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Modification of morphological traits of common beans through gamma-ray irradiation: analysis of three consecutive generations

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ABSTRACT

The objectives of this investigation were to study the effects of different levels of gamma-rays on some morphological characteristics of a nearly-white seed coat color bean (*Phaseolus vulgaris* L.) cultivar, and to determine the radiation level which would generate the greatest genetic variability. Breeder seeds of EMGOPA 201 - "Ouro" cv, a beige seed coat color cultivar, were submitted to gamma-ray irradiation (^{60}Co). Treatments consisted of eight levels of radiation: 0, 10, 15, 20, 25, 30, 35 and 40 Krad. The experimental design was a randomized complete block with four replications. In the field, plots consisted of 100 seeds. The following data were collected: percent germination, plant height, final stand, plant yield and yield components, number of chlorotic and albino mutants, leaf mutants, growth habit alterations, earliness, seed coat color, seed coat brightness, halo color, seed size and format. Among traits greatest variations were observed for seed morphology. Seed coat color varied from completely white to a dark-brownish color. Halo color was also modified from yellow (normal) to pink. Brightness of seeds varied from opaque to bright. Seed varied from squared to rounded, and from very small to large. Treatments with 20 and 25 Krad generated the greatest variability for several morphological traits from the M_1 to M_3 generations, a dosage equivalent to the LD_{50} observed in the M_1 generation. Traits such as percent germination, plant height and some yield components were highly and negatively affected by increasing levels of radiation. Modification of yield components as well as many unusual characteristics with late onset were observed in advanced generations, suggesting that late selection would also be useful.

INTRODUCTION

Alternative techniques to expand the genetic background of common beans (*Phaseolus vulgaris* L.) are of paramount importance for the improvement of this species. Mutagenesis, although random and uncontrollable, can induce modifications in desired traits (Al-Rubeai, 1982; Carneiro *et al.*, 1987; Guimarães, 1988; Sena, 1990; Paula, 1992).

Parameters generally used to determine radiosensitivity are percent germination, seedling survival, plant yield, plant height and overall plant growth during population development (Marcos Filho and Godoy, 1971). In common beans, the target tissue most frequently used is the seed (Carneiro *et al.*, 1987; Sena, 1990), and gamma-ray irradiation is the preferred mutagenic method, as it promotes DNA or chromosomal breakage, followed by random recombination (Tulmann Neto, 1990). The ideal level of radiation, according to Tulmann Neto (1990), is obtained by using the LD_{50} (lethal dose - 50%) for the M_1 generation,

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promoting 50% reduction in germination and plant height in comparison with the control treatment.

Gamma-ray irradiation levels used in beans vary from 0.1 Krad (Marcos Filho and Godoy, 1971) to 48 Krad (Marcos Filho *et al.*, 1972). Carneiro *et al.* (1987), using increasing levels (0 to 20 Krad) of gamma radiation (^{60}Co) in the cultivar "Milionário 1732", concluded that radiation effects were small for all levels used, suggesting that greater levels would be necessary to induce variation. Marcos Filho *et al.* (1972) and Silva *et al.* (1983), however, obtained variability in bean yield with lower levels of exposure to gamma-rays.

Marcos Filho and Godoy (1971), Silva *et al.* (1983), Alvarez (1983), Albertoni *et al.* (1987), and Carneiro *et al.* (1987) used germinability and yield parameters to determine mutagenic effects. Silva *et al.* (1983) also found that 20 to 30 Krad most affected seedling survival and number of seeds.

MATERIAL AND METHODS

The cultivar used in this investigation, "EMGOPA 201-OURO", has the following characteristics: small rounded seed, beige seed coat color, yellowish-orange halo, type II growth habit, good resistance to bean rust (*Uromices phaseolus* var. *typica*), and moderate resistance to leaf angular spot (*Isariopsis griseola* S.). Breeder seed stock with high genetic purity was obtained from the Empresa Goiana de Pesquisa Agropecuária (EMGOPA).

Seeds were submitted to gamma-ray irradiation (^{60}Co) at the Centro Nacional de Energia Nuclear na Agricultura (CENA), Piracicaba, SP. Treatments consisted of eight levels of radiation: 0, 10, 15, 20, 25, 30, 35 and 40 Krad. The control treatment (0 Krad) was packed and sent to CENA, together with the other treatments. The experimental design utilized was a randomized complete block with four replications. In the field, plots consisted of three rows two m. long, sowed with 100 seeds at a rate of 15 seeds per m. Three consecutive generations after irradiation treatment were planted in isolated areas throughout the summer of 1991, the winter of 1992, and the summer of 1993, at the Experimental Farm "Capim Branco", Universidade Federal de Uberlândia.

Data collected from the M_1 generation (first generation after treatment) were: percent germination 15 days after planting, plant height 30 days after germination, number of pods per plant, 50-seed weight, number of plants at harvest, number of productive plants (plants at the end of the vegetative cycle that produced seeds), and yield per plot.

M_2 seeds were planted in winter 1992. Each M_2 plant originated a M_3 family. Ninety-nine plants were chosen to be analyzed at the M_3 generation, based on phenotypic alterations, such as seed coat color, growth habit, 60-seed weight, plant architecture, leaf morphology and yield. Sixty seeds of each M_3 family were planted in summer 1993.

For the M_2 and M_3 generations the following data were collected: germination rates, number of chlorotic and albino mutants, number of leaf mutants, occurrence of growth habit alterations, earliness, plant yield, and yield components, and observations on seed coat color, seed coat brightness, halo color, seed size, and format. Confirmation of the highest variability obtained by the LD_{50} in the M_1 generation was done by analyses of the advanced generations.

Analysis of variance was performed for the M_1 generation. Mean comparisons were obtained using Tukey's procedure. Data were analyzed using SAS for Personal Computer Software, version 6.0 (1985).

RESULTS AND DISCUSSION

M_1 generation

Germination and final stand were highly influenced by increasing levels of radiation (Table I). Although these observations do not support the findings of Marcos Filho and Godoy (1971) and Carneiro *et al.* (1987), they agree with the ones by Marcos Filho *et al.* (1972), Alvarez (1983) and Sena (1990).

Plant height 30 days after germination was highly influenced by radiation treatments, correlating negatively with increasing levels of gamma-ray irradiation. This was also detected by Tulmann Neto and Ando (1971) and Sena (1990). Although no differences were observed among treatments for the 50-seed weight average, the low values obtained were possibly due to the high incidence of bean golden mosaic virus, which also caused a severe loss of seed quality.

Treatments with 20 and 25 Krad of radiation promoted a typical LD_{50} response for plant height and percent germination. The 20 and 25 Krad treatments caused a reduction of around 50% in germination and plant height, both important to determine the radiosensitivity of the plant population. Percent germination for the 10-Krad treatment was not significantly different from the 0-Krad treatment (control), but was superior to the other treatments (Table I). This result was also observed by Tulmann

Table I - Germination, plant height, yield and yield components observed in the M_1 generation of the common bean line EMGOPA 201 - Ouro after treatment with seven levels of gamma-ray irradiation, Uberlândia, 1991/92.

Treatment	% Germination*		Plant height		Pods/ plant	Stand (no. of plants)		50-Seed weight	Yield
(Krad)	Mean	Relative	(cm)	(%)	(no.)	Final	Productive	(g)	(g/plot)
0	91.0 a	100.0	23.7 a	100.0	4.20 a	80.8 a	59.0 a**	5.00 a	65.50 a
10	92.8 a	101.9	20.6 ab	86.0	1.72 a	81.5 a	41.5 b	5.50 a	38.34 b
15	88.6 ab	96.7	16.4 bc	69.2	1.56 a	60.3 b	25.3 c	4.33 a	26.59 bc
20	76.3 b	83.8	11.0 cd	46.4	1.26 a	21.0 c	4.5 d	3.36 a	6.73 cd
25	52.3 c	57.4	11.8 cd	49.8	2.24 a	8.5 d	5.0 d	4.93 a	7.43 cd
30	19.0 d	20.9	10.0 d	42.1	2.71 a	2.0 d	1.0 d	4.38 a	1.88 cd
35	10.0 d	11.0	9.7 d	40.9	3.26 a	3.5 d	1.5 d	4.71 a	1.42 d
40	8.3 d	9.1	7.5 d	31.6	2.26 a	2.0 d	0.8 d	4.00 a	1.63 d

*Germination data were analyzed as transformed data by arcsin $(x+1)^{1/2}$.

** Means in the same column followed by the same letter were not significantly different at the 0.05 level of probability, according to Tukey's test.

Neto and Ando (1971) for four other cultivars, with the same level of radiation.

Plant yield and productive stand were highly and negatively influenced by increasing levels of radiation (Table I). The number of pods per plant did not vary significantly, although all radiation treatments had values below that of the control treatment. An unexpected number of pods in the 35-Krad treatment (3.26 pods/plant) was due to a plant that produced a large number of pods (N=38), which influenced the average. This result could be a consequence of the radiation treatment itself, or be due to the lower number of plants that survived the treatment, thus decreasing competition amongst plants. There are similar results in the literature (Carneiro *et al.*, 1987 and Sena, 1990).

M_2 generation

Leaf mutants included the following aberrant types: plants with rounded leaflets, narrow and elongated stems, and plants with abnormal leaflets (two, four or five). Most of these mutants manifested their phenotypes only at the first leaflet stage, with the exception of the "long and narrow leaf" trait that persisted throughout the whole plant cycle. This character was observed in the 10-Krad treatment (Table II). Chlorotic mutants, in most cases, are yellowish plants that cannot survive, classified by Sena (1990) as *xantha*, and this is a very frequent trait detected in other mutagenesis studies (Al-Rubeai, 1982; Barbosa *et al.*, 1988; Sena, 1990). The frequency of these mutants in

Table II - Occurrence and frequency of mutants observed in the M_2 generation and M_3 seeds after treatment with seven levels of gamma-ray irradiation of common bean line EMGOPA 201 - Ouro cv., Uberlândia, 1992.

Treat. (Krad)	Plant cycle	Growth habit	Growth habit/ cycle	Plant sterility	Erect plants	Dehiscent pods	Long narrow leaf	Chlorotic plants	Small seeds	Squared seeds	Large seeds	White seeds	Pink halo	Dark color seed	Bright seed coat	Number of plants	% mutants
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	02	301	0.7
10	03	15	07	01	01	01	01	02	01	01	01	02	-	01	-	727	5.1
15	03	03	01	02	01	-	-	-	03	01	02	10	-	06	17	553	8.9
20	-	03	-	-	-	-	-	-	01	-	10	01	-	01	04	115	17.4
25	-	04	-	-	01	-	-	-	-	-	02	05	01	-	-	157	8.3
30	-	03	-	-	-	-	-	-	-	-	01	-	-	-	-	21	19.1
35	-	-	-	-	-	-	-	-	-	-	-	-	-	01	-	89	1.1
40	-	01	-	-	-	-	-	-	03	-	-	02	-	02	-	32	25.0

*The symbol "-" indicates no observations for the specific trait.

the M_2 generation was low (0.275%). It was observed in only two plants, descendents of the 10-Krad treatment.

The cultivar utilized in this study has a type II growth habit - indeterminate growth (Silva and Moraes, 1987); a few M_2 plants, however, changed to type III. In addition, this cultivar plant architecture is definitely bushy, with a tendency to prostrate, but erect types were observed, after the radiation treatments with 10, 15 and 25 Krad. Although the planting was done during a period when cultivars normally show prolonged cycles (Vieira, 1991), some M_2 plants had longer cycles than the control, especially in the 10- and 15-Krad treatments. It was also observed that three plants had normal development, but one could not flower and the other two were male sterile. One plant formed seedless pods, and the others had partial seed abortion during pod development (Table II). These aberrant plant types were also observed by Sena (1990).

Based on the average yield for control plants (0 Krad), plant yield differences were only considered for those mutants with total seeds per plant weighing above 35 grams. Plants with very high yield (data not shown) were detected in all treatments. Data for yield were not conclusive, because variation was also high among control plants. Low germination probably decreased the competition among plants for nutrients and light. The average weight of 60 seeds ranged from 7.9 to 17.1 g (data not shown). Part of such variation might have come from seed size and format alterations (Table II).

Among all traits observed, seed morphology alterations were the ones which varied the most. Seed coat color varied from completely white to a dark-brownish color (normal color is light beige). Halo color was also modified from yellow (normal) to pink, especially in the 25-Krad treatment. Brightness of seeds varied from opaque to bright coat. Seed size and format alterations included: rounded and squared seeds (oval format is the normal type) and small and large seeds (medium to small size is the normal type).

For the M_1 generation, seeds irradiated with 20 and 25 Krad gave the greatest variability, according to the LD_{50} (lethal dose 50); however, at the M_2 generation, the 30- and 40-Krad treatments showed the highest frequency of mutations (Table II). High levels of radiation, however, would not be recommended due to the drastic reductions in population size which they impart (Table I), diminishing the chances of obtaining useful plants. Tulmann Neto (1990) recommends that, after inducing mutagenesis, a very large population should be grown to detect favorable mutants. Treatments with 15, 20 and 25 Krad presented intermediate frequencies of mutations (Table II), which associated with higher numbers of surviving plants, would increase the probability of detecting and selecting desirable phenotypes. The 10-Krad treatment would not be recommended because of its lower frequency of mutations, making very large populations necessary. Similarly, the 35-Krad treatments should not be utilized due to the substantially reduced population size, consequently also reducing the probability to detect favorable mutants.

M_3 generation

The high incidence of bean golden mosaic virus caused a severe plant dwarfism, altering the growth habit as well as seed size and growth cycle. However, interesting mutations in leaf and seed morphology, plant chlorosis, halo color and seed coat color were observed, demonstrating in some cases a delayed reaction following irradiation.

All detected leaf morphological alterations, such as ''long and narrow leaf'' in one M_2 plant (15-Krad treatment), were characterized as epigenetic traits, because they did not persist to the next generation (M_3). Many plants, however, had a late onset of a few unusual traits, such as three cotyledons and three leaflets instead of two in both cases, and completely chlorotic plants (Table III). A generalized plant chlorosis was observed for treatments with 15, 20 and 40 Krad, reaching 75% plant chlorosis for the 40-Krad treatment (Table III). These plants died before the secondary leaf stage, or soon after germination. It was also observed that progeny of the 10-Krad treatment had a decreased plant height. A high incidence of mutation for several traits was observed (data not shown), especially for the 20, 25, and 40-Krad treatments with frequencies higher than 50%. The following modified traits were considered as fixed characteristics: small squared seeds (10-Krad treatment), halo color (25-Krad treatments), and the seed coat color alteration (10- to 30-Krad treatments) from nearly-white to brownish color, with a few seeds with black or brown spots.

The results suggest that mutation breeding programs should breed additional generations to possibly identify new useful genotypes, instead of selecting only in the first two generations. It also suggests that 20 to 25 Krad are appropriate levels of radiation, although this varies with cultivar, size of seed lot and seed physiological conditions. Considering all traits, the greatest variability was observed for seed morphology. Traits such as percent germination, plant height and some yield components were highly and negatively affected by increasing levels of radiation.

Table III - Frequency of mutations and occurrence of some unusual traits in the M_3 generation (M_3 plants and M_4 seeds) for seven levels of gamma-ray irradiation in the common bean line EMGOPA 201 - Ouro, Uberlândia, 1993.

Treatments (Krad)	Number of progenies	Frequency of mutant progenies %	Chlorotic plants no.	No. of plants with 3 cotyledons and 3 leaflets
0	07*	0**	0	0
10	20	40	0	4
15	30	30	2	6
20	15	53	22	3
25	12	50	0	1
30	05	40	0	3
35	02	0	0	0
40	08	75	7	0

*Ninety-two mutant progenies and seven control plants were chosen according to mutations observed in the M_2 generation to proceed to the next generation.

**Frequency of mutations was obtained by couting the number of plants with morphological alterations (specified in Table II) within each family.

RESUMO

Embora muitos problemas originem-se da mutagênese induzida, é importante determinar o nível de radiação ideal para que se obtenha o máximo de variabilidade associada à viabilidade da semente. Nenhuma pesquisa foi encontrada na literatura utilizando mutagênese em cultivares de feijão com tegumento claro. Por esta razão, os objetivos desta investigação foram estudar os efeitos de diferentes níveis de radiação gama em algumas características morfológicas de uma cultivar com tegumento bege e determinar qual nível de radiação induz maior variabilidade. Sementes básicas da cultivar "EMGOPA 201 - Ouro", com tegumento da semente de cor bege, foram submetidas à radiação gama (^{60}Co) no Centro Nacional de Energia Nuclear na Agricultura (CENA), Piracicaba, SP. Os tratamentos consistiram de oito níveis de radiação: 0, 10, 15, 20, 25, 30, 35 e 40 Krad. O experimento foi conduzido em blocos casualizados com quatro repetições. No campo, as parcelas consistiram de 100 sementes. A testemunha (0 Krad) foi conduzida similarmente aos outros tratamentos em todas as gerações para previnir possíveis efeitos ambientais em lotes diferentes de sementes. Os seguintes dados foram coletados: porcentagem de germinação, altura de plantas, estande final, produtividade e seus componentes, número de plantas cloróticas e albinas, número de mutantes foliares, alterações do hábito de crescimento, precocidade, cor do tegumento da semente, brilho da semente, cor do halo da semente, tamanho e formato da semente. Entre todas as características observadas, as alterações da morfologia da semente foram as que mais variaram. A cor do tegumento variou de completamente branco para marron-escuro. A cor do halo foi modificada de amarelo para rosa. O brilho da semente variou de opaco a brilhante. O tamanho e o formato da semente sofreram modificações, variando de sementes quadradas a arredondadas e de sementes pequenas a grandes. Em geral, os tratamentos com 20 e 25 Krad de radiação geraram maior variabilidade nas gerações M_1 a M_3 para vários caracteres morfológicos, semelhante ao observado na geração M_1 para a DL_{50}. Características tais como germinabilidade, altura de plantas e alguns componentes de produtividade foram afetados negativamente de forma significativa pelos crescentes níveis de radiação. Muitos caracteres incomuns se manifestaram tardiamente em gerações avançadas, inclusive modificações dos componentes de produtividade, sugerindo que a seleção de populações após mutagênese deveria também ser realizada em gerações avançadas.

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