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INDUCED MUTATIONS IN THE COMMON BEAN, *Phaseolus vulgaris* L., AFFECTING FLOWER COLOR AND SEED CHARACTERISTICS

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ABSTRACT

Gamma ray doses of 0 (control), 6, 12, 18, 24, and 30 krads were applied to dormant seeds of *Phaseolus vulgaris* L., cv. Milionário 1732 (BAT 65). Mutations affecting flower and seedcoat color, and other seed characteristics were detected in the M_2 generation. Highest mutation frequencies were induced with 24 krads. Mutants with white flowers, different seedcoat colors, shiny seedcoat, and altered size and shape of seeds were obtained. At least two mutated loci affecting seedcoat color were involved in four of the six segregating M_2 progenies.

INTRODUCTION

Mutation induction is an important complementary method of breeding annual diploid self-fertilizing species. Mutants in beans (*Phaseolus vulgaris* L.) have been obtained that have improved grain yield (Gotoh, 1968; Rubaihayo, 1975; Hussein and Disouki, 1979; Al-Rubeai, 1982), protein content (Safari, 1973; Rubaihayo, 1975; Hussein and Disouki, 1979), resistance to diseases (Fadl, 1983; Micke, 1983) and altered seed characteristics, such as color, brightness, and size (Gotoh, 1968; Swarup and Gill, 1968; Moh, 1969; Safari, 1973; Rubaihayo, 1975; Hussein and Disouki, 1976; Saito *et al.*, 1980; Al-Rubeai, 1982; Carneiro, 1986; Barbosa *et al.*, 1988). Seed characteristics are important because they determine market acceptance (Vieira, 1967).

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Promising results have been reported from our laboratory following ethyl-methanesulfonate (EMS) treatment of seeds of a high yielding bean variety (Carneiro, 1986; Barbosa *et al.*, 1988; Guimarães *et al.*, 1989). Using the same variety, Carneiro *et al.* (1987) reported the effects of gamma rays on M_1 plants grown from treated seeds. However, because they did not grow the M_2 generation, they did not report the occurrence of mutants. Thus, the objectives of this study were to determine the frequency and types of mutants affecting flower color and seed characteristics in the M_2 generation following treatment of seeds of that variety with gamma rays.

MATERIAL AND METHODS

The variety used was Milionário 1732 (BAT 65), which has violet flowers and black seeds. Dormant seeds (ca. 13% moisture) were exposed to 0 (control), 6, 12, 18, 24, and 30 krads of gamma rays from a Co^{60} source at the Centro de Energia Nuclear na Agricultura, Piracicaba, SP. The gamma-ray-treated seeds were planted in the field at Viçosa, MG, with the treatments arranged in a randomized complete block design with six replications. Each plot contained 50 plants spaced 20 cm in two rows spaced 60 cm.

Each M_1 plant was harvested separately. For the 18-krad treatment, 20 seeds were taken from each M_1 branch. When an M_1 branch yielded less than 20 seeds all seeds were used. For the other treatments, a maximum of 70 seeds was taken from each M_1 plant. Seeds from each M_1 branch or each M_1 plant were planted in a single row. Seventy seeds from the unirradiated control population were planted in each of 50 rows interspersed in the field. Spacing and the experimental area were the same as those used to grow the M_1 plants.

Flower color mutants were identified in the M_2 generation. Each M_2 plant row was harvested separately. Mutants affecting color, shine, size, and shape of seeds were determined by opening six to eight pods from each M_2 plant. When a mutant was found all seeds were saved.

RESULTS AND DISCUSSION

Mutants affecting flower and seedcoat color

The 24-krad treatment induced the highest frequency of flower and seedcoat color mutations (Table I). Only white flower mutants were found. The six M_1 plants that segregated for flower color in M_2 also segregated for seedcoat color. The mutant seedcoat phenotypes and their frequencies in M_2 are presented in Table II. The mutant phenotypes varied from white to beige and several hues of brown, with or without stripes. White seedcoat was found in only one segregating M_2 progeny (L8) and was always associated with plants having white flowers. This is probably a case of mutation (point or deletion) affecting the basic dominant gene *P.* According to Prakken (1972), *pp* plants have white flowers and white seedcoat. In the other five segregating M_2 progenies the mutant seedcoat color was found in plants having either white or violet flowers. These may have resulted from mutations in color (complementary) or in intensifying genes (Prakken, 1972).

Table I - Frequencies of M_2 progenies segregating for flower and seedcoat color mutants.

Radiation dose (krad)	No. of treated seeds	No. of surviving M_1 plants	M_2 progenies segregating for flower and seedcoat color		
			No.	Percent of Treated seeds	Percent of Surviving M_1 plants
0	300	276	0	0	0
6	300	265	2	0.67	0.75
12	300	265	0	0	0
18	300	251	1	0.33	0.83
24	300	121	3	1.00	1.19
30	300	23	0	0	0

Due to the multicellular nature of the seed embryo, a mutated M_1 plant usually has a chimerical structure, with the mutated sector reduced in size in comparison with the normal sector. A deficiency of the mutant phenotype is thus expected and is usually found in M_2. This is illustrated by the segregating progenies L8 and L258 (Table II). The segregation ratio of 3 normal: 1 mutant is expected when a single cell that is heterozygous for the mutant allele gives rise to an M_1 plant, so that the whole plant will be non-chimerical. An excess of mutant phenotypes was found in three M_2 progenies. On the assumption that the respective M_1 plants were non-chimerical, and combining all mutant phenotypes from each progeny, a ratio of 9 normal: 7 mutants fits the data for progenies L666-L668 (χ^2 = 1.524; P = 0.30 - 0.20), L537 (χ^2 = 1.050; P = 0.50 - 0.30), and L579 (χ^2 = 0.698; P = 0.50 - 0.30). This would require simultaneous mutations in two independent and complementary loci. The different mutant seedcoat colors found in each of four of the six segregating progenies (Table II) clearly indicate that mutations occurred in at least two seedcoat color genes. This seems to be an important factor contributing to underestimation of mutation frequencies presented in Table I. Other factors were considered by Barbosa *et al.* (1988) who reported similar results after treating seeds of the same variety with EMS. The simultaneous mutation of several genes does not seem to be a rare phenomenon in mutation induction experiments (Gottschalk, 1987).

Table II - Segregation for normal (black) and mutant seedcoat phenotypes in M_2 progenies.

Radiation dose (krad)	Identification of M_2 progeny	No. of M_2 plants having seedcoat phenotype		Mutant seedcoat color
		Normal	Mutant	
0	All	2674	0	-
6	L8	50	5	White
	L258	38	3	Yellowish brown
12	All	11802	0	-
18	L666-668	20	14	Beige with brown or black stripes; grayish beige; different hues of brown with or without stripes
24	L537	10	13	Beige with brown or black stripes; different hues of brown with brown stripes or without stripes
	L538	11	3	Beige with brown and black stripes; yellowish brown with brown stripes
	L579	28	16	Beige with black stripes; grayish beige with brown stripes; light reddish beige with brown stripes; different hues of brown
30	All	585	0	-

Striped seedcoat mutants were found in four of the six segregating progenies (Table II). Barbosa *et al.* (1988) and Barbosa and Guimarães (1989) proposed that such striped mutants were caused by the activation, by the mutagen, of controlling elements that exist in the bean chromosomes.

Mutants affecting seed shape and size

Nine M_2 progenies segregated for different seed size and shape. Two progenies were grown from 12-krad treated seeds, four from the 18-krad treated seeds, and three from 24-krad treated seeds. Some of the mutants had thinner and longer seeds, others had smaller seeds (12 to 14 g/100 seeds), and still others had larger seeds (28 to 30 g/100 seeds). Seed weight of 100 seeds of the parental variety was 16 to 18 g (Vieira *et al.*, 1983). Large-seeded bean mutants have been reported by Gotoh (1968) and Carneiro (1986). Large-seeded mutants have also been induced in other legumes (Gottschalk and Wolff, 1983).

Shiny seedcoat mutant

In the progeny of the 24-krad treatment a single M_2 plant produced seeds with strong seedcoat shine. Carneiro (1986) reported two similar mutants induced by EMS in the same variety. Shiny seedcoat has been known to be conditioned by a single dominant gene (Prakken, 1934; Moh and Alan, 1964). Shiny seedcoat is one of the rare cases of dominant mutations as it has been estimated (Gottschalk and Wolff, 1983) that about 99% of all mutations are recessive.

The results presented here show that gamma irradiation of bean seeds may be efficiently used to induce changes in seed characteristics, supporting previous results (Gotoh, 1968; Moh, 1971; Hussein and Disouki, 1976; Al-Rubeai, 1982). Several different mutants were obtained in a relatively short time. The agronomic potential of these mutants should be evaluated as was done by Guimarães *et al.* (1989) with EMS-induced seedcoat color mutants in the same variety. Even those mutants with lower yielding ability are of value because they may be used to incorporate desired changes in the original variety by the conventional backcrossing method.

RESUMO

Doses de radiação gama de 0 (controle), 6, 12, 18, 24 e 30 krads foram aplicadas em sementes de *Phaseolus vulgaris* L., cv. Milionário 1732 (BAT 65). Mutações afetando a cor das flores e do tegumento e outras características das sementes foram detectadas na geração M_2. Maior frequência de mutações foi induzida com 24 krads. Foram obtidos mutantes com flores brancas, tegumentos de várias cores, brilho no tegumento e

com modificações no tamanho e forma das sementes. Pelo menos dois locos afetando a cor do tegumento foram mutados em cada uma das quatro progênies de um total de seis que segregaram na geração M_2.

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