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EFFECT OF GAMMA IRRADIATED SEEDS OF *Phaseolus vulgaris* ON THE GENOSYMBIOTIC EFFICIENCY OF *Rhizobium leguminosarum* BIOVAR PHASEOLI AFFECTING VEGETATIVE AND SOME CHEMICAL TRAITS IN *Phaseolus vulgairs*

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ABSTRACT

Seeds from two varieties of common bean (*Phaseolus vulgaris*) were irradiated with four doses of gamma rays to induce mutations affecting *Rhizobium* symbiosis, as the development of N_2-fixing nodules requires a coordinated expression of genes of both symbiotic partners. Symbiotic response to irradiation affecting leguminous host was measured in two generations using wild type strain of rhizobia . Both doses of 20 and 40 Krad revealed higher symbiotic efficiency in M_1 and M_2 populations, respectively. Inoculation with rhizobia was shown to decrease the deleterious effect of radiation below that of uninoculated ones. Gamma irradiation disrupted the normal ontogeny leading to reduction the development of new tissues in some vegetative traits. Leaf area was markedly reduced by gamma irradiation, and was suitable for radiosensitivity assay depending on the growth of genotypes. Leaf area was significantly affected by both doses of gamma irradiation and biofertilization among M_1 and M_2 populations. Protein content and chlorophyll concentrations in most of the inoculated plants was higher than uninoculated ones. Plants inoculated with rhizobia produced higher seed protein content than uninoculated ones. The interaction between varieties by biofertilization significantly affected shoot nitrogen content among M_1 and M_2 populations. Pod productivity in both generations was markedly affected by plant genotypes, doses of gamma rays and biofertilization. Reductions in seed and pod yields / plant were observed in M_1 and M_2 populations as dosages of gamma rays progressed. The results indicated that plant is most sensitive to gamma irradiation from the time it begins to develop its reproductive capability until after fertilization when embryo development begins. Irradiation was used in this study to induce genetic variation in common bean to enhance the response of rhizobia and the legume host to increase N_2 fixation .

Keywords: Chlorophyll, gamma rays, *Phaseolus vulgaris*, *Rhizobium*, symbiotic efficiency .

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) represents the main source of protein for Egyptian people. Enhancement of its nitrogen-fixing capacity is thus a major agronomic goal. The capacity to fix N_2 is variable among genotypes of common bean, ranging from 4 to 59% nitrogen derived from the atmosphere (Hardarson *et al.*, 1993). Breeding programmes with the objective of enhancing nitrogen fixation and yield of common bean are based exploiting this variability. Crop responses to inoculation with selected strains of *Rhizobium* are often low, frequently due to the high competitive ability of native rhizobia. *Rhizobium leguminosarum* is normally present in soils in numbers ranging from 10^3 to 10^5 cells per gram of soil (Jensen *et al.*, 1985). The indigenous populations contain ineffective or poorly effective, as well as, highly effective strains (Jensen, 1987). The ineffective strains may be as competitive for nodule formation on the legume hosts as the effective ones (Amarger, 1981), inoculation with effective strains could increase the crop yield. A bean crop may accumulate more than 300 kg N ha^{-1} of which 60 to 70% may be derived from the symbiotic relationship with *Rhizobium leguminosarum* (Amarger, 1981). Genetic information in *Rhizobium spp.* is usually distributed among the chromosome and different large plasmids. *Rhizobium leguminosarum* bv. *Phaseoli* CFN42 contains six plasmids, ranging in size from 200 (p42*a*) to 600 (p42*f*) kb. (Brom *et al.*, 1991). This study was designed to investigate the effects of gamma irradiation-treated seeds on induction of genetic variation leading to improvement of the symbiotic performance developed between the wild type strain of *Rhizobium leguminosarum* biovar *phaseoli* and their host plant of *Phaseolus* beans, affecting the vegetative, chemical traits and yield components of *Phaseolus* beans.

MATERIALS AND METHODS

I. Genetic materials :

seeds of two varieties of common bean (*Phaseolus vulgaris*) namely Polesta 68 and Giza 91 were kindly provided by Vegetable Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The wild type strain of *Rhizobium leguminosarum* bv. *phaseoli* USDA3644 , obtained from the United States Department of Agriculture, USDA , was used in this study to inoculate common bean. This strain was grown in yeast extract mannitol medium (YEM) according to Allen (1959).

II. Field experiment:

Dry seeds of the two *Phaseolus vulgaris* cultivars were subjected to 10, 20, 30 and 40 Krad of gamma radiation from a cobalt-60 source at the Radioisotope Center, Madenet Naser, Cairo, Egypt. Treated seeds were grown in randomized complete block (RCB) with three replications . Each treatment combination was planted in plot of ten rows, where each row was 4.0 m in length and 0.7 meter in width . A distance of 30 cm was maintained between the plants. Plants were thinned after 3-4 days of germination to two plants per hill. The plants of M_1 and M_2 generations were inoculated after germination with rhizobia suspension (10^8 cells/ml) for two times with the rate of one ml/plant with seven days interval in between . Several traits were observed to assess the dose response, such as , nitrogen fixation, chlorophyll concentration, plant dry weight, number of branches per plant, number of pods per plant, pollen abortion, leaf area and weight of seeds per plant. The M_1 and M_2 generations were grown in the field experiments at the farm in El-Lowsey village, Kafer Saad Center, Domyate Governorate, during the summer season of 2000 and 2001. At maturity, 18 guarded plants were selected at random from each treatment and data were recorded for number of pods per plant, and yield per plant . In addition , the other observations were recorded at 45 days of planting.

In M_2 study, seeds from each treatment in M1 generation were bulked. Random samples from each bulked treatment were planted in an RCB design with three replications. Rows were the same as in M_1 study and the traits in M_1 were recorded in M_2 populations at the same age of plants used before in M_1 study.

III. Inoculation:

Cultures in mid-log phase growing in nutrient broth of YEM were used for inoculation (Kucey, 1989). Seeds were surface-sterilized with 10% ethanol solution (Dobert and Blevins, 1993) and washed three times with sterilized distilled water. Five-surface-sterilized seeds were then planted in each hill using a randomized complete block design with three replications. Plants were thinned after 3-4 days of germination to two plants per hill. The plants were watered to field capacity with water as needed until harvest. The plants were inoculated after germination with rhizobia suspension (10^8 cells/ml) for two times with the rate of one ml/plant, seven days between each of them. The plants were fertilized by phosphorus at the rate of 80 kg/feddan

1. Symbiotic efficiency and vegetative traits:

After four weeks from planting, the plants of three replications, each containing two plants from each plot were removed and washed by tap water. Then, the shoots and roots were separated, dried and weighted.

Symbiotic efficiency (SE) was calculated as follows:

SE = Shoot dry weight (SDW) of inoculated plants / SDW of non-inoculated plants.

The different parts of plant (shoots and roots) were oven dried at 70°C to constant weight. The dry weights of shoots and roots per plant were calculated for each inoculation treatment. Leaf area / plant was determined using the fresh weight method. The leaves were cleaned from dust and then weighed. Certain known disks were taken from the leaves with a cork puncher and weighed. The leaf area was calculated according to the following formula:

$$\text{Leaf area in dm}^2 = \frac{\text{Fresh weight of leaf}}{\text{Fresh weight of disk}} \times \text{Leaf area of disks in dm}^2$$

For the determination of chlorophyll concentrations, leaf tissue was collected at random and was put in 10 ml of 80% methanol overnight in the dark, and then the extract was read at 650 and 665 O.D. using a spectrophotometer (Spekoll 11, Carl Zeiss). Chlorophyll concentrations was calculated according to Markinney's (1941) formula as follows :

mg chlorophyll a / g tissue = 12.7 (A 663) – 2.69 (A 645) X V / 1000 X W

mg chlorophyll b / g tissue = 22.9 (A645) – (A663) X V / 1000 X W

Where , A = Absorbance at specific wavelengths ,

V = Final volume of chlorophyll extract

and W = Fresh weight of tissue extracted

2. Determination of nitrogen content in plant and seeds:

Nitrogen content in both dried plant materials and seeds was determined by the wet digestion of dried and finely pulverized plant material using the macrokjeldahl method (Jakson, 1973). Samples of 0.20 gm dry material were digested by sulphoric and perchloric acids. Distillation was carried out using 40% NaOH, and ammonium was received in 4% boric acid solution. The distillation were then titrated with 0.041 N HCl using the mixed methyl red-bromocresol green indicator. Nitrogen concentration was determined according to Burris and Wilson (1957). Nitrogen and protein percentage was calculated on weight basis according to Jackson (1973) as follows:-

$$N\,\% = \text{Volume of acid used} \times \text{acid molarity} \times \frac{0.014}{\text{Sample weight}} \times \frac{\text{Total volume of sample}}{\text{Volume of used sample}} \times 100$$

Total nitrogen content (mg / plant) = N_2 % x dry weight of plant x 100

Crude protein in plant tissue and seeds (%) = N% x 6.25.

3. Yield and its components

Immature green pods were continuously harvested when they reached suitable maturity stage. The following traits were recorded, average number of pods per plant, average weight of green pods per plant, pod length and number of branches per plant.

4. Pollen abortion:

Pollen was collected from plants in each exposure rate and stained with acetocarmine: pollen abortion was estimated from a total of 400 pollen grains scored in four different fields of the slide according to Monti and Danini (1968).

5. Yield response % to inoculation:

Utilization efficiency of phosphorus and nitrogen were calculated concerning the seed yield per plant, measured in the sample of four randomly collected plants . Yield response (%) to *Rhizobium* inoculation was expressed as follows:

$$\frac{\text{Grain yield with } \textit{Rhizobium} - \text{Grain yield without } \textit{Rhizobium}}{\text{Grain yield without } \textit{Rhizobium}} \times 100$$

Statistical analysis:

The data were subjected to the analysis of variance of factorial arrangement in a randomized complete block design with the general linear model (GLM) procedure for repeated measures of SAS (1995).

RESULTS AND DISCUSSION

1. Effect of gamma-irradiated *phaseolus vulgaris* on symbiotic efficiency :

Early steps in the establishment of the nitrogen-fixing symbiosis between rhizobia and legumes involve an exchange of signals between the two partners (Long, 1996). Roots of host plants secrete flavonoids, which activate the products of rhizobial regulatory *nod D* genes and induce the expression to bacterial nodulation (*nod, nol,* and *noe*) genes (Long, 1996). Most Nod proteins are involved in the synthesis and excretion of signal molecules, called Nod factors (NFs), which elicit a number of symbiotic responses on host plants (Schultz and Kondorosi, 1998). The results presented in Table (1) revealed that symbiotic efficiency was higher at 20 and 40 krad in both varieties in M_1 and M_2 generations, respectively. This indicated that the dose of 20 Krad revealed higher symbiotic efficiency in M_1 generation of both common bean cultivars. In addition, the dose of 40 krad revealed higher symbiotic efficiency in M_2 generation.The results summarized in M_2 generation (Table 2) revealed that non of the source of variances showed any significant effect on symbiotic efficiency . Laranjo *et al.* (2001) reported that rhizobia isolates with a single plasmid showed a significantly higher symbiotic efficiency in chickpea (*Cicer arietinum*). This agrees with the hypothesis that extra symbiotic genes are pseudogenes, or are activated under different conditions, as already suggested for the presence of extra copies of *nif* genes *in Rhodopseudomonas capsulate* (Scolnik and Haselkorn, 1984).

Table 1: Effect of gamma - irradiated *Phaseolus vulgaris* on symbiotic efficiency ratio developmented in common bean in two generations.

Traits	Var.	Gen.	Doses (K rad)					L.S.D. At 0.05
			0	10	20	30	40	
Symbiotic efficiency ratio	I	M_1	1.06 ±0.325	1.126 ±0.186	1.226 ±0.41	0.793 ±391	0.69 ±0.237	0.522
		M_2	0.993 ±0.119	1.20 ±0.141	1.11 ±0.020	1.946 ±307	2.94 ±0.39	1.786
	II	M_1	1.37 ±0.73	1.320 ±0.882	1.65 ±0.086	1.14 ±0.121	1.03 ±137	0.522
		M_2	1.06 ±0.168	1.343 ±0.115	1.24 ±0.415	1.35 ±1.24	2.66 ±1.52	1.786

M_1, M_2, = Irradiated parental population and first resulted generation, respectively. I, II = Variety Polesta 68 and Giza 6, respectively. var. = Varieties, Gen. = Generations, a, b = Inoculated and uninoculated plants, respectively.

Table 2: Analysis of variance and mean squares of symbiotic efficiency affected by gamma irradiation in *Phaseolus vulgaris* .

Source Of variance	df	Symbiotic efficiency			
		M_1		M_2	
		MS	Pr > F	MS	Pr > F
Varieties (Var.)	1	0.771	0.056^{NS}	0.022	0.920^{NS}
Doses (Dos.)	4	0.314	0.195^{NS}	3.217	0.250^{NS}
Var.x Dos.	4	0.010	0.993^{NS}	0.054	0.998^{NS}
Error	20	0.1880		2.199	

M_1, M_2, = Irradiated parental population and first resulted generation, respectively.

2. Effect of gamma- irradiated *phaseolus vulgaris* on the symbiotic performance of rhizobia and their effects on vegetative parameters of common bean:

The results presented in Table (3) showed that all vegetative growth traits revealed significant differences between rhizobia inoculated and uninoculated plants for most doses of gamma irradiated *Phaseolus vulgaris* in M_1 and M_2 generations. The number of tillers per plant in variety I, for example ranged between 2.06 – 5 and 1.33 – 5 in inoculated and uninoculated plants, respectively. All vegetative growth traits were reduced significantly by gamma irradiation, compared with non-irradiated plants . The disruption of normal morphogenesis of branches / plant was also show at the higher doses of gamma irradiated plants (40 Krad), among M_1 and M_2 generations in both varieties of common bean. This indicated that irradiation generally had a depressing effect on the number of branches / plant, especially at higher doses of gamma irradiation (40 Krad). Radiation treatments with increasing doses led to reduction in the development of new tissues. From the foregoing discussion, it is apparent that the marked decrease in the number of tillers / plant was the direct consequence of vegetative growth and development reduction, which subsequently affected reproductive structures. This resulted in further decrease in the number of flowers and pods / plant.

The doses of gamma irradiation affect to significantly reduced shoot and root dry weight among both varieties in M_1 and M_2 populations. This indicated that growth rate was generally reduced when *Phaseolus vulgaris* were subjected to ionizing radiation. Van't Hof and Sparrow (1965), in a similar study , found that irradiation decreased the number of proliferating cells thereby reducing the growth rate. The decrease in total biomass affected by gamma irradiation were due to depressing effect on stems, leaves and number of tillers per plant. The present results are in agreement with Teramura and Murali (1986), who reported that the total dry weight of Essex cultivar of soybean was reduced upon exposure to UVB. The results also showed lower growth rate in irradiated plant roots in relative to unirradiated plants with increasing exposure rate. The present results are in agreement with the results obtained by Al-Rubeai and Godward (1981), who found that there was a clear difference in gamma-radio-sensitivity between the parental varieties of *Phaseolus vulgaris* L. as increased by survival, seedling height, root length and pollen stainability.

Table 3: Effect of gamma - irradiated *Phaseolus vulgaris* on the mean of vegetative traits due to symbiotic performance of rhizobia in common bean among two generations.

Traits	Var.	Gen.	Doses (Krad) 0		10		20		30		40		L.S.D. at 0.05
			a	B	A	b	A	b	A	b	a	B	
Number of tillers / plant	I	M_1	5.00±1.00	4.00±1.00	4.33±1.15	3.66±0.57	3.66±0.57	3.00±1.73	3.33±0.57	3.00±1.00	2.66±1.50	3.00±1.00	0.567
		M_2	4.00±1.00	5.00±1.00	3.66±0.57	3.66±0.57	3.00±1.00	2.66±0.57	2.66±0.57	1.66±0.57	2.00±1.00	1.33±0.57	0.380
	II	M_1	5.66±2.08	5.00±1.00	5.00±1.00	4.66±1.52	4.66±1.52	4.00±1.00	3.66±0.57	3.00±1.00	2.66±1.15	2.33±0.57	0.567
		M_2	4.00±1.00	4.66±1.00	3.66±0.57	4.00±0.00	3.33±0.57	3.33±0.57	2.60±1.00	1.66±0.57	1.00±0.00	1.33±0.57	0.380
Root dry weight (g/plant)	I	M_1	0.590±0.085	0.230±0.028	0.450±0.050	0.206±0.037	0.383±0.076	0.176±0.005	0.393±0.076	0.150±0.050	0.300±0.050	0.156±0.044	0.037
		M_2	0.226±0.032	0.530±0.250	0.186±0.010	0.400±0.100	0.130±0.020	0.330±0.050	0.103±0.005	0.200±0.100	0.090±0.010	0.133±0.051	0.036
	II	M_1	0.416±0.373	0.370±0.037	0.266±0.057	0.250±0.019	0.166±0.057	0.193±0.010	0.086±0.015	0.130±0.020	0.053±0.015	0.090±0.010	0.037
		M_2	0.233±0.040	0.350±0.035	0.186±0.015	0.216±0.028	0.133±0.015	0.203±0.015	0.113±0.011	0.193±0.011	0.106±0.11	0.170±0.200	0.036
Shoot dry weight (g/plant)	I	M_1	4.03±0.55	3.76±0.49	3.33±0.57	2.93±0.305	2.90±0.650	2.430.404	1.56±0.635	2.26±1.10	1.10±0.10	1.80±0.79	0.307
		M_2	3.06±0.51	3.06±0.150	3.30±0.36	2.73±0.05	2.26±0.025	2.03±0.208	1.73±0.11	1.56±0.32	0.46±0.35	0.53±0.37	0.164
	II	M_1	3.06±0.152	2.66±1.26	3.20±0.655	2.13±0.90	2.13±0.35	1.26±0.020	1.56±0.208	1.36±0.57	1.166±0.115	1.133±0.058	0.307
		M_2	3.06±0.152	2.66±0.128	3.20±0.125	3.20±0.057	2.13±0.10	1.26±0.37	1.56±0.208	1.366±0.208	1.6660.115	1.3330.057	0.164
Leaf Area	I	M_1	14.00±1.00	12.66±1.52	12.66±2.08	12.662.08	12.00±2.00	10.00±1.00	10.66±2.51	8.00±1.00	7.00±1.00	6.06±1.00	0.753
		M_2	13.66±1.52	13.00±1.00	14.33±3.05	11.33±1.52	11.33±1.15	9.00±1.00	9.66±1.50	6.33±1.57	7.33±0.57	6.66±1.154	0.797
	II	M_1	13.00±1.00	12.66±1.52	12.33±1.52	11.66±1.52	11.66±1.52	9.00±1.00	9.00±1.00	8.00±1.00	6.66±1.52	7.00±1.00	0.753
		M_2	16.66±1.52	16.00±1.00	15.33±2.51	14.00±1.00	18.33±1.52	11.66±1.52	11.00±2.00	10.66±1.527	8.33±1.527	8.00±1.00	0.797

M_1, M_2, = Irradiated parental population and first resulted generation, respectively. I, II = Variety Polesta 68 and Giza 6, respectively.
var. = Varieties, Gen. = Generations, a, b = Inoculated and uninoculated plants, respectively.

M_1, M_2, = Irradiated parental population and first resulted generation, respectively. I, II = Variety Polesta 68 and Giza 6, respectively.
var. = Varieties, Gen. = Generations, a, b = Inoculated and uninoculated plants, respectively.

In both cultivars of common bean used in this study, the doses of gamma irradiation markedly reduced leaf area, but in most cases, it was higher in inoculated plants than uninoculated ones. These results are in agreement with those obtained by Mark *et al.* (1997), who found that all *Phaseolus vulgaris* L examined showed significant reduction in height of up to 31.8% in most growth phases under intensive UV-B, however, fresh and dry weight and leaf area were reduced under intense UV-B in the cultivars. In general, the leaf area of irradiated plants was much less than that of control, and also irradiation generally had a depressing effect on leaf area. Also, it is evident that leaf area was suitable for radiosensitivity assay depending on the growth of genotypes, leading to notable leaf area being markedly affected by gamma irradiation. The results indicated that maximal reduction in stem length occurred when plants were irradiated by 40 Krad. This are in agreement with Van't Hof and Sparrow (1965), who found that irradiation had no effect on the duration of the mitotic cycle, but that irradiation decreased the number of proliferating cells, thereby reducing the growth rate.

The mean squares concerning number of tillers / plant (Table 4) revealed that gamma irradiation appeared to have significant effect on the number of tillers / plant among M_1 and M_2 populations, more than all other sources of variance. The effect of gamma irradiation on total plant biomass may be dependent on the concentration of gamma irradiation absorbing compounds that attenuate incoming radiation and effectively limit damage to cellular components including the genetic material. The results obtained here are in agreement with Huystee and Cherry (1967), who found that exposure of peanut seeds to 250 and 500 Krad of x-rays reduced germination by 30 and 50 percent, respectively. The same x-rays dosages inhibited seedling growth by as much as 60 percent. Conversely, sensitivity of all cultivars of cowpea may reside in their inability to increase or even maintain levels of radiation-absorbing compounds under elevated gamma irradiation. It is notable that irradiation, generally, had a depressing effect on number of branches / plant.

The mean squares of root dry weight among M_1 and M_2 populations showed that varieties, doses of gamma irradiation, biofertilization had highly significant effect on root dry weight among both generations. In addition, the interaction between variety x biofertilization shown significant effect in M_1 population, rather than their effect in M_2 population. The interaction between doses effect of gamma irradiation x biofertilization showed significant effect in M_2 population. The results obtained here indicated a disruption of normal morphogenesis in the irradiated plant, which affected growth of new tissues in the roots.

The mean squares of shoot dry weight revealed significant effect of genotypes (varieties) and doses of gamma irradiation among M_1 and M_2 populations on the growth of common bean. Biofertilization also showed significant effect on dry weight in M_2 population rather than in M_1 population. This are due to the disruption of normal morphogenesis in the irradiated plant which affected growth of new tissues in the shoots concerning number of branches / plant, number of leaves, and leaf area. The results obtained here are in agreement with Donini (1967), who assumed that the higher

Table 4 : Analysis of variance and mean squares of vegetative traits affected by gamma - irradiated *Phaseolus vulgaris* and symbiotic performance of rhizobia.

Source of variance	df	Number of tillers / plant				Root dry weight				Shoot dry weight			
		M_1		M_2		M_1		M_2		M_1		M_2	
		MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
Varieties (Var.)	1	3.75	0.082^{NS}	0.066	0.729^{NS}	0.150	0.001**	0.027	0.22^{NS}	6.20	0.001**	5.52	0.0001**
Doses (Dos.)	4	9.64	0.001**	18.18	0.001**	0.119	0.001**	0.080	0.001**	8.75	0.001**	13.14	0.0001**
Biofertilization (Fer)	1	3.75	0.08^{NS}	0.00	1.00^{NS}	0.193	0.001**	0.225	0.0001**	0.79	0.137^{NS}	0.77	0.0082**
Var.x Dos.	4	0.25	0.52^{NS}	0.483	0.048^{NS}	0.010	0.095^{NS}	0.080	0.152**	1.33	0.439^{NS}	0.60	0.006*
Var.x Fer.	1	0.016	0.906^{NS}	0.60	0.30^{NS}	0.227	0.001**	0.033	0.008**	1.204	0.06^{NS}	0.32	0.57^{NS}
Dos. x Fer.	4	0.291	0.910^{NS}	0.91	0.17^{NS}	0.009	0.148^{NS}	0.010	0.08^{NS}	0.75	0.089^{NS}	0.114	0.035^{NS}
Var.x Dos.x Fer	4	0.141	0.974^{NS}	0.183	0.85^{NS}	0.019	0.822^{NS}	0.060	0.24^{NS}	0.063	0.93^{NS}	0.012	0.97^{NS}
Error	40	1.183		0.55		0.005		0.048		0.346			

radiosensitivity of chronically irradiated plants of *Pinus halepensis* compared to *P. pinea* can also be due to the longer duration of the mitotic cycle in the former species.

Doses of gamma irradiation and biofertilization showed a significant effect on leaf area in M_1 population, rather than other sources of variation (Table 5). However, plant genotypes (varieties), doses of gamma irradiation, biofertilization and the interaction between varieties x biofertilization showed a significant effect on leaf area in M_2 population, rather than other sources of variation. This was in agreement with Sheverov *et al.* (1992), who found that the lowest radiation dose slightly stimulated the growth in pea seedlings.

Leaf area was similar significantly affected in M_1 and M_2 populations by the doses of gamma irradiation and biofertilization. Although, there were no significant differences between leaf area in several samples of wild bean and the two improved cultivars evaluated by Pena-Valdivia *et al.* (1997), the smaller leaf size of bean was evident in this study due to radiation. Leaf area efficiency depends mainly on the photosynthetic activity, but other factors such as maximum duration of photosynthetic activity of a leaf, the leaf biomass accumulation and its partitioning are also important (Evans, 1993).

Table 5 : Analysis of variance and mean squares of leaf area affected by gamma-irradiated *Phaseolus vulgaris* and symbiotic performance of rhizobia.

Source Of variance	df	Leaf area			
		M_1		M_2	
		MS	Pr > F	MS	Pr > F
Varieties (Var.)	1	1.66	0.376^{NS}	74.81	0.001**
Doses (Dos.)	4	85.89	0.001**	113.44	0.001**
Biofertilization (Fer)	1	17.06	0.00067**	8.81	0.05*
Var.x Dos.	4	0.62	0.87^{NS}	9.191	0.72^{NS}
Var.x Fer.	1	1.66	0.376^{NS}	30.81	0.008**
Dos. x Fer.	4	2.77	0.274^{NS}	0.85	0.83^{NS}
Var.x Dos.x Fer	4	0.875	0.793^{NS}	1.108	0.75^{NS}
Error	40	2.08		2.23	

Tabulated F at 0.05 and 0.01 probability levels are equal 4.08 and 7.31, respectively at df 1/40. It was equal 2.60 and 3.83 at the same probability levels, respectively at df 4/40.

3. Effect of gamma-irradiated common bean under the effect of inoculated rhizobia on pollen viability and some chemical traits of *phaseolus vulgaris*:

Pollen collected from plants at each exposure rate and stained with acetocarmine clearly showed a decrease in stainability with increasing exposure rate (Table 6). The decrease in pollen stainability with increasing exposure rate indicated the increase in pollen abortion. In general, plants inoculated with rhizobia revealed high tolerance to gamma irradiation than uninoculated plants, because of significant differences obtained between both of them at most exposure rates. As shown from the results, pollen abortion depended on the dose of exposure rate only. For all controls, a normal pollen fertility was found in variety I (between 81 and 86 percent), while variety II

Table 6: Effect of gamma - irradiated *Phaseolus vulgaris* on pollen viability and some chemical traits as affected by symbiotic performance of rhizobia with common bean in two generations.

Traits	Var.	Gen.	Doses (Krad)										L.S.D. at 0.05
			0		10		20		30		40		
			a	B	A	b	A	b	A	b	a	B	
Pollen stainability ratio	I	M_1	0.860 ±0.13	0.810 ±0.085	0.713 ±0.153	0.613 ±0.242	0.590 ±0.030	0.640 ±0.520	0.530 ±0.010	0.583 ±0.126	0.383 ±0.040	0.450 ±0.050	0.047
		M_2	0.813 ±0.020	0.806 ±0.086	0.666 ±0.076	0.800 ±0.05	0.490 ±0.177	0.590 ±0.050	0.573 ±0.125	0.450 ±0.020	0.423 ±0.083	0.306 ±0.005	0.056
	II	M_1	0.793 ±0.097	0.920 ±0.043	0.753 ±0.085	0.720 ±0.085	0.720 ±0.051	0.610 ±0.030	0.553 ±0.045	0.550 ±0.030	0.296 ±0.025	0.320 ±0.026	0.047
		M_2	0.860 ±0.036	0.560 ±0.310	0.730 ±0.055	0.746 ±0.055	0.696 ±0.133	0.680 ±0.105	0.526 ±0.115	0.476 ±0.075	0.330 ±0.051	0.276 ±0.075	0.056
Seed protein ratio	I	M_1	0.206 ±0.005	0.186 ±0.015	0.196 ±0.005	0.170 ±0.010	0.173 ±0.005	0.160 ±0.020	0.146 ±0.005	0.143 ±0.030	0.126 ±0.005	0.123 ±0.025	0.009
		M_2	0.206 ±0.005	0.200 ±0.010	0.180 ±0.010	0.186 ±0.005	0.170 ±0.110	0.170 ±0.010	0.150 ±0.01	0.150 ±0.01	0.140 ±0.01	0.126 ±0.152	0.005
	II	M_1	0.206 ±0.015	0.186 ±0.015	0.180 ±0.01	0.163 ±0.015	0.146 ±0.01	0.150 ±0.026	0.136 ±0.026	0.143 ±0.015	0.123 ±0.015	0.126 ±0.020	0.009
		M_2	0.203 ±0.050	0.193 ±0.005	0.176 ±0.005	0.170 ±0.010	0.145 ±0.050	0.163 ±0.005	0.136 ±0.020	0.143 ±0.005	0.123 ±0.010	0.120 ±0.100	0.005
Ratio of shoot nitrogen	I	M_1	0.047 ±0.025	0.043 ±0.005	0.043 ±0.005	0.043 ±0.007	0.040 ±0.005	0.035 ±0.005	0.033 ±0.005	0.028 ±0.007	0.030 ±0.005	0.025 ±0.005	0.0032
		M_2	0.051 ±0.002	0.050 ±0.005	0.038 ±0.007	0.045 ±0.005	0.036 ±0.007	0.037 ±0.0025	0.031 ±0.002	0.030 ±0.003	0.023 ±0.002	0.023 ±0.002	0.0043
	II	M_1	0.045 ±0.005	0.041 ±0.005	0.041 ±0.007	0.040 ±0.005	0.036 ±0.007	0.035 ±0.005	0.033 ±0.002	0.030 ±0.005	0.026 ±0.011	0.028 ±0.005	0.0032
		M_2	0.054 ±0.005	0.048 ±0.007	0.046 ±0.003	0.045 ±0.005	0.040 ±0.004	0.035 ±0.001	0.035 ±0.005	0.028 ±0.010	0.031 ±0.002	0.023 ±0.005	0.0043

M_1, M_2, = Irradiated parental population and first resulted generation, respectively. I, II = Variety Polesta 68 and Giza 6, respectively.
var. = Varieties, Gen. = Generations, a, b = Inoculated and uninoculated plants, respectively.

showed lower pollen fertility which ranged between 56 and 92 percent. Pollen abortion and/or fertility seems the best parameter among those analyzed for several reasons. First of all, the percentage of pollen fertility for a given exposure rate does not change in flowers collected at different times, the total exposure received by the plant having no influence (Monti and Donini, 1968). This was already shown in chronic irradiation experiments in *Capsella* (Devreux, 1963). Furthermore, the controls are quite homogeneous (from 19 to 14 percent pollen abortion) in variety I. Therefore, this parameters was sensitive to small increases in the exposure rate. The high sensitivity of this parameter is probably due to a more direct estimation of the radiation damage, the analysis being performed at the cellular level. The results obtained here are in agreement with Evans and Sparrow (1961) and Donini (1967). who assumed that the higher radiosensitivity of chronically irradiated plants of *Pinus halepensis* compared to *P. pinea* can also be due to the longer duration of the mitotic cycle in the former species.

Legumes and rhizobia can grow and reproduce independently, but neither usually fixes atmospheric N_2 alone (Sprent and Minllin, 1985). Among both varieties of *Phaseolus vulgaris*, most of inoculated plants showed significant increase in percent of seed protein and nitrogen in shoots, both traits were decreased with increasing exposure doses of gamma irradiation. Fixed N_2 results from the cumulative action of factors such as specific nodule activity, leghemoglobin concentration, nitrogenase activity and total leghemoglobin, which fluctuate, following seasonal profiles (Rennie and Kemp, 1984). Wolyn *et al.* (1989) reported that the high-fixing line of *Phaseolus vulgaris* L. had significantly more nodule mass than the low fixing line. The results at most exposure doses indicated that inoculation with rhizobia inocula improved N_2 fixation than non-inoculated treatment. The total N yield results presented here show a wide variation between the various treatments. Danso *et al* (1987) found that the range of actual amounts fixed was from 17.0 to 132.1 kg N/ha, and the proportion fixed varied from 6.0 to 44.6%. Several reports have shown that N_2 fixed by a *Rhizobium* strain is strongly influenced by the host plant (Danso *et al.*, 1987), and that nitrogen fixation supporting traits often vary among different hosts.

The concentrations of chlorophyll a and b was greatly decreased in the treated plants with gamma irradiation as compared to the controls (Table 7) . This was mainly due to the effect of gamma irradiation which decreased chlorophyll content, as reported before (Strid and porra , 1990). Chlorophyll concentrations in most inoculated plants were higher than uninoculated ones. This indicated that the synthesis of these pigments proceeds faster and was tolerant to gamma irradiation under the effect of inoculated rhizobia than uninoculated ones during the recovery period. Changes in chlorophyll content and chlorophyll a/b ratio due to gamma irradiation are well documented. Several studies have indicated that changes in mRNA turnover of the chlorophyll a/b binding protein are responsible for reduction in total chlorophyll content (MacKerness *et al.*, 1997). On the other hand, in most cases plants inoculated with rhizobia produced more N and protein contents than uninoculated ones. The increase in N content in inoculated plants could be important for the germination of their seeds (Iwanzih *et al.* , 1983). The

Table 7: Effect of gamma - irradiated *Phaseolus vulgaris* on chlorophyll concentrations as affected by symbiotic performance of rhizobia with common bean in two generations.

Traits	Var.	Gen.	Doses (Krad) 0 a	0 b	10 A	10 b	20 A	20 b	30 A	30 b	40 a	40 B	L.S.D. at 0.05
Chlorophyll a concentration (mg/g tissue)	I	M_1	0.276 ±0.253	0.203 ±0.075	0.233 ±0.010	0.163 ±0.010	0.196 ±0.010	0.140 ±0.010	0.18 ±0.010	0.130 ±0.010	0.143 ±0.040	0.103 ±0.010	0.015 8
		M_2	0.243 ±0.030	0.236 ±0.056	0.233 ±0.010	0.160 ±0.010	0.186 ±0.011	0.133 ±0.005	0.170 ±0.010	0.106 ±0.015	0.113 ±0.032	0.060 ±0.043	0.015
	II	M_1	0.256 ±0.035	0.255 '±0.075	0.236 ±0.011	0.160 ±0. 17	0.196 ±0.011	0.140 ±0.010	0.180 ±0.010	0.133 ±0.010	0.143 ±0.103	0.103 ±0.015	0.015 8
		M_2	0.276 ±0.005	0.253 ±0.075	0.233 ±0.015	0.163 ±0.015	0.196 ±0.010	0.140 ±0.00	0.180 ±0.010	0.133 ±0.015	0.143 ±0.040	0.100 ±0.020	0.015
Chlorophyll b concentration (mg/g tissue)	I	M_1	0.203 ±0.015	0.210 ±0.010	0.160 ±0. 36	0.186 ±0.010	0.146 ±0.005	0.150 ±0.010	0.130 ±0.010	0.133 ±0.011	0.110 ±0.010	0.110 ±0.010	0.008
		M_2	0.203 ±0.015	0.210 ±0.050	0.146 ±0.037	0.180 ±0.010	0.150 ±0.010	0.150 ±0.010	0.130 ±0.010	0.130 ±0.010	0.110 ±0.010	0.110 ±0.010	0.017
	II	M_1	0.276 ±0.015	0.250 ±0.020	0.216 ±0.023	0.206 ±0.015	0.173 ±0.020	0.203 ±0.025	0.163 ±0.011	0.150 ±0.020	0.106 ±0.011	0.011 ±0.010	0.008
		M_2	0.250 ±0.036	0.246 ±0.025	0.223 ±0.064	0.206 ±0.010	0.236 ±0.040	0.166 ±0.015	0.153 ±0.035	0.116 ±0.015	0.113 ±0.015	0.076 ±0.005	0.013 7
		M_2	0.215 ±0.168	0.250 ±0.020	0.296 ±0.010	0.186 ±0.020	0.152 ±0.080	0.180 ±0.010	0.223 ±0.030	0.140 ±0.010	0.106 ±0.010	0.110 ±0.020	0.323

M_1, M_2, = Irradiated parental population and first resulted generation, respectively. I, II = Variety Polesta 68 and Giza 6, respectively.
var. = Varieties, Gen. = Generations, a, b = Inoculated and uninoculated plants, respectively.

same authors found an increase in protein content after UV-B irradiation which they explained by the increased content of protective pigments.

The doses of gamma irradiation showed significant effect on pollen viability (Table 8) among both populations of M_1 and M_2 plants. Among M_2 generation alone the interaction between doses of gamma irradiation x biofertilization, as well as, varieties x doses of gamma irradiation x biofertilization showed significant effect on pollen viability . A genetic control of sensitivity in higher plants has been found or presumed to occur in both chronic (Scarascia *et al.*, 1963) and acute (Davies, 1962) irradiation experiments. In acute x-irradiation of pea seeds, it was presumed that the recessive genes *r* and *le* increase the radiosensitivity (Gelin *et al.*, 1958). In this study, the genotypic constitution of two varieties was not found to influence the radiation response. Pollen viability appeared to be a statistically useful parameter of radiation response because of the high number of pollen grains per flower. Therefore, this parameters was sensitive to small increases in the exposure rate. The high sensitivity of this parameters is probably due to a more direct estimation of the radiation damage, the analysis being performed at the cellular level.

Table 8 : Analysis of variance and mean squares of pollen viability and some chemical traits affected by gamma irradiated *Phaseolus vulgaris* and symbiotic performance of rhizobia.

Source of variance	df	Pollen viability				Seed protein content			
		M_1		M_2		M_1		M_2	
		MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
Varieties (Var.)	1	0.001	0.639^{NS}	0.008	0.83^{NS}	0.007	0.123^{NS}	0.001	0.004**
Doses (Dos.)	4	0.381	0.001**	0.373	0.0001**	0.009	0.001**	0.009	0.001**
Biofertilization (Fer)	1	0.001	0.966^{NS}	0.005	0.51^{NS}	0.0012	0.049*	0.001	0.17^{NS}
Var.x Dos.	4	0.015	0.138^{NS}	0.026	0.083^{NS}	0.001	0.63^{NS}	0.001	0.83^{NS}
Var.x Fer.	1	0.003	8.31^{NS}	0.005	0.488^{NS}	0.002	0.33^{NS}	0.004	0.53^{NS}
Dos. x Fer.	4	0.0065	0.53^{NS}	0.030	0.05*	0.003	0.30^{NS}	0.001	0.22^{NS}
Var.x Dos.x Fer	4	0.0131	0.190^{NS}	0.22	0.129^{NS}	0.002	0.98^{NS}	0.001	0.43^{NS}
Error	40	0.008		0.011		0.002		0.001	

Tabulated F at 0.05 and 0.01 probability levels are equal 4.08 and 7.31, respectively at df 1/40. It was equal 2.60 and 3.83 at the same probability levels, respectively at df 4/40.

Table 9: Analysis of variance and mean squares of some chemical traits affected by gamma irradiated *Phaseolus vulgaris* and symbiotic performance of rhizobia.

Source of variance	df	Nitrogen ratio In the shoots				Chlorophyll a concentration			
		M_1		M_2		M_1		M_2	
		MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
Varieties (Var.)	1	0.00001	0.490^{NS}	0.00006	0.13^{NS}	0.004	0.82^{NS}	0.005	0.017*
Doses (Dos.)	4	0.0006	0.001**	0.0012	0.0001**	0.031	0.001**	0.036	0.001**
Biofertilization (Fer)	1	0.0001	0.087^{NS}	0.0007	0.09^{NS}	0.032	0.001**	0.034	0.001**
Var.x Dos.	4	0.00006	0.95^{NS}	0.0001	0.817^{NS}	0.006	0.990^{NS}	0.004	0.74**
Var.x Fer.	1	0.00018	0.49^{NS}	0.0001	0.0273*	0.0004	0.832^{NS}	0.00	1.00^{NS}
Dos. x Fer.	4	0.0006	0.95^{NS}	0.0002	1.00^{NS}	0.0014	0.191^{NS}	0.001	0.26^{NS}
Var.x Dos.x Fer	4	0.00007	0.94^{NS}	0.0001	0.99^{NS}	0.007	0.987^{NS}	0.001	0.95^{NS}
Error	40	0.0001		0.0002		0.009		0.088	

Tabulated F at 0.05 and 0.01 probability levels are equal 4.08 and 7.31, respectively at df 1/40. It was equal 2.60 and 3.83 at the same probability levels, respectively at df 4/40.

Protein percent was significantly affected by doses of gamma irradiation and biofertilization among M_1 population, as well as, by genotypes (varieties) and doses of gamma rays in M_2 population. This indicated that the doses of gamma rays were shown to be more effective on protein content of seeds in M_1 and M_2 populations. The effects of applied rhizobia on the N content of the seeds clearly indicated that N accumulations may be greater in roots than in stems, leaves or seeds, whereas the N_2 fixing system promoted greater N accumulations in the root and shoot systems than in the seeds (Rai, 1992). The results indicated that changes in seed protein content indicated that this trait was affected by gamma irradiation. The production of higher protein in the seeds revealed that inoculation with rhizobia have a positive effect on seed protein content .

The results summarized in this study indicated that the doses of gamma irradiation showed significant effect on the ratio of nitrogen content in shoot and root dry weight among M_1 and M_2 populations (Table 9) . The interaction between genotypes x biofertilization showed significant effect on the percent of nitrogen in shoots and roots of M_2 population. These results are in agreement with those found by Moawad *et al.* (1998), who found that inoculation of lentil (*Lens culinaris*) with different *R. leguminosarum* strains did not induce significant increases in dry matter yield and N content of lentils as compared to the uninoculated controls. These results support the finding of Abdel-Daiem *et al.* (1988) and Mukhtar and Abu-Naib (1988), who found no significant differences in plant dry weight or N-content in faba beans following inoculation with *Rhizobium* in soil having native *R. leguminosarum* population. Perhaps, the lack of response of common bean to inoculation is attributable to the low competitive ability of inoculant strains used. It is, therefore, apparent that inoculation of common bean with selected highly effective and competitive rhizobial strains is needed. The differences in N_2-fixation effect between M_1 and M_2 populations could be attributed to ecological factors in the ecosystem from, which the strain was applied or may be related to the genetic differences resulted by gamma rays between M_1 and M_2 populations. This is important for a better management of the host / rhizobia system. In this study, the irradiated seeds of *Phaseolus vulgaris* were used to induce heterogeneity in the host for possible better rhizobia response. This have been successfully used for best strain response with the host plant.

Doses of gamma irradiation and biofertilization showed highly significant effect on the concentration of chlorophyll a among M_1 and M_2 populations. In addition, plant genotypes (varieties) revealed the same trend in M_2 population only . As shown from these results, plant genotypes, doses of gamma irradiation and the interaction between plant genotypes x biofertilization, showed significant effect in chlorophyll b concentration among M_1 and M_2 populations (Table 10). In addition, biofertilization and the interaction between plant genotypes x biofertilization showed the same trend of their significant effect on chlorophyll b concentration among M_2 population only . The results obtained here are in agreement with Teramura and Sullivan (1994), who found that the effects of UV-B radiation decreased photosynthesis and biomass production and reductions in chlorophyll concentration. The resulting increase in UV-B radiation at the Earth's surface (Blumthaler and Ambach, 1990) can induce damaging and non-damaging photomorphogenetic responses in higher plants. Photosynthesis is impaired by UV-B in most species (Teremura and Sullivan, 1994). Recent research indicates that disruption of the thylakoid membrane and decreases in mRNA transcripts for the chloroplast proteins together with direct effects on photosystem II cause the reduction (Chow *et al.*, 1990). Reduced chlorophyll contents have often been reported by Deckmyn and Impens (1995). The results indicated that protein percent in whole plants (shoot + root) was significantly affected by the doses of gamma rays among M_1 and M_2 populations. Although, it was affected in M_2 generation by the interaction between variety x doses of gamma rays. In addition, N_2 uptake was significantly affected among M_1 and M_2 populations by; plant genotypes, doses of gamma rays, and biofertilization.

Table 10: Analysis of variance and mean squares of chlorophyll b concentration affected by gamma irradiated *Phaseolus vulgaris* and symbiotic performance of rhizobia.

Source of variance	df	Chlorophyll b concentration			
		M1	Pr>F	M2	Pr>F
Varieties (Var.)	1	0.015	0.001**	0.01	0.001**
Doses (Dos.)	4	0.027	0.0001**	0.02	0.001**
Biofertilization (Fer)	1	0.004	0.700 NS	0.02	0.06 NS
Var.x Dos.	4	0.001	0.0016**	0.0027	0.045*
Var.x Fer.	1	0.003	0.251 NS	0.006	0.0029**
Dos. x Fer.	4	0.003	0.350 NS	0.09	0.23 NS
Var.x Dos.x Fer	4	0.004	0.168 NS	0.003	0.67 NS
Error	40				

Tabulated F at 0.05 and 0.01 probability levels are equal 4.08 and 7.31, respectively at df 1/40. It was equal 2.60 and 3.83 at the same probability levels, respectively at df 4/40.

4. Effect of rhizobia inoculation on the yield components of gamma irradiated *Phaseolus vulgaris*:

In most treatments (Table 11), inoculated plants produced higher seed yield/plant and number of pods/ plant than uninoculated ones. In addition, the production of seed yield / plant and number of pods / plant were decreased at higher exposure doses of gamma irradiation relative to unirradiated plants in control experiment.The results pointed out that the low yield shown in inoculated plants are due to smaller seed size, less seed weight and fewer seeds per pod, especially in the treatment giving lower seed yield / plant which produced high pods / plant. The greater number of seeds per plant did not improve the yield because it had very low seed weight per pod. The results demonstrated that seed size was an important component of yield. However, White and Gonzalez (1990) found that the relationship between yield and seed size could be positive depending on the growing environment, while Bayuelo-Jimenez *et al.* (1999) found no statistically significant correlation between yield and seed size in both the domesticated and wild bean. The differences in pod number and length of pods, as well as, the similarity in pod wall biomass between inoculated and uninoculated plants indicated that a compensatory effect and a different pattern of photoassimilate distribution probably existed between these structures. The number of pods per plant is considered as one of the most important yield components in the case of grain legumes (Adams and Britz, 1992), and it has been recommended as a selection criterion for high seed yield (Safari, 1978). It can be concluded that the best yielding variety tended to fix the highest N_2 as shown by Wolyn *et al.* (1991) , who reported a positive correlation between seed yield and whole plant fixed N_2. Cregan and Van Berkum (1984) suggested that increased grain N yields should be obtained by selecting for higher N accumulation, while maintaining the nitrogen harvest index (% of plant N in the seed), because N uptake was thought to be independent of N remobilization and translocation to the seeds.

Yield data of all irradiated plants were significantly affected by gamma irradiation related to seed weight / plant and number of pods / plant (which seemed to contribute more to yield than did number of seeds per pods or seed weight). This conclusion agreed with Grafius (1964). Yield, a more radiation sensitive end point than stem length, is a composite result of the sensitivity of various processes that are initiated at different stages of the plant's life cycle. The present results indicated that the plant is most sensitive to gamma irradiation from the time it begins to develop its reproductive capability until after fertilization when embryo development begins.

The effect of *Rhizobium* on yield was tested in gamma irradiated-common bean under field conditions. The best response of irradiated plants to rhizobia inoculation of variety I was shown at the doses of 20, and 10 Krad in M_1 and M_2 generations, respectively. In addition, the dose of 40 krad which induced the best variation in common bean responded well to inoculation among M_1 and M_2 generations. It is also evident from the present results that an efficient symbiotic system may be beneficial for seed production through N_2 fixation. Although, the best cultivar variation / strain combination used in the present study responded well to inoculation and significant seed yields were obtained. Therefore, it is important to select plant genotypes and *Rhizobium spp.* strains for the best symbiotic system in order to produce high seed yields by N_2 fixation. This suggests that any breeding programme for *P. vulgaris* should include, as a high priority, selection for high N_2 fixation levels under stress conditions and normal soils. The present results are in agreement with Rai (1992), who reported that only two *Phaseolus vulgaris* genotypes (HUR 137 and VL 63) and two *Rhizobium spp.* strains (ND_1 and ND_2) produced maximum nodulation, nitrogenase activity, plant N content and grain yields in saline-sodic soil . Selection of favourably interacting symbionts is necessary for N economy, so that bean yields can be increased by the application of an active symbiotic system.

Both plant genotypes and doses of gamma irradiation showed significant effect on seed yield / plant among M_1 and M_2 populations (Table 12). Although, the interaction between plant genotypes x doses of gamma irradiation, plant genotypes x biofertilization, plant genotypes x doses of gamma irradiation x biofertilization showed significant effect on seed yield / plant among M_2 population, However, biofertilization in M_1 population showed significant effect on seed yield / plant. This was in agreement with the results reported by Cregan and Van Berkum (1984), who suggested that physiological and biochemical attributes related to N metabolism should be incorporated in selection for increased seed yield. In addition, DuBois and Burris (1986) reported that selection for increased levels of N_2 fixation in common beans grown under N-limiting conditions should contribute to improving seed yield potential, since fixed N_2 has been shown to be accumulated preferentially in the seeds.

Table 11: Effect of gamma - irradiated *Phaseolus vulgaris* on the mean of yield components traits affected by symbiotic performance of rhizobia with common bean among two generations.

Traits	Var.	Gen.	Doses (Krad) 0 a	0 b	10 A	10 b	20 a	20 b	30 a	30 b	40 a	40 b	L.S.D. at 0.05
Seed yield (g) / plant	I	M_1	22.66 ±3.05	27.00 ±8.15	18.33 ±1.52	18.33 ±3.51	17.39 ±2.08	25.66 ±3.21	18.00 ±1.00	18.00 ±1.00	12.00 ±1.00	13.00 ±2.00	1.506
		M_2	23.60 ±3.21	25.00 ±5.00	19.00 ±1.73	13.66 ±2.30	14.00 ±1.00	11.66 ±1.52	12 56 ±2.08	10.00 ±2.00	6.66 ±1.52	8.00 ±2.00	1.416
	II	M_1	18.00 ±1.00	20.00 ±1.00	18.66 ±1.00	18.66 ±1.52	15.33 ±0.577	17.66 ±0.57	11.66 ±2.08	15.00 ±1.00	11.33 ±5.85	10.63 ±1.52	1.506
		M_2	12.33 ±2.09	16.66 ±2.88	7.10 ±1.75	13.33 ±5.77	5.33 ±0.057	12.66 ±2.309	6.00 ±1.00	10.66 ±3.031	10.66 ±3.21	6.66 ±3.05	1.416
Number of pods / plant	I	M_1	11.33 ±7.31	8.33 ±1.53	8.66 ±0.57	7.33 ±0.57	7.00 ±1.00	6.06 ±0.57	5.66 ±0.57	5.66 ±0.57	4.33 ±1.52	4.33 ±1.52	1.05
		M_2	14.33 ±2.23	11.66 ±3.51	17.00 ±1.00	8.33 ±1.52	13.00 ±3.60	6.66 ±0.057	8.00 ±1.00	5.00 ±1.00	3.00 ±1.00	2.33 ±0.57	0.387
	II	M_1	18.05 ±7.21	17.33 ±14.50	16.66 ±1.52	13.66 ±1.52	12.04 ±1.00	10.33 ±1.52	13.00 ±4.35	7.66 ±0.57	6.66 ±1.52	6.00 ±1.03	1.05
		M_2	18.66 ±3.21	17.33 ±14.508	15.33 ±1.51	13.66 ±1.52	12.00 ±10.33	10.33 1.52	13.00 4.35	7.66 ±0.577	6.33 ±1.15	6.00 ±1.00	0.387
Ratio of yield response to inoculation		M_1	0.243 ± 0.037		0.140 ± 0.036		0.363 ± 0.159		0.000 ± 0.000		0.066 ± 0.115		0.093
		M_2	0.0433 ± 0.075		0.4000 ± 0.121		0.316 ± 0.175		0.383 ± 0.425		0.220 ± 0.255		0.253
		M_1	0.126 ± 0.132		0.066 ± 0.028		0.123 ± 0.063		0.213 ± 0.155		0.480 ± 0.250		0.093
		M_2	0.230 ± 0.236		0.373 ± 0.316		0.560 ± 0.072		0.370 ± 0.321		0.653 ± 0.736		0.253

M_1, M_2, = Irradiated parental population and first resulted generation, respectively. I, II = Variety Polesta 68 and Giza 6, respectively.
var. = Varieties, Gen. = Generations, a, b = Inoculated and uninoculated plants, respectively.

Table 12: Analysis of variance and mean squares of seed yield per plant affected by gamma - irradiated *Phaseolus vulgaris* and symbiotic performance of rhizobia.

Source of variance	df	Seed yield / plant				Number of pods / plant			
		M_1		M_2		M_1		M_2	
		MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
Varieties (Var.)	1	173.4	0.0001**	275.2	0.0001**	416.06	0.001**	144.15	0.0001**
Doses (Dos.)	4	176.20	0.0001**	234.35	0.0001**	131.05	0.001**	227.55	0.0001**
Biofertilization (Fer)	1	68.26	0.006**	17.6	0.130^{NS}	41.6	0.0027**	150.41	0.001**
Var.x Dos.	4	18.94	0.078^{NS}	50.5	0.003**	17.941	0.0048**	6.77	0.46^{NS}
Var.x Fer.	1	5.40	0.425^{NS}	102.7	0.006**	8.06	0.166^{NS}	18.15	0.126^{NS}
Dos. x Fer.	4	13.97	0.174^{NS}	8.52	0.34^{NS}	2.79	0.60^{NS}	10.62	0.24^{NS}
Var.x Dos.x Fer	4	9.10	0.373^{NS}	33.34	0.0039**	4.77	0.336^{NS}	10.10	0.26^{NS}
Error	40	8.33		7.37		4.06		7.45	

Tabulated F at 0.05 and 0.01 probability levels are equal 4.08 and 7.31, respectively at df 1/40. It was equal 2.60 and 3.83 at the same probability levels, respectively at df 4/40.

The results summarized in this study revealed that pod productivity in common bean is markedly affected by plant genotypes, doses of gamma irradiation (environmental factors) and biofertilization among M_1 and M_2 populations. In addition, the interaction between plant genotypes x doses of gamma irradiation showed significant effect on pod production in M_1 population rather than in M_2. The present results are in agreement with those obtained by Popsecu (1998), who found that the effect of bacterial strains was statistically significant for grain yields calculated for all experimental cycles. The same authors also found significant influences were exerted by other individual factors, namely locations and years, and by the interaction between bacterial strains and locations in two of the four cycles, and by the interaction between bacterial strains and years. The data obtained in this study suggested that induction of genetic variation in common bean and inoculation of seeds with selected strains is needed for beneficial interaction even in the presence of large native rhizobial populations, which are characteristic for Egyptian soils.

As shown from the results presented in Table 13 the interaction between doses of gamma irradiation x plant genotypes (varieties) showed significant effect on the yield response to inoculation in M_1 population. So, if highly effective genotypes of *Phaseolus vulgaris* are developed in the future, and inoculated with effective strains of *Rhizobium leguminosarum*, it should be possible to increase the nitrogen fixation and the yield of common bean. This indicated that it may be possible to select or develop plant genotypes and watch them with selected rhizobial strains that may increase the yield.

In conclusion, although common bean (*Phaseolus vulgaris*) plants are sensitive to gamma rays, even under conditions of biofertilization with rhizobia where reductions in vegetative biomass production, flowering and chlorophyll concentrations are found, the yield of the cultivars used was increased by inoculation of irradiated plants with rhizobia as compared with uninoculated ones . Changes in plant growth rate, N content and chlorophyll concentration indicated that leaf morphology was changed by gamma rays. Root exudates from some irradiated plants may stimulate the symbiotic efficiency of rhizobia followed by infection, typically through root hairs.

Table 13: Analysis of variance and mean squares of yield response (%) to inoculation with rhizobia affected by gamma irradiated *Phaseolus vulgaris* and symbiotic performance of rhizobia.

Source of variance	df	Yield response			
		M_1		M_2	
		MS	Pr > F	MS	Pr > F
Varieties (Var.)	1	0.0116	0.390^{NS}	0.203	0.190^{NS}
Doses (Dos.)	4	0.0359	0.085^{NS}	0.094	0.509^{NS}
Var.x Dos.	4	0.106	0.001**	0.551	0.736^{NS}
Error	20	0.0150		0.110	

Tabulated F at 0.05 and 0.01 probability levels are equal 4.35 and 8.10, respectively at df 1/20. It was equal 2.87 and 4.43 at the same probability levels, respectively at df 4/20.

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تأثير معاملة بذور الفاصوليا بأشعة جاما على كفاءة تكافل الرايزوبيا مع نباتات الفاصوليا من خلال العلاقة الجينية بين الميكروب والعائل البقولى وأثر ذلك على الصفات الخضرية وبعض الصفات الكيماوية فى الفاصوليا

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تمت معاملة بذور صنفين من الفاصوليا بأربع جرعات مختلفة من أشعة جاما بغرض إستحداث طفرات فى هذا العائل البقولى تؤثر على الكفاءة التكافلية لرايزوبيا الفاصوليا وذلك لأن هذه الكفاءة يلزمها تعبير خطى للجينات على مستوى طرفى هذه العلاقة وهما الرايزوبيا كميكروب غير مميز النواة والفاصوليا كعائل بقولى ذو نواة مميزة وقد تم قياس إستجابة هذه الكفاءة التكافلية بين الميكروب والنباتات المعاملة بأشعة جاما على مدى جيلين بإستخدام السلالة البرية للرايزوبيا. وقد أظهرت النتائج أن الجرعات ٢٠ ، ٤٠ كيلو راد من أشعة جاما قد أظهرت أعلى معدل من العلاقة التكافلية على مستوى عشائر نباتات جيل الآباء والجيل الأول على الترتيب. وقد إتضح من النتائج أن النباتات الملقحة بالرايزوبيا كانت أكثر تحملاً للأثر الضار لأشعة جاما مقارنة بالنباتات غير الملقحة بها. كما أثرت أشعة جاما على الإخلال من التشكل والنمو الطبيعى للنباتات التى عوملت بالإشعاع ، وقد ترتب على هذا إنخفاض معدل تكوين الأنسجة النباتية الجديدة مؤثرة بذلك على الصفات الخضرية للنباتات. وقد إنخفضت المساحة الورقية إنخفاضاً ملحوظاً بفعل أشعة جاما ، وبذلك فإن المساحة الورقية تعد من الصفات المناسبة جداً لقياس حساسية النباتات للإشعاع فهى تعتمد على نمو التراكيب الوراثية للنباتات. وتعكس النتائج أيضا مدى تأثر المساحة الورقية تأثيراً معنوياً بواسطة كل من جرعات التعريض لأشعة جاما والتسميد الحيوى على مستوى عشائر نباتات جيل الآباء والجيل الأول . أظهرت معظم النباتات المعاملة بالإشعاع والملقحة بالرايزوبيا محتوى مرتفع من كل من البروتين فى البذور والمجموع الخضرى وتركيزات الكلورفيل فى أوراق النباتات مقارنة بالنباتات غير الملقحة بالرايزوبيا والمعاملة بالإشعاع. أثر التفاعل بين كل من الأصناف والتسميد الحيوى تأثيراً معنوياً على محتوى المجموع الخضرى من النيتروجين على مستوى عشائر نباتات جيل الآباء والجيل الأول . وقد تأثر معنوياً إنتاج النباتات من القرون الخضراء على مستوى عشائر نباتات جيل الآباء والجيل الأول بالتركيب الوراثى للنباتات ، جرعات التعريض لأشعة جاما ، التسميد الحيوى بالرايزوبيا. وقد أظهرت النتائج أيضا أن الإنخفاض فى إنتاج النبات للبذور والقرون الخضراء على مستوى عشائر نباتات جيل الآباء والجيل الأول قد إزداد بزيادة الجرعة المستخدمة من الإشعاع. إن نتائج هذه الدراسة تعكس أن النباتات تعد من أكثر الكائنات حساسية لأشعة جاما منذ وقت تكوين البذور وحتى وصول النباتات إلى مرحلة المقدرة على الإزهار والإثمار وتكوين البذور وحتى بعد عملية الإخصاب وتكوين الأجنة. إستخدمت أشعة جاما فى هذه الدراسة بغرض إحداث إختلافات وراثية فى الفاصوليا كأحد العوائل البقولية الهامة فى الزراعات الحقلية فى مصر لتكوين ورؤية أفضل علاقة تكافلية تستجيب فيها الرايزوبيا لعائلها البقولى بغرض زيادة معدل تثبيت النيتروجين الجوى من خلال الإختلافات الوراثية المستخدمة فى الفاصوليا.