infected plants) compared with untreated ones (healthy plants). Theses results are in agreement with those recorded by some investigators (Abdou *et al* 2001, Shalaby *et al* 2001, El-Fiki *et al* 2004 and Mahmoud 2004).

Protein electrophoresis

The electrophoretic banding patterns of proteins extracted from the leaves of the three sesame cultivars and their genotypes in M_3 under CABMV stress (infected plants = I) and unstressed conditions (healthy plant = H) are shown in Fig. (4). The total number of bands and the molecular weight of both lightest and heaviest bands for three sesame cultivars irradiated with different gamma ray doses for both uninfected or infected with CABMV in M3 generation were illustrated in Table (6).

The results of SDS-PAGE revealed a different number of bands according to the genotype, the irradiation dose and the infection status, with different molecular weights (MW) ranging from 145.95 to 19.07 KDa (Giza 32 genotypes), from 141.23 to 17.85 KDa (Toshky 1 genotypes) and from

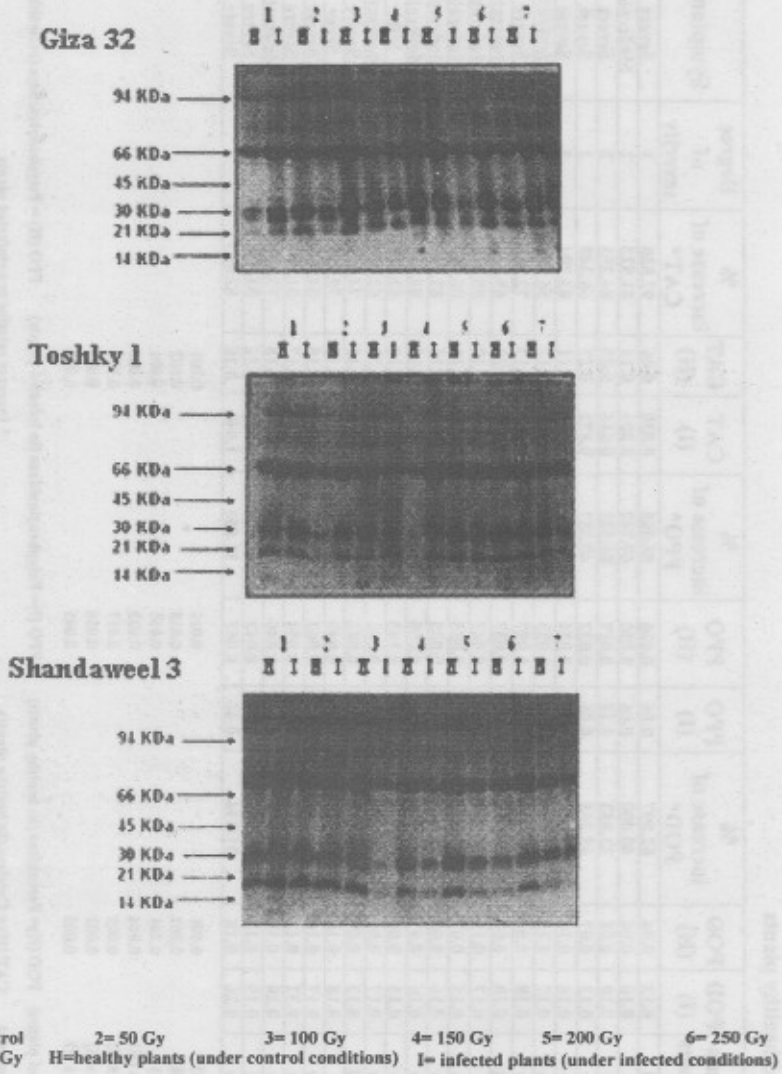
Table 5. Effect of CABMV on enzymes activity (minute/g), degree of severity and observed symptoms of 21 sesame genotypes combined across M_2 and M_3 generations derived from irradiated three sesame cultivars with gamma ray doses, for infected and healthy plants

Cultivars	Gamma ray doses	POD (I)	POD (H)	% increase of POD*	PPO (I)	PPO (H)	% increase of PPO*	CAT (I)	CAT (H)	% increase of CAT*	Degree of severity	Symptoms
Giza 32	control	0.12	0.04	62.097	0.06	0.026	58.065	0.830	0.06	92.530	3	Severe
	50 Gy	0.10	0.06	40.000	0.06	0.020	67.742	0.893	0.16	81.523	2	Moderate
	100 Gy	0.10	0.08	23.853	0.12	0.017	86.822	0.616	0.09	84.253	3	Severe
	150 Gy	0.13	0.07	45.255	0.06	0.032	48.387	0.872	0.27	68.349	3	Severe
	200 Gy	0.14	0.14	3.448	0.05	0.034	41.379	0.670	0.11	83.284	3	Severe
	250 Gy	0.17	0.13	19.883	0.07	0.023	67.143	0.887	0.12	86.020	2	Moderate
	300 Gy	0.18	0.07	61.290	0.11	0.057	49.107	0.478	0.13	72.385	3	Severe
Toshky 1	control	0.10	0.05	44.554	0.14	0.037	74.658	0.958	0.28	69.833	2	Moderate
	50 Gy	0.17	0.15	12.000	0.07	0.042	46.835	0.742	0.10	85.849	2	Moderate
	100 Gy	0.15	0.07	53.642	0.10	0.025	75.248	0.825	0.11	86.303	2	Moderate
	150 Gy	0.16	0.05	66.667	0.03	0.030	3.226	0.644	0.11	82.764	2	Moderate
	200 Gy	0.16	0.07	56.970	0.11	0.055	51.754	0.687	0.10	84.571	2	Moderate
	250 Gy	0.15	0.06	57.233	0.04	0.143	-225.000	0.642	0.10	83.022	1	Mild
	300 Gy	0.17	0.06	64.205	0.40	0.066	83.784	0.754	0.27	63.793	2	Moderate
Shandaweel 3	control	0.13	0.09	26.119	0.09	0.042	56.250	0.589	0.15	74.024	3	Severe
	50 Gy	0.18	0.16	7.735	0.19	0.040	79.167	0.757	0.15	79.128	3	Sever
	100 Gy	0.17	0.15	12.209	0.08	0.062	27.907	0.977	0.15	84.033	3	Severe
	150 Gy	0.32	0.12	61.250	0.06	0.054	14.286	0.912	0.17	81.031	3	Severe
	200 Gy	0.20	0.15	22.927	0.06	0.046	33.333	0.891	0.18	79.125	2	Moderate
	250 Gy	0.19	0.13	30.769	0.08	0.042	50.000	1.029	0.16	83.576	3	Severe
	300 Gy	0.26	0.18	31.034	0.06	0.061	11.594	1.049	0.18	82.650	3	Severe

LSD 5%				
Genotype (G)	0.001	0.012	0.001	
Gamma ray doses (R)	0.202	0.018	0.002	
Infection status (I)	0.201	0.010	0.001	
(G) x (R)	0.004	0.032	0.003	
(G) x (I)	0.002	0.017	0.002	
(R) x (I)	0.003	0.026	0.003	
(G) x (R) x (I)	0.005	0.045	0.005	

POD (I)= Peroxidase (in infected plants) POD (H)= Peroxidase (in healthy plants) PPO (I)= Polyphenyloxidase (in infected plants) PPO (H) = Polyphenyloxidase (in healthy plants)

CAT (I)= Catalase (in infected plants) CAT (H) = Catalase (in healthy plants) * Increase relative to uninfected plants.



1= control 2= 50 Gy 3= 100 Gy 4= 150 Gy 5= 200 Gy 6= 250 Gy
7= 300 Gy H=healthy plants (under control conditions) I= infected plants (under infected conditions)

Fig. 4. SDS-protein banding patterns for leaf proteins of the irradiated and non-irradiated genotypes of the three sesame cultivars under control and CABMV infection in M3 generation.

Table 6. The total number of bands and the molecular weight of both lightest and heaviest band for three sesame cultivars irradiated with different gamma ray doses for both uninfected (H) or infected (I) with CABMV in M_3 generation.

Cultivars	Gamma ray doses	Infection statuses	Max. MW. (KDa)	Min. MW. (KDa)	Total number of bands
Giza 32	control	H	142.81	24.39	7
		I	144.89	24.57	13
	50 Gy	H	143.85	24.75	10
		I	145.95	24.57	12
	100 Gy	H	142.81	24.57	9
		I	142.81	26.23	8
	150 Gy	H	140.76	26.04	6
		I	140.76	19.63	13
	200 Gy	H	140.76	27.19	10
		I	141.78	26.42	6
	250 Gy	H	140.76	19.07	11
		I	103.84	26.42	6
	300 Gy	H	138.73	19.49	12
		I	106.89	20.21	9
Toshky 1	control	H	140.10	19.35	13
		I	136.75	24.44	12
	50 Gy	H	98.32	24.05	4
		I	140.10	24.05	9
	100 Gy	H	138.97	17.85	13
		I	141.23	19.35	10
	150 Gy	H	76.61	23.66	4
		I	136.75	24.05	10
	200 Gy	H	136.75	23.85	9
		I	136.75	17.85	10
	250 Gy	H	134.57	23.47	11
		I	135.66	24.24	11
	300 Gy	H	133.49	24.63	8
		I	133.49	24.44	7
Shandaweel 3	control	H	121.65	21.69	7
		I	121.65	16.30	12
	50 Gy	H	120.45	12.69	8
		I	120.45	21.27	7
	100 Gy	H	118.10	20.85	7
		I	115.80	27.21	3
	150 Gy	H	115.80	20.05	4
		I	115.80	19.66	4
	200 Gy	H	114.66	19.66	6
		I	113.54	19.66	4
	250 Gy	H	112.43	19.85	4
		I	112.43	20.65	4
	300 Gy	H	114.66	21.48	4
		I	64.12	30.93	3

121.65 to 12.69 KDa (Shandaweel 3 genotypes), which were not necessarily present in all genotypes. Data showed four, one and four common bands (monomorphic), in Giza 32, Toshky 1 and Shandaweel 3 genotypes,, respectively, while the remaining bands were polymorphic.

The SDS-protein banding pattern of the genotypes developed *via* irradiation and its parental cultivars (control) under the control conditions and the viral infection with CBAMV stress conditions (Table 6) was found to be useful in identifying the induction of variations in the genotypes developed *via* irradiation. Under the control condition, the number of bands of all genotypes developed *via* irradiation increased over Giza 32 genotype (control), except those developed *via* irradiation with 150 Gy. The opposite was true for Toshky 1 and Shandaweel 3 cultivars, where the number of bands decreased in all genotypes except Shandaweel 3 genotype that produced from irradiation with 50Gy. Although irradiation does not significantly alter the chemical composition of proteins, changes are observed in their secondary and tertiary structures. When proteins are irradiated, several types of reactions can occur. One type of reaction leads to the breaking of a small number of peptide bonds to form polypeptides of shorter length than the original protein. Radiation damage can also lead to aggregation or cross linking of individual polypeptide chains which will result in protein denaturation. These changes are similar to those that occur as a result of heating. A third type of reaction that can occur involves the reaction of amino acids in the polypeptide chain with the free radicals from water, without the breaking of peptide bonds, Lorenz (1975), Nawar (1978), Diehl (1995) and Choi and Hwang (1997).

On the other hand, under the viral stress conditions, the total number of protein bands was decreased compared with the normal (control) condition in Giza 32 genotypes (100, 200, 250 and 300 Gy.) and Toshky 1 genotypes (control, 100 and 300 Gy.) and Shandaweel 3 genotypes (50, 100, 200 and 300 Gy.). While, the number of bands in Toshky 1 genotype developed *via* irradiation with 250 Gy and Shandaweel 3 genotypes developed *via* irradiation with 150 and 250 Gy. didn't change neither under the infected conditions nor under the control one.

From all previous data presented in Tables (5 and 6) and Fig. (5) we can conclude that proteins with molecular weight 82.0 and 38.0 KDa found only under infected conditions were found as proteins associated marker with mild to CBAMV symptoms as in Toshky 1 genotype developed *via* irradiation with 250 Gy (which showed high POD and CAT activities and low PPO activity under infected conditions).

These results are in harmony with those obtained by Krupinska *et al* (2002) in barley plants. They found that a systemic acquired resistance associated with the expression of defense genes such as pathogenesis related gene (Hv S40) was strongly expressed exclusively in the infected leaf. In

addition, the lesion response into tobacco containing the N-gene has been associated with the presence of a protein with anti-viral properties named inhibitor of virus replication (IVR) and has molecular weight of 23 KDa (Matthews 1981). Mahmoud (2004), stated that the correlation between induced resistance and some biochemical changes in plant tissues like increased the activity of enzymes and appear of new polypeptides protein has become a model in the study of plant diseases resistance, this biochemical changes became a marker to inducer resistance.

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المظمات الوراثية الكيميائية الحيوية لمستويات المقاومة لفيروس موزيك اللوبيا المنقول بالمن فى السمسم المشعع بأشعة جاما

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تم لأول مرة ملاحظة وجود فيروس موزيك اللوبيا المنقول بالمن على نباتات السمسم في مصر. وتم عزل الفيروس من نباتات السمسم المصابة طبيعيا والمنزرعة في مركز البحوث الزراعية بالجيزة والتي شوهدت عليها أعراض موزيك وتلزم وتشوهات في الأوراق، ثم تنقية عزلة الفيروس بيولوجيا وتعريفها والتأكد منها باختبار الأليزا غير المباشر (اختبار الارتباط المناعي على غشاء النتروسليلوز) باستخدام الأنتيسيرم المتخصص للعزلة وتم حقن هذه العزلة لنباتات السمسم في الجيل الطفوري الثاني والثالث. تم تشعيع ثلاثة أصناف من السمسم هي جيزة 32 وتوشكى 1 و شندويل 3 بجرعات صفر و50 و100و 150و 200و 250و 300 جراى من أشعة جاما بمعدل تشعيع (بث) 0.8 جراى/ دقيقة. وتم زراعتهم في مركز البحوث الزراعية بالجيزة في ثلاث أجيال متعاقبة. في الجيل الطفوري الثاني وجدت بعض الاختلافات المعنوية بين الأصناف الثلاثة بغض النظر عن الجرعات الإشعاعية المستخدمة والحقن الميكانيكي بعزلة الفيروس بالنسبة لطول النبات و ارتفاع أول كبسولة وعدد الفروع ومحصول البذور للنبات ومحتوى الزيت كما كانت التفاعلات كلها معنوية. أظهرت النتائج أن المعاملة 150 جراى أدت لزيادة معظم الصفات المدروسة في الجيل الطفوري الثاني للسمسم كان التفاعل بين الأصناف ومعاملات الإشعاع معنوية لكل الصفات المدروسة مشيرا إلى استجابات مختلفة للأصناف المدروسة للمعاملة بأشعة جاما في الجيل الطفوري الثالث وبعد أنتخاب10% من النباتات المقاومة نسبيا للفيروس في الجيل الطفوري الثاني، أظهرت التحليلات الإحصائية أن الفروق بين الأصناف كانت معنوية لصفات طول النبات وارتفاع أول كبسولة ومحصول البذور. أدت كل معاملات الإشعاع لزيادة محصول البذور/نبات بالمقارنة بالكنترول. أن الانتخاب للمقاومة لفيروس موزيك اللوبيا المنقول بالمن في الجيل الطفوري الثاني كان له تأثير إيجابي على المقاومة للفيروس في الجيل الطفوري الثالث بجانب التأثير الإيجابي على المحصول ومكوناته ومحتوى الزيت. كما تم ملاحظة انخفاض في كل الأصناف المدروسة تقريبا كنتيجة للإصابة الفيروسية.

تم دراسة تأثير الفيروس على نشاط ثلاث إنزيمات البيروكسيديز والبولى فينول أوكسيديز والكتاليز في أحدى وعشرون تركيب وراثي للسمسم في الجيل الطفوري الثاني والثالث (مجتمعين) والناتجة من التشعيع بأشعة جاما.

أظهر تحليل التباين للنشاط الأنزيمي لختلافات معنوية لكل الإنزيمات المدروسة وحالة الإصابة الفيروسية والأصناف وتفاعلاتهم. زاد النشاط الأنزيمي لكل الأنزيمات المدروسة تقريبا في كل التركيب الوراثي الناتج من التشعيع مقارنة بالكنترول، في كل من حالات الإصابة وغير الإصابة فيما عدا أنزيم الكتاليز في التراكيب الوراثية الناتج من تشعيع الصنف توشكى 1 تحت كلا من ظروف العدوى وعدم العدوى. وعلى الجانب الأخر زادت كل من الأنزيمات المدروسة تحت ظروف العدوى مقارنة بظروف الكنترول (غير مصابة) والعكس صحيح بالنسبة لنشاط أنزيم البولى فنيل أوكسيديز في التركيب الوراثي الناتج من تشعيع الصنف توشكى بجرعة إشعاعية 250

جراى وفى نفس الوقت فإن هذا التركيب الوراثي كان الأكثر مقاومة والذي أظهر أعراض متوسطة الإصابة في الحقل (mild) .

أظهر التفريد الكهربائي لنماذج البروتين (SDS-PAGE) والمعزولة من أوراق المسلم المصابة وغير المصابة و التراكيب الوراثية الناتجة من التشعيع اختلافات في عدد الحزم البروتينية تبعا للصنف وجرعات الإشعاع وحالة الإصابة بأوزان جزيئية متباينة. أظهرت النتائج وجود أربعة و واحد وأربعة حزم شائعة (monomorphic) في التركيب الوراثية الصنف جيزة 2تو توشكى 1 و شندويل 3 بالترتيب. وكان التفريد الكهربائي لنماذج البروتين مفيدا فى أظهار واستحداث التباينات في التراكيب الوراثية الناتجة من التشعيع بأشعة جاما. يمكن أن نخلص إلى أن حزمتي البروتين ذو الوزن الجزيئي 82 و 38 كيلو دالتون والتي وجدنا فقط تحت ظروف الإصابة الفيروسية يمكن اعتبارهما بروتين مصاحب للأعراض المتوسطة (mild) الفيروس موزيك اللوبيا المنقول بالمن في التركيب الوراثى الناتج من تشعيع الصنف توشكى بجرعة 250 جراى.