

## THE ASSOCIATION OF BOTANICAL TRAITS WITH MOLECULAR MARKERS USING INDUCED MUTANTS OF FABA BEAN

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### ABSTRACT

Twenty-four faba bean mutants were selected and treated with gamma rays doses of 30 and 60 Gy and ethyl methane sulfonate (EMS) at conc. of 0.15 and 0.30%. The effect of mutagens was studied using a number of parameters, such as morphological growth characters, physiological parameters, flowering, yield attributes and the genetic characterization of all protein and DNA molecular markers based on SDS-PAGE RAPD analysis. The data showed that the induced mutants by either gamma rays or EMS tended to have higher values in most growth characters and the maximum increase was achieved by the mutant lines No. 7, 14, 24, 25, 18, 19 and 26. Mutant lines which were produced by (EMS) were more effective in increasing the concentrations of photosynthetic pigments, Chl. b, carotenoids and total chlorophylls than that produced by gamma ray. Transpiration rate (TR) exceeded the control in all mutants produced by gamma radiation except for mutant line No. 18. On the other hand, mutants No. 5, 12, 20, 23, 24 and 25 which were produced by EMS also exceeded the control. Mutant lines produced by gamma rays were more effective than that produced by (EMS). As for shelling percentages, the mutant lines No. 7, 8, 9, 21, 23 and 18 showed a significant increase while the mutant lines No. 1, 4, 19, and 26 showed considerable lower values than the control. The increase in yield and yield components using physical and chemical mutagens was noticed. It was possible to associate the specific polymorphic DNA fragments of about 3000 bp and 200 bp in size which were generated by the random primers (A1, A5 and A3) for Gy or EMS mutants markers might be of great importance in future programs of *Vicia faba* plants breeding and selection for mutants, with both high yield and early flowering.

### INTRODUCTION

The importance of Field bean as a good source of protein is a known fact. All over the world as well as in Egypt, extensive efforts are continuously done for increasing its productivity, by the means of induced mutations. Many investigators studied the high yield mutants (Al-Hamdany *et al.*, 1998 and Atia *et al.*, 1995), the high value nutritive mutants (Farag, 1999 and Selim *et al.*, 2002).

Several investigators studied the physical mutagen agents such as gamma rays and chemical agents such as EMS (ethyl methane sulfonate) and many others for crop improvement (Matta *et al.*, 1981, Mal, 1982, Habib *et al.*, 1988, Abdelsalam *et al.*, 1993, Hassan 1996, and Selim *et al.*, 2002).

Many changes in morphological, physiological, and genetical behaviour of many traits has been observed when gamma rays and chemical mutagen agents were used. Al-Hamdany et al., (1998), Atia et al., (1995) and Selim et al., (2002) found changes in plant height, root length, leaf area, growth habit, maturity and flowering behaviour as well as yield components in mutants induced by gamma rays and EMS. Also, mutagens were used to induce mutants that varied in chlorophyll and carotenoids concentrations (Selim et al., 2002 and Datta, 1999).

The improvement of *Vicia faba* productivity is a result of using new disciplines of biotechnology such as gene targeting, genome mapping and the possibility of gene exchange between different organisms overcoming the genetic barriers. The development of molecular markers, based on SDS-protein and RAPD constitute one of the important tools of genetic studies revealing the genetic diversity and relatedness among mutants either in populations or individuals. These molecular parameters also permit the prediction of characteristics of different mutants and the pre-selection of the best genotypes (Stuber, 1992).

Polymerase Chain Reaction (PCR) based genetic markers and random amplified polymorphic DNA (RAPD) markers have been shortly introduced and gained widespread application in genetics and plant breeding. (Williams et al., 1990).

The application of biotechnology in crop improvement is generally achieved by large-scale and rapid production of genetically uniform plants through the use of EMS. Many commercially important plants, including faba bean have been also used for the selection of novel and improved plant varieties, with increased yields, faster growth and disease resistance (Glick and Pastemak, 1994). Hence, this investigation was carried out to study the changes that occurred in some growth traits, photosynthetic pigments, plant water relations, flowering, fruiting and yield attributes in the induced mutants from cv. Giza-2 of faba bean by gamma radiation and EMS. The study will investigate the genetic characterization of all protein and DNA molecular markers based on SDS-PAGE RAPD analysis.

## MATERIALS AND METHODS

Pots experiment was carried out during the season of 2004/2005 to study the variations in some growth, yield and physiological aspects as well as to analyze the biochemical genetic aspects and DNA fingerprinting in some faba bean mutants induced by gamma-radiation and EMS. This study was done as follows:

### Mutant induction:

Years ago, faba bean seeds (*Vicia faba*, L., cv. Giza-2) were divided into three batches. The 1<sup>st</sup> seed batch was divided into two parts and irradiated by gamma rays using the doses; 30 and 60 Gy. Seeds in the 2<sup>nd</sup> batch were divided to two equal parts to be submerged separately in freshly prepared EMS (Ethyl methane sulfonate) solutions at concentrations of 0.15 and 0.30% at room temperature for 4 hrs. The 3<sup>rd</sup> batch was used as a control (untreated seeds) by submerging seeds in water (the solvent used in EMS

dilution) for 4hrs. At the Experimental Farm of the Egyptian Atomic Energy Authority Inshas, the treated and untreated seeds were sown to obtain M1 seeds which were planted to give the M2 generation. Seeds of the M2 from the selected plants were sown to give the M3 which gave the M4 generation seeds. The M4 selected plants were sown to give the M5 generation. In the Agricultural Botany Dept. Faculty of Agriculture, Menoufiya University, A.R.E., seeds of M5 generation were sown for many following seasons to give a sequence of generations. In the season of 2004/2005, twenty-four faba bean mutants were selected from the latest generation and their characteristics are presented in Table 1.

**Table 1: Characteristics of the twenty-four selected faba mutants selected in the 2004/2005 season.**

Mutant No.	Characteristics	Designation mutant mark	Mutant agent
1	Violet Medium seed	V.M. 0.15	0.15%EMS
2	Dark Brown Medium seed	D.B.M. 60	60 Gy
3	Brown Medium seed	B. M. 30	30 Gy
4	Violet seed	V. 0.15	0.15%EMS
5	Violet Small seed	V.S. 30	0.30%EMS
6	Furrow Violet seed	F.V. 30	0.30%EMS
7	DarkViolet seed	D.V. 0.15	0.15%EMS
8	Dark Violet M. seed	D.V. M. 0.15	0.15%EMS
9	Violet Medium seed	V. M. 0.15	0.15%EMS
11	Dark Brown seed	D.B. 0.15	0.15%EMS
12	Light Violet seed	L.V. 0.15	0.15%EMS
14	Light Brown seed	L.B. 0.15	0.15%EMS
15	Furrow. White seed	F.W. 0.15	0.15%EMS
16	Smooth- Light Brown seed	Sm. L.B. 30	30 Gy
17	Brown Large seed	B.L. 30	30 Gy
18	Smooth Brown Large seed	Sm. B.L. 60	60 Gy
19	Brown Large seed	B. L. 60	60 Gy
20	Dark Brown P.Blackseed	D.B.P.B. 15	EMS
21	Smooth Brown seed	Sm. B. 30	0.15%EMS
22	Light Violet seed	L.V. 15	30 Gy
23	White seed	W. 15	0.15%EMS
24	Light Violet seed	L.V. 15	0.15%EMS
25	Dark Violet seed	D.V. 15	0.15%EMS
26	Furrow Brown Large seed	F.B.L. 60	60 Gy
Control(13)	Brown M. seed	B. M. seed	

**Sowing and agriculture practices:**

In the growing season 2004/2005, four seeds of each mutant from the above-mentioned twenty-four faba bean mutants beside the control seeds (untreated) were sown in pots 30-cm inner diameter. Pots were filled with 7kg clay loam soil (pH= 8.00, O.M. (%)= 1.88, EC= 0.63 dm<sup>-1</sup>, TSS (%)= 0.17). The seeds were inoculated with strains of Rhizobia (*Rhizobium leguminosarum*) before sowing. Pots were arranged in four replicates according to randomized complete blocks design. Each replication was

represented by four pots. The seedlings were thinned to three plants per pot after twenty-one days from sowing date. All recommended agriculture practices were done during the growing season.

**Samples:**

After eighty-five days from sowing, samples of three plants were taken randomly from each mutant and the control. The following measurements were recorded: plant height (cm.), number of branches /plant, number of leaves /plant, root dry weight (gm.), stem dry weight (gm.), leaves dry weight (gm.), total leaf area (dm<sup>2</sup>/plant) and shoot/root ratio. Photosynthetic pigments were extracted from fresh leaves using acetone 80% (Wettstein, 1958), then calculated as mg./g dry weight. The total water content (TWC, %) and transpiration rate (mg. cm<sup>-2</sup>.h<sup>-1</sup>) were measured according to Kreeb (1990).

At the flowering stage and harvest time, flowers number/plant, pods number/plant, pods set (%) and the total shedding percentage, pod yield (gm./plant), straw yield (gm./plant) and shelling percentage were recorded.

Statistical analysis of the data was done according to Gomez and Gomez, (1984) using of the COSTAT C Statistical package (American Computer Program), to compare means and the LSD test at 5% level of probability.

**Protein electrophoresis:**

SDS polyacrylamide gel electrophoresis (SDS-PAGE) was used to detect the protein banding patterns of 1 gm leaves of faba bean samples from all mutants according to the method of Laemmli (1970), as modified by Studier (1973). Molecular weight estimates were calculated using a simple basic program analysis (GEL works ID) software UVP Company GEL Documentation system using SDS molecular weight marker of Sigma 70 L Kit (97000KD to 14200KD).

**Polymerase Chain Reaction (PCR) procedures:**

DNA purification was performed using 10-30 mg of fresh leaves in liquid nitrogen. The procedure suggested by Sambrook *et al.*, (1989) and polymerase chain reaction (PCR) was conducted to detect random amplified polymorphic DNA (RAPD) markers using eight random 10-mer oligoprimers as showed in Table (2) according to Williams *et al.*, (1990).

**Table 2: Code numbers and sequences of the 10-mer random primers used in the present study.**

Primer	Sequence
A1*	5' CAGGCCCTTC 3'
A2	5' TGCCGAGCTG 3'
A3*	5' GGCTGCAGAA 3'
A5*	5' GGCTGCAGAA 3'
K1	5' ACGGTACCAG 3'
K4*	5' TGCTGCAGGT 3'
O1	5' GGCACGTAAG 3'
O4	5' AAGTCCGCTC 3'
Z11*	5' CTCAGTCGCA 3'

\* primers annealed with the present DNA materials.

## RESULTS AND DISCUSSION

### 1. Growth characters:

Data presented in Table 3 indicated that mutants lines No. 7, 14, 20, 24, 25, 18, 19 and 26 showed an increase in plant height compared with the control. The increase reached the level of significance for mutant lines No. 24, 25, 19 and 26. The percentages of increase were about 27%, 27%, 22% and 27%, respectively in comparison with the control. On the other hand, the mutant lines No. 1, 4, 5, 8, 12, 21 and 2 showed a significant decrease in plant height in comparison with the control plants.

Mutant lines No. 7, 14, 21, 24, 25, 16, 18, and 28 produced an increase in number of branches/plant and the increase was only significant for mutant line No.18 and the percentage of increase was about 64%. On the contrast, the mutant lines No. 6, 2 and 22 showed a significant decrease in this trait in comparison with the control plants.

Number of leaves/plant was significantly increased in the mutants lines No. 7, 9, 14, 25, 18 and 19, but it was decreased in mutants No. 8 and 2 in comparison with the control.

All mutant lines except mutant No. 14 and 26 showed a significant decrease in root dry weight in comparison with the control. The same trend was seen in stem dry weight except for the mutant lines No. 18, 19 and 26 that which showed a significant increase in comparison with the control. Leaves dry weight tended to decrease except for mutant lines No. 9, 14, 21, 24, 18, 19, 22 and 26 which showed a significant increase in comparison with the control. The heaviest leaf dry weight was produced from mutants No. 9, 24, 18 and 26. The increase of leaves dry weight may be due to the increase in leaves number of plants in those mutants.

Total leaf area as well as the shoot/ root ratio had the same trend as observed for dry weight of leaves. The maximum increase was observed in the mutant lines No. 9, 11, 12 and 14, meanwhile the maximum decrease was seen in the mutants No. 8, 15 and 23.

From the above results, it could be concluded that faba bean mutants induced either by gamma rays or EMS tended to have higher values in most growth characters and the maximum increase was achieved by the mutant lines No. 7, 14, 24, 25, 18, 19 and 26. Similar results were reported by Charbajck and Nabulsi (1999), Surender- Kumar *et al.*, (1999) Al- Hamdany *et al.*, (1998), Waghmare and Mehra (1998), Sushil- Kumar *et al.*, (1998), Thiede *et al.*, (1995) Sagan *et al.*, (1995) and Selim *et al.*, (2002). The stimulating (at low dose of 4 KR) or the inhibitory (at high dose of 10 KR) effect on the growth may ascribe to the hormonal balance (the ratio of stimulators (IAA, GA and cytokinins)/ inhibitors (ABA)) as reported by Rabie *et al.*, (1996).

Table 3: Some growth characteristics in some faba bean mutants induced by gamma radiation and EMS.

Mut. No.	Treat.	Plant height (Cm.)	Growth characters							S/R Ratio
			Branches No./plant	Leaves No./plant	Root DW (g)	Stem DW (g)	Leaves DW (g)	T. leaf area (m <sup>2</sup> /p)		
1	0.15%EMS	58.67	4.00	69.67	1.60	15.73	17.14	75.68	20.54	
4	0.15%EMS	70.67	5.33	71.33	1.23	10.10	19.97	77.53	24.45	
5	0.30%EMS	64.67	4.67	62.33	1.30	6.57	12.84	74.36	14.93	
6	0.30%EMS	73.33	2.67	63.33	0.80	7.57	20.65	119.56	35.27	
7	0.15%EMS	85.67	6.33	98.33	1.90	12.07	23.60	109.69	16.77	
8	0.15%EMS	55.33	3.33	46.33	1.30	2.93	8.34	52.61	8.67	
9	0.15%EMS	78.33	5.33	119.00	1.97	7.47	35.70	252.22	21.91	
11	0.15%EMS	71.67	3.67	72.67	1.70	9.73	24.27	171.48	20.00	
12	0.15%EMS	65.33	5.67	79.00	2.13	8.57	18.96	109.80	12.92	
14	0.15%EMS	98.00	6.67	109.00	4.60	13.23	27.69	163.00	8.89	
15	0.15%EMS	61.00	4.67	61.00	1.60	6.77	13.05	41.92	12.39	
20	0.15%EMS	86.33	4.67	75.67	2.70	13.77	25.73	67.32	14.63	
21	0.15%EMS	68.67	6.00	72.67	2.90	8.93	28.63	74.92	12.95	
23	0.15%EMS	83.33	4.00	78.33	1.73	11.40	15.67	36.89	15.65	
24	0.15%EMS	104.00	6.33	79.67	2.60	16.77	35.05	88.45	19.93	
25	0.15%EMS	104.00	6.33	91.33	2.30	12.93	26.12	76.89	16.98	
13	Control	81.67	5.67	72.67	3.03	15.00	24.71	61.04	13.10	
LSD 5%		16.48	1.40	18.36	0.90	3.71	3.28	25.21	3.29	
2	60 GY	58.67	3.33	50.00	0.60	3.23	11.70	28.50	24.88	
3	30 GY	72.67	4.67	62.33	1.10	7.33	16.58	39.31	21.74	
16	30 GY	74.33	6.00	70.33	1.53	8.77	25.32	60.03	22.28	
17	30 GY	74.67	3.67	65.33	1.57	8.10	14.37	34.07	14.31	
18	60 GY	93.00	9.33	102.67	2.33	23.60	40.45	95.90	27.49	
19	60 GY	100.00	4.33	85.53	3.13	26.50	27.37	66.68	17.21	
22	30 GY	78.67	3.33	72.67	1.40	7.67	28.63	67.88	25.93	
26	60 GY	104.00	6.67	79.67	5.10	24.77	49.87	118.24	11.64	
13	Control	81.67	5.67	72.67	3.03	15.00	24.71	61.04	13.10	
LSD 5%		12.60	1.68	11.61	0.62	3.10	9.28	14.21	3.80	

## **2. Some physiological aspects:**

### **2.1. Photosynthetic pigments:**

Data presented in Table 4 showed that the concentrations of chlorophyll a, chlorophyll b, total chlorophylls and carotenoids in all mutant lines induced by EMS (except the mutant lines No. 20, 21, 23, 24 and 25) were significantly increased in comparison with the control. On the contrast, all the mutant lines which were produced by gamma rays except mutants No. 3 and 18 tended to be decreased in this trait comparable with the control.

From these results, it could be concluded that mutant lines which were produced by (EMS) as a chemical mutagen were more effective in increasing the concentrations of photosynthetic pigments than that produced by gamma ray. The higher concentrations were recorded by the mutant lines No. 1, 4, 5, 6, 7, 8, 9, 11, 12, 14 and 15 which gave: 4.97, 6.16, 6.61, 7.71, 7.89, 10.73, 9.77, 11.39, 10.46, 7.92 and 4.14, respectively, comparable with the control which showed 3.38. The same trend was seen for Chl. b, carotenoids and total chlorophylls.

These results are in agreement with those obtained by Seyyedi et al. (1999), Datta (1999), Ismaeil (1995), Hussein et al (1995), Selim and Atia (1996b), Yousef *et al.*, (1996); Selim and El Bana (2001) and Selim *et al.*, (2002). The changes in leaf chlorophylls and carotenoids concentration which resulted from different treatments could be interpreted on the basis of the widely and diversely effect of gamma radiation on many enzymes, genes and hormones through many aspects of growth that could be altered (Chiscon, 1962, Rabie *et al.*, 1996).

### **2.2. Leaf water relations:**

Data presented in Table 4 indicated that the total water content (TWC) in leaves of faba bean mutants No. 5, 7, 23, 24, 25, 2, 16, 17, 19, and 26 was higher, whereas the other mutants was lower in comparison to the control. As for transpiration rate (TR). Data which are presented in Table 4 showed that TR exceeded the control in all mutants produced by gamma radiation except for mutant line No. 18. On the other hand, mutants No. 5, 12, 20, 23, 24 and 25 which were produced by EMS also exceeded the control.

From these results, it could be concluded that the mutant lines produced by gamma rays were more effective than that produced by (EMS). These results are in agreement with those obtained by Selim *et al.*, (2002).

Table 4: Photosynthetic pigments concentration and water relations in some of faba bean mutants induced by Gamma radiation and EMS.

Mut No.	Treat.	Photosynthetic pigments							Water relations			
		Chl. a mg/g	Chl. b mg/g	Carotene mg/g	Chl. a+b mg/g	Chl. a/b	Chl. a+b/Car	T-total water content (%)	Transpiration rate (mg/cm <sup>2</sup> /hr)	Total water content (%)		
1	0.15%EMS	4.97	1.44	1.38	6.41	3.44	4.64	83.94	0.99	83.94		
4	0.15%EMS	6.16	1.91	1.66	8.07	3.23	4.86	85.48	0.48	85.48		
5	0.30%EMS	6.61	2.34	1.84	8.95	2.82	4.87	90.00	2.79	90.00		
6	0.30%EMS	7.71	2.34	2.11	10.05	3.29	4.76	83.51	0.46	83.51		
7	0.15%EMS	7.89	2.36	2.07	10.24	3.35	4.96	89.61	1.36	89.61		
8	0.15%EMS	10.73	3.87	2.90	14.60	2.77	5.04	85.19	0.46	85.19		
9	0.15%EMS	9.77	3.06	2.72	12.82	3.19	4.72	82.05	0.30	82.05		
11	0.15%EMS	11.39	3.77	3.21	15.15	3.02	4.73	80.01	0.05	80.01		
12	0.15%EMS	10.48	3.75	2.65	14.23	2.79	5.37	84.96	1.98	84.96		
14	0.15%EMS	7.92	3.38	2.16	11.30	2.36	5.24	85.40	0.31	85.40		
15	0.15%EMS	4.14	1.55	1.20	5.69	2.68	4.72	82.25	1.02	82.25		
20	0.15%EMS	1.76	0.87	0.50	2.63	2.03	5.27	84.36	2.02	84.36		
21	0.15%EMS	2.14	0.84	0.65	2.98	2.57	4.56	84.07	1.18	84.07		
23	0.15%EMS	3.47	0.97	1.00	4.44	3.57	4.46	87.31	4.66	87.31		
24	0.15%EMS	1.73	0.72	0.59	2.46	2.39	4.19	88.71	1.80	88.71		
25	0.15%EMS	2.00	1.01	0.62	3.01	1.98	4.88	56.48	1.88	56.48		
13	Control	3.38	1.06	0.88	4.44	3.19	5.04	85.46	1.53	85.46		
2	60 GY	3.01	1.00	0.87	4.01	3.00	4.62	87.96	2.75	87.96		
3	30 GY	3.57	1.39	0.96	4.96	2.56	5.15	85.07	5.77	85.07		
16	30 GY	1.37	0.48	0.43	1.85	2.87	4.3	86.74	1.62	86.74		
17	30 GY	2.03	0.63	0.65	2.66	3.25	4.10	87.47	10.81	87.47		
18	60 GY	3.76	1.24	1.00	4.99	3.03	4.99	82.30	1.10	82.30		
19	60 GY	2.88	1.17	0.76	4.05	2.47	5.34	87.37	1.54	87.37		
22	30 GY	1.36	0.67	0.44	2.03	2.01	4.66	85.40	4.03	85.40		
26	60 GY	2.16	0.60	0.62	2.76	3.62	4.45	90.09	2.57	90.09		
13	Control	3.38	1.06	0.88	4.44	3.19	5.04	85.46	1.53	85.46		



### **3- Flowering and yield attributes:**

Data presented in Table 5 showed that mutant lines No. 144,18, 19 and 9 and 14(in descending order) produced the greatest number of flowers/plant whereas mutants No. 2, 8 and 15 produced the lowest number of flowers in comparison with that number of the control. Either the increase or decrease in No. of flowers/plant reached the level of significance in 16 out of the 24 mutant lines in comparison with the control. Data in the same Table showed that pod number of plant was significantly increased in the mutants No. 9 and 18, but the increase did not reach the level of significance in the mutants No. 25 and 26. The percentage of pod set was significantly increased in the mutant lines No. 4, 7, 9, 15, 16, 25 and 26 in comparison with the control. Total shedding percentage of flowers and pods/plant tended to be significantly decreased in the mutants No. 4, 9, 18, 25, and 26, whereas it was significantly increased in 14 out of the 24 mutants in comparison with the control. Pod yield as well as seed yield / plant tended to increase in same mutants, whereas it was decreased in the others. The increase in pod and seed yield reached the level of significance in the mutant lines No. 9, 14, 25, 18 and 19, but it was decreased in the mutant lines No.1, 4, 5, 6, 7, 8, 12, 15, 20, 21, 2, 3, 16, 17, 22 and 26. Kumar and Mishra (1999) found that the mutagenic treatments (gamma rays 10, 20, 30 and 40 KR) and diethyl sulfate (DES) 0.25, 0.50, 0.75 and 1 % separately on green gram caused an increases in pollen sterility and number of days to flowering.

As for shelling percentages, the mutant lines No. 7, 8, 9,21, 23 and 18 showed a significant increase while the mutant lines No. 1, 4 , 19, and 26 showed considerable lower values than the control .However, the increase in yield and yield components in faba bean using physical and chemical mutagens was seen by several investigators (Atia *et al.*, 1995; Kumar *et al.*, 1995; Kumeri, 1996; Al-Hamdany *et al.*, 1998, Kumar and Mishra, 1999; Bordoloi and Talukdar, 1999 and Selim *et al.*, 2002 )

From these results, it could be concluded that the mutant lines of faba bean give a high yield that could be obtained using physical and chemical mutagens and these mutant lines will be evaluated in the next generations.

### **4. Biochemical Genetic Analysis:**

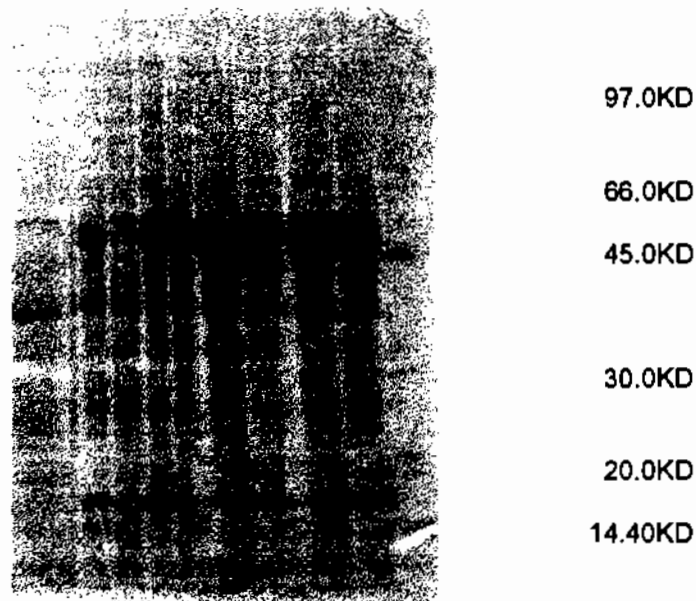
Figures 1 and 2 demonstrated the presence of variable changes in the protein banding patterns in *M Vicia faba* plants whose parents were previously treated with EMS either 0.15 or 0.30% as a chemical mutagen and with gamma- irradiation (30Gy or 60Gy) as physical mutagen. These changes included differences in band intensities and the appearance of new bands and subfractionation. These changes could be attributed to occurrence of mutational events (Muller and Gottschalk, 1973); Hussein and El-Abidin Salam,1985 and Abdelsalam *et al.*, 1993). However, most changes in banding patterns showed an increase in the high molecular weight region in both 30Gy and 60Gy radiation treatments. 60Gy mutants (2, 19 and 26) showed 6 more bands while 30Gy mutants (3,17 and 22) showed 4 more bands when compared to the control.

**Table 5: Yield characters in some faba bean mutants induced by gamma radiation and EMS.**

Mut.No.	Treat.	Flowers No./ Plant	Pods No./ plant	Pods set (%)	Total shedding (%)	Pod yield (g/plant.)	Seed yield (g/plant)	Straw yield (g/plant)	Shelling (%)
1	0.15%EMS	143.50	21.80	17.14	84.81	34.56	24.14	19.1	69.85
4	0.15%EMS	163.63	38.75	25.52	76.32	34.95	24.90	22.2	56.56
5	0.30%EMS	131.85	22.33	19.21	83.06	23.43	17.50	14.83	74.69
6	0.30%EMS	126.89	22.00	21.73	82.66	37.17	29.19	16.06	78.53
7	0.15%EMS	181.02	33.72	24.47	81.37	35.09	30.43	14.39	86.72
8	0.15%EMS	93.00	12.86	16.90	86.17	22.85	18.34	13.23	80.26
9	0.15%EMS	212.00	48.00	30.12	77.36	57.60	50.47	33.37	87.82
11	0.15%EMS	163.52	27.14	21.67	83.40	43.76	34.33	23.44	78.45
12	0.15%EMS	188.41	34.17	19.37	81.86	34.68	26.62	18.33	76.76
14	0.15%EMS	273.00	36.33	16.75	86.69	66.48	51.73	32.58	77.81
15	0.15%EMS	96.25	19.25	24.68	80.00	20.70	15.68	18.53	75.75
20	0.15%EMS	166.60	30.20	18.97	81.87	49.10	37.14	25.20	75.64
21	0.15%EMS	163.63	18.33	12.83	88.80	26.89	22.53	18.46	83.79
23	0.15%EMS	138.25	29.25	22.16	78.84	49.03	42.81	14.89	87.31
24	0.15%EMS	184.63	33.25	18.42	81.99	47.13	35.80	31.33	75.96
25	0.15%EMS	162.40	40.40	27.98	75.12	60.82	44.28	30.12	72.80
13	Control	180.71	37.38	21.79	79.31	53.57	39.08	27.44	72.95
LSD 5%		33.34	7.82	2.21	1.11	8.16	7.02	8.21	2.55
2	60 GY	74.66	15.17	23.21	79.68	23.80	17.17	13.73	72.14
3	30 GY	126.88	17.50	17.77	86.21	25.28	19.20	13.36	75.95
16	30 GY	124.85	26.50	22.43	78.77	30.32	21.73	15.77	71.67
17	30 GY	128.21	21.50	18.53	83.23	34.59	26.95	16.41	77.91
18	60 GY	238.00	73.00	37.87	69.33	93.74	79.07	49.40	84.35
19	60 GY	213.50	40.86	23.76	80.86	74.77	48.13	35.13	64.37
22	30 GY	133.60	20.75	14.60	84.47	29.78	21.02	18.92	70.58
26	60 GY	154.00	37.40	35.06	75.71	51.68	34.14	38.63	66.06
13	Control	180.71	37.38	21.79	79.31	53.57	39.08	27.44	72.95
LSD 5%		23.53	8.95	5.35	3.14	11.19	6.70	15.23	4.83

Also, 0.30 EMS treatment mutants (5, 6) showed 7 more bands and 0.15% treatment with EMS mutants (4, 7, 9, 11 and 24) showed 5 bands more than the control. Abdelsalam *et al.*, (1993) and Gamal EL-Din *et al.*, (1988) reported that the genes encoding protein bands in the high molecular weight region comprising vicilin and legumin subunits were more sensitive to the mutagenic induction due to pesticide treatments. Changes in band intensities could be explained on the basis of occurrence of mutational events in the regulatory systems that control the concerned structural genes. This would lead to constitutive protein production or to attenuation or complete suppression for the concerned genes. This would produce either an increase or decrease in band intensity, or complete band disappearance (Hussein *et al.*, 1985 ; Hassan, 1996).

Bands sub-fractionation of low molecular weight appeared in the two treatments of EMS also in gamma rays mutants which could be explained on the basis of the occurrence of gene duplications event, followed by point mutation in one or more of the duplicated genes that encode for a particular band. Therefore, four bands would be formed, two of them with the original molecular weight, while the other two were changed two. Few changes were recorded among the control sample. This could be attributed to the occurrence of few spontaneous mutational events.



**Figure 1: SDS-protein electrophoretic banding patterns of radiation induced faba bean mutant lines.**

Production of mutant high protein content was stated by Nayeem et al (1999) on wheat; Farag (1999) on sunflower; Apama-Das *et al.*, (1999) on potato. The decline in protein concentration might be attributed to the depression in RNA synthesis as reported by Selim and El-Bana (2001). Selim and Atia (1996a) found that treating wheat grains with irradiation doses of 5 and 10 kR significantly increased the concentration of DNA, RNA total nucleic acids as well as total protein in leaves. Doses over 10kr caused a gradually reduction in its concentrations.

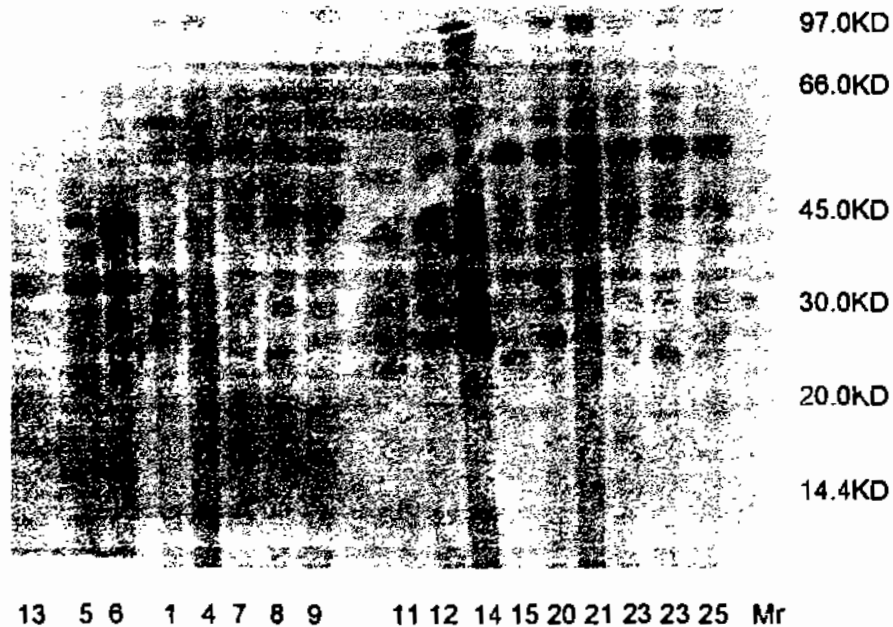


Figure 2: SDS-protein electrophoretic banding patterns of EMS induced faba bean mutant lines.

##### 5. DNA fingerprinting:

Genetic characterization of EMS, radiation and control plants, based on RAPD markers, indicated the presence of some genetic differences among the different faba bean mutant plants under this study. Nine random 10-mer primers were used for RAPD-PCR amplification of genomic DNA samples from control (13, 3), 0.15% EMS mutants (4, 7, 9, 11, 24), 0.30% EMS mutants (5, 6) and 30Gy radiation mutants (3, 17, 22) and 60Gy radiation mutants (2, 19, 26). Only five primers succeeded in amplifying the present faba bean DNA samples and generated various fragments differing in molecular size measured as base pairs (bp), while the other primers failed.

Figure 3A showed agarose-gel electrophoretic separations of DNA fragments amplified using primer A5. The control (13) plants generated five adjacent fragments with molecular sizes ranged from 3000 to 400 bp. 06Gy mutants (2, 19 and 22) showed nine fragments with molecular sizes ranging from 3000 to 200 bp showing four fragments more than the control (two fragments with size of about 1000 bp and another two small fragments equal

or less to 400 bp). 30Gy mutants (3, 17 and 22) showed seven fragments with molecular size ranged from 2500 to 400 bp. Mutants of 0.3% treatment with EMS (5, 6) showed five fragments with sizes from 2500 to 800 bp. Mutants of 0.15% treatment with EMS (4, 7, 9, 11 and 24) showed four fragments with molecular size of about 2000 to 800 bp (Table 6).

Figure 3B and Table 6 showed the fragments generated by primer (Z11) where polymorphic patterns could be noticed. The control (13) revealed six fragments ranged from 2000 bp . to 800 bp, Meanwhile, the 60Gy mutants (2, 19 and 22) showed nine fragments with molecular size of about 4000 to 400 bp with three fragments which have higher molecular sizes (2500 bp) than in the control. 30 Gy mutants (3, 17 and 22) showed six fragments with molecular size ranging from 3000 to 400 bp. Mutants of 0.3% EMS (5 and 6) showed four fragments ranging in size from 2000 to 400 bp. Meanwhile 0.15% EMS mutants (4, 7, 9, 11 and 24) showed common five fragments ranged in size from 1500 to 200 bp.

Data in Figure 3C and Table 6 showed six fragments amplified using primer A1 in both the mutant samples and the control, Meanwhile, mutants of both the 60Gy and 30Gy generated different fragments than those of the control. The control plants revealed six fragments, ranged from 2500 bp, to 400 bp. On the other hand, all the 6 mutants of the 30 Gy and 60 Gy mutants showed six similar fragments. Both the 0.15% and 0.3% EMS mutants had 5 similar size fragments out of six and only differed in one fragment as seen in Table 6.

Figure 3D showed the fragments amplified using primer K4 where polymorphic patterns could be noticed. The control revealed four fragments with molecular size ranging from 1500 to 800 bp. Both mutants induced by 60 Gy and 30 Gy treatments generated five fragments with molecular size of about 3000 to 800 bp with only larger fragment than those in the control. 0.15 and 0.30% EMS mutants showed six fragments with molecular size of about 4000 to 400 bp with two fragments larger than 2500 bp which are larger than those observed in the control as noticed in Table 6.

Figure 3E showed a clear gel of the fragments generated by primer A3 using the faba bean genomic DNA samples of mutant plants and the control. Control (13) showed a total of four identifiable fragments with different size, ranging from about 2000 to 1000 bp. Each of the 60Gy mutants (2, 19, 26) and 30 Gy mutants (3, 17, 22) showed a total of eight fragments ranging in size from about 3000 to 200 bp, with four more fragments than in the control, which had two small fragments with less than 400 bp in size and another two fragments with more than 2000 bp in size. Mutants of the 0.3% EMS (5, 6), had similar fragments as in mutants of either 60 Gy or 30 Gy treatment, while 0.15% EMS mutants (4, 7, 9, 11, 24) showed six fragments ranging in size from about 2500 to 400 bp Table 6.

In the present study, it was possible to associate the specific polymorphic DNA fragments of about 3000 bp and 200 bp in size which were generated by the random primers (A1, A5 and A3) for Gy or EMS mutants (Fig., 1A, 1B, 1E). Such markers might be of great importance in future programs of *Vicia faba* plants breeding and selection for mutants, with both highly yield and early flowering .

**Table 6: DNA fragments numbers and sizes, PCR amplified using random primers of EMS and Radiation treated**

Primers	MW	Control	0.15% MS					0.3% MS					30Gy					60Gy																							
			4	7	9	11	24	5	6	3	17	22	2	19	26	4	7	9	11	24	5	6	3	17	22	2	19	26													
A6	3000	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	2500	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	2000	1	2	2	2	2	-	-	-	-	-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2		
	1500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	1000	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Z11	400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	200	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
	Total	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
	4000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2000	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	1500	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	1000	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	800	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Total	6	6	5	9	11	24	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6		
	13	4	7	9	11	24	5	6	3	17	22	2	19	26	4	7	9	11	24	5	6	3	17	22	2	19	26	4	7	9	11	24	5	6	3	17	22	2	19	26	

Table 6: "Cont.":

Primers	MW	Control	0.15% MS						0.3% MS			30Gy			60Gy			
			4	7	9	11	24	5	6	3	17	22	2	19	26			
A1	3000				1					1	1							
	2500	1			2					2	2	1	1	1	1	1		
	2000	1																
	1500	1								1	1	1	1	1	1	1		
	1000	1								1	1	1	1	1	1	1		
	800									2	2	2	2	2	2	2		
	400	2																
200												1	1	1	1	1	1	
Total		6			6					6	6	6	6	6	6	6	6	6
K4	4000		1		1					1	1	1	1	1	1	1		
	3000																	
	2500		2		2					2	2	2	2	2	2	2		
	2000											1	1	1	1	1	1	1
	1500	2										1	1	1	1	1	1	1
	1000	1								1	1	1	1	1	1	1	1	1
	800	1								1	1	1	1	1	1	1	1	1
400									1	1	1	1	1	1	1	1	1	
200																		
Total		4	6	6	6			6	6	6	6	5	5	5	5	5	5	5

Table 6: "Cont." :

Primer	MW	Contro	0.15% MS						0.3% MS			30Gy			60Gy			
			4	7	9	11	24	5	6	3	17	22	2	19	26			
A 3	3000	13																
	2500	-	1															
	2000	1	1						1	1	1	1	1	1	1	1	1	1
	1500	2	1						2	2	2	2	2	2	2	2	2	2
	1000	1	1						1	1	1	1	1	1	1	1	1	1
	800		1															
	400		1															
	200								2	2	2	2	2	2	2	2	2	2
	Total	4	6						6	6	6	6	6	6	6	6	6	6
	400	-			1				1	1	1	1	1	1	1	1	1	1
	200	-			1				-	-	-	-	-	-	-	-	-	-
	Total	6		5					4	4	4	4	4	4	4	4	4	4



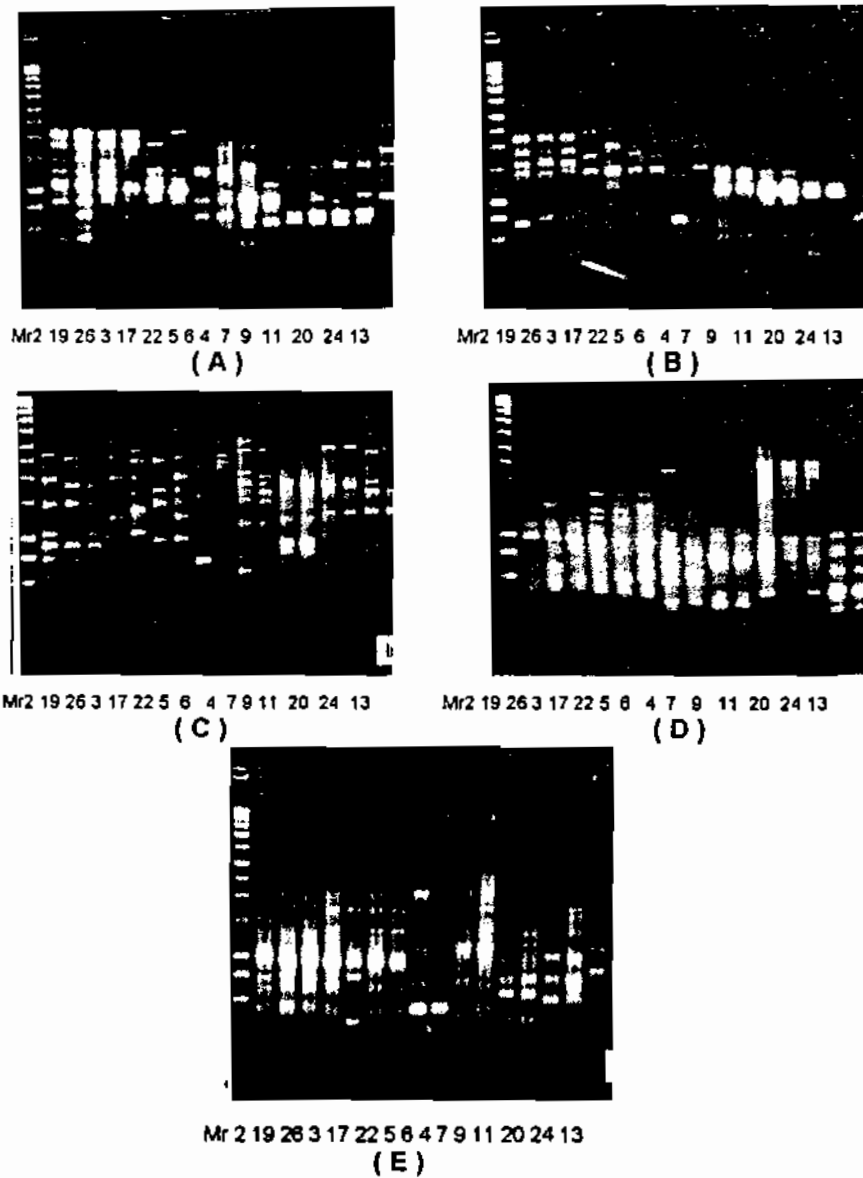


Figure. 3: Photograph of agarose gel separation of *Vicia faba* DNA profiled for EMS and radiation.

(A): primer A6 (5' GGCTGCAGAA 3')

(B): primer Z11 (5' CTCAGTCGCA 3')

(C): primer A1 (5' CAGGCCCTTC 3')

(D): primer K4 (5' TGCTGCAGGT 3')

(E): primer A3 (5' GGCTGCAGAA 3')

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## الترباط بين الصفات النباتية والمعطات الجزيئية لبعض الطفرات المستحدثة فى الفول البلدى

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تم اختيار أربعة وعشرين طفرة ناتجة من الفول البلدى صنف جيزة-٢ ٢ المعامل بجرعات من اشعة جاما ( ٦٠ و ٣٠ جراى ) وتركيزات مختلفة من مادة الاثيل ميثان سلفونات ( ٠,١٥% و ٠,٣٠% ) وذلك من خلال برنامج التربية لهذا الصنف حيث تم الحصول على الطفرات الأربعة والعشرون وذلك لدراسة التغيرات الناتجة من استخدام هذه الطفرات الطبيعية والكيميائية من خلال دراسة صفات النمو الخضري والترهيب وبعض الصفات الفسيولوجية وصفات المحصول والقيمة الغذائية للبذور مقارنة بالكنترول الغير معامل وكذلك دراسة المعطات الوراثية للبروتين وال دن أ وذلك من خلال RAPD ، SDS ، PAGE. - وقد أوضحت النتائج ما يلى :

- ١ - كانت الطفرات الحادثة سواء باستخدام اشعة جاما او مادة الاثيل ميثان سلفونات تميل الى ان تكون ذات قيمة عالية لمعظم صفات النمو الخضري وتتفوق على الكنترول وقد تحققت زيادة عالية بالنسبة للطفرات : ٧ ، ١٤ ، ٢٤ ، ٢٥ ، ١٨ ، ١٩ ، ٢٦ .
- ٢ - تفوقت الطفرات التي نتجت من استخدام مادة الاثيل ميثان سلفونات فى زيادة تركيز الصبغات التمثيلية وكذلك الكلوروفيل ب والكاروتينويدات والكلوروفيلات الكلية عن تلك الناتجة من استخدام اشعة جاما .
- ٣ - كان معدل الفتح اعلى لكل الطفرات الناتجة من استخدام اشعة جاما عن الكنترول عدا الطفرة رقم ١٨ ، وعلى الجانب الاخر تفوقت الطفرات : ٥ ، ١٢ ، ٢٠ ، ٢٣ ، ٢٤ ، ٢٥ الناتجة عن طريق مادة الاثيل ميثان سلفونات على الكنترول وكانت الطفرات الناتجة من اشعة جاما اعلى من الطفرات الناتجة من استخدام مادة الاثيل ميثان سلفونات.
- ٤ - أظهرت السلالات ٧ ، ٨ ، ٩ ، ٢١ ، ٢٣ ، ١٨ زيادة معنوية بالنسبة لنسبة التقشير بينما حازت الطفرات : ١ ، ٤ ، ١٩ ، ٢٦ على قيم منخفضة عن الكنترول .
- ٧ - أظهرت البيانات وجود زيادة فى المحصول ومكوناته سواء باستخدام الطفرات الطبيعية أو الكيميائية .
- ٨ - أوضحت البيانات انه من الممكن استخدام شذايا الدن أ بحجم 200bp،3000bp ناتجة من استخدام البادئ العشوائى A1,A5,A3 كمعطات جزيئية بالنسبة للطفرات الناتجة عن طريق الـ Gy أو مادة الاثيل ميثان سلفونات لتمييز صفات المحصول العالى والترهيب المبكر فى الفول البلدى.