

INHERITANCE OF SELF-COLORED AND STRIPED SEEDCOAT MUTANTS INDUCED IN *Phaseolus vulgaris* L.*

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ABSTRACT

The seedcoat color inheritance of ten ethyl methanesulfonate (EMS)-induced mutants in *Phaseolus vulgaris* L. was determined in the F₂ generation from crosses of each mutant with the original black-seeded variety. All mutants behaved as recessives. Monogenic inheritance was found for six mutants with uniformly colored seedcoats. There is evidence that one of these was caused by deletion. The F₂ generation from crosses between two other self-colored mutants or two variegated mutants with the parent variety segregated in the ratio 9 black: 3 yellowish-brown: 4 beige, indicating two mutated loci. Evidence is presented favoring the hypothesis that EMS-activated transposable elements, not yet described in *Phaseolus*, are the cause of stripes in variegated mutants.

INTRODUCTION

The seedcoat color is an important trait determining the acceptance of a bean (*Phaseolus vulgaris* L.) variety by growers and consumers (Vieira, 1967; Moh, 1969, 1971; Tulmann Neto *et al.*, 1980). As a result of an investigation designed to change the seedcoat color of a high-yielding variety, various seedcoat color mutants have been induced (Carneiro, 1986; Barbosa *et al.*, 1988). The agronomic potential of some of those mutants has been reported by Guimarães *et al.* (1989). The present study was undertaken to determine their mode of inheritance.

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MATERIAL AND METHODS

The ten mutants used were induced in the black-seeded variety Milionário 1732 and are of independent M_1 origin. Mutant M_3 seeds were propagated for one or two generations to confirm their non-segregating nature. All lines are true breeding with the exception of L252-15 which segregates for black/brown stripes on an otherwise true breeding beige background color of seedcoat.

Hybridizations were made in the greenhouse according to Buishand's (1956) friction method. Milionário 1732 was used as the male parent in crosses with each mutant line. Most of the mutants used give origin to green hypocotyl seedlings which is recessive to red hypocotyl (Lamprecht, 1935), typical of Milionário 1732. Thus, all putative F_1 seedlings having green hypocotyl should be derived from undesired self-pollinations and could be discarded. When red hypocotyl mutants (L573-6 and L582-10) were crossed to the parent variety, discarding of progenies derived from self-pollinations could only be made after examination of F_2 seed phenotypes. The bean seedcoat is of maternal origin, its phenotype being determined by the female parent genotype. The reason for using Milionário 1732 as the male parent was the need of a genetic marker that enabled the identification and discarding of progenies originated from selfings. All heterozygous F_1 plants should yield black F_2 seeds, as black seedcoat is conditioned by several dominant genes (Kooiman, 1931; Prakken, 1972). Mutant-colored seeds would result from self-pollinations and should be eliminated. Progenies from crosses involving these two mutant lines were the only ones among which plants derived from self-pollinations were found. This confirmed the efficiency of selection based on hypocotyl color.

One thousand and five hundred black F_2 seeds from each cross were planted in the field in a row 150 m long at Viçosa, MG. Plants from each F_2 progeny were harvested individually and the seedcoat color was determined after threshing two or three pods per plant.

RESULTS AND DISCUSSION

Segregation data from F_2 progenies of crosses involving eight mutants and Milionário 1732 are given in Table I. Five F_2 progenies segregated according to the ratio 3 black (normal): 1 mutant, indicating recessiveness of mutant phenotype and monogenic inheritance. When L763-2 was used in the cross there was a significant deviation ($P < .01$) from 3:1 due to deficiency of the mutant class. Plants of L763-2 are characterized by their small size and low yielding ability (Guimarães *et al.*, 1989). At maturity, the F_2 progeny from the cross L763-2 x Milionário 1732 was composed of normal-height plants, like Milionário 1732, and small plants, like L763-2. All normal-height plants produced black seeds, whereas all small plants yield mutant

Table 1 - Segregation for seedcoat color in F₂ progenies from crosses between mutant seedcoat color lines and the parent variety.

Designation	Mutant line	No. of F ₂ plants with seedcoat color				Expected ratio	χ ²	P
		Seedcoat color	Black	Yellowish-brown	Beige			
L22-1	Beige	770	246	338	9:3:4	0.34	0.80-0.90	
L573-6	Beige	1039	—	323	3:1	1.20	0.20-0.30	
L582-10	Beige	1044	—	351	3:1	0.02	0.80-0.90	
L630-6	Beige	796	260	338	9:3:4	0.50	0.70-0.80	
L669-1	Yellowish-brown	986	316	—	3:1	0.37	0.50-0.70	
L676-1	Yellowish-brown	989	324	—	3:1	0.07	0.70-0.80	
L681-6	Beige	1013	—	298	3:1	3.60	0.05-0.10	
L763-2	Beige	1030	—	265	3:1	14.21	<0.01	

seeds. In no case was this association disrupted. If different mutational events had affected genes conditioning seedcoat color and plant development, recombinant classes would have been detected among the 1295 F_2 plants (Table I), unless the mutated genes were very tightly linked. Absence of recombinants, deficiency of mutant phenotype, and multiple phenotypic effects observed L763-2 provide strong evidence that this mutant resulted from deletion of a chromosome segment containing genes affecting seedcoat color and plant development. Deficiency of the recessive class is known to be caused by lower transmission of male gametes carrying the deletion when competing with normal gametes (Burnham, 1962). Of course, this does not imply that the other mutants used are the result of point mutations. One might argue that the mutated gene(s) in L763-2 has pleiotropic effects on plant development. In such a case, other mutants with similar effect on seedcoat color would be expected to affect plant development also, but none was found to do so. In addition, deficiency of the recessive class would not be expected. According to Gottschalk (1968), simultaneous mutations of closely linked genes are frequently confounded with pleiotropic gene action.

The F_2 progenies from crosses between either L22-1 or L630-6 and Milionário 1732 showed a good fit to the expected ratio of 9 black:3 yellowish brown:4 beige (Table I). This indicates that each mutant resulted from two independent, recessive mutations. For the sake of convenience, let the affected genes be called A/a and B/b , without any reference to known genes in the literature. When both dominants are present ($A-B-$), each of them participate in a different step of a biochemical pathway leading to black pigment synthesis. Allele A , say, would be responsible for some step leading to the transformation of a beige precursor to a yellowish-brown intermediate, and B would act afterwards in the transformation of the intermediate to black pigment. When only dominant A is present ($A-bb$), the seedcoat is yellowish-brown due to a block in the second step. In the absence of A ($aaB-$ or $aabb$) the seedcoat is beige. Thus, aa is epistatic to B . The main reason for reviewing the genetical-physiological basis for the 9:3:4 ratio relates to the interpretation of the data discussed below. As shown in Table II, five phenotypic classes were observed in the F_2 generation from crosses between L252-15 or L742-2 with the parent variety. If the yellowish-brown classes are combined irrespective of presence/absence of stripes and the beige classes are combined disregarding stripe color, the data (Table II) fit the ratio of 9 black:3 yellowish-brown:4 beige, as discussed above for the data (Table I) related to mutant lines L22-1 and L630-6. As early as 1909; Emerson (cited by Lamprecht, 1947) assumed that the variegated seedcoat character in beans was controlled by two genes Y and Z , tightly linked. According to Tjebbes and Kooiman (cited by Koiman, 1931), stripes are caused by gene S completely linked to gene B which is now designated by C (Nakayama and Saito, 1975). Lamprecht (1947) proposed that striped seedcoat was due to allele R^{st} of the R gene linked to gene C with about 8.0 percent

recombination. However, Feenstra (1960) and Nakayama (cited by Prakken, 1972) found no recombinants and refer to a single locus *C*. Prakken (1972), on the other hand, found a few recombinant classes but considers a complex locus *C* as responsible for stripes. The striped pattern produced by Lamprecht's R^{st} is very similar to that found in L252-15 and L742-2.

Table II - Segregation for seedcoat color in F_2 progenies from crosses between striped seedcoat mutant lines and the parent variety.

Mutant line		No. of F_2 plants with seedcoat color					χ^2 (9:3:4) ¹	P
		Black	Yellowish- brown with brown stripes (a)	Yellowish- brown (b)	Beige with black stripes (c)	Beige with brown stripes (d)		
Designation	Seedcoat color							
L252-15	Beige with either black or brown stripes	792	137	84	276	93	7.482	0.02-0.05
L742-2	Beige with brown stripes	766	143	116	233	98	0.291	0.80-0.90

¹ The 9:3:4 ratio was tested by combining (a) with (b) and (c) with (d).

Analogous to the striped seedcoat in beans is the variegated pericarp (seedcoat) of the maize kernel controlled by the *P* locus. Emerson (1929) suggested, as he did for variegated beans, that mutability at the *P* locus was influenced by a linked gene or genes. Later, Brink and Nilan (1952) postulated that the unstable allele for variegation was actually a stable allele P^{RR} plus an accessory element, which they called *Mp* (Modulator), that inhibits normal gene function. Loss of *Mp* from the locus in some cells caused "mutation" from colorless to colored streaks. Barclay and Brink (1954) showed that *Mp* corresponds to *Ac* (Activator) of McClintock's (1951) *Ac-Ds* system. Pigment variegation of the maize kernel is the most conspicuous feature of the presence of active transposable elements and allowed the identification of the best characterized systems.

The striking resemblance between the variegated seedcoat in beans and in maize kernel led Barbosa *et al.* (1988) to suggest that their striped bean mutants were induced by EMS through the activation of transposable elements. These elements may exist cryptically in the chromosomes (Fedoroff *et al.*, 1983; Freeling, 1984; McClintock, 1984; Döring, 1985) and may have their action triggered by genomic stress (McClintock, 1984) caused by bridge-breakage-fusion cycle (McClintock, 1951; Doerschug, 1973), irradiation (Peterson, 1953; Neuffer, 1966; Bianchi *et al.*, 1969; Mottinger, 1973; Walbot, 1988), and virus infection (Friedemann and Peterson, 1982). Additional indirect evidence supporting the hypothesis of Barbosa *et al.* (1988) is provided by the occasional occurrence of bean seeds having seedcoats almost completely colored, except for small areas of light colored tissue, among seeds of pure breeding striped mutant lines. In these cases, the seedcoat color is the same as that of the stripes common to the line. This is analogous to Emerson's (1917) early descriptions of almost self-colored maize seeds (having small light streaks) on variegated ears. Such phenotypes are due to very early loss of the element from the locus. The time during development when the apparent "reversions" to nonmutant phenotype occur is associated to what McClintock (1951) called "change in state" of the element.

Segregation for stripe color (Table II) may be explained on the basis of gene activation and inactivation. Let the two loci involved be called V/V^{ph} and F/f , again without any reference to known genes in the literature. V^{ph} stands for the inactivated form of V due to the insertion of the element ph (from *Phaseolus*). Genes V/V^{ph} and F/f would act similarly to the genes A/a and B/b , respectively, previously considered. Genotype $V-F-$ produces black and $VVff$ yellowish-brown seedcoat. $V^{ph}V^{ph}F-$ conditions beige background color with black stripes. Black stripes are produced in seedcoat sectors containing cells in which ph was displaced from the V locus. Brown stripes on beige background appears in $V^{ph}V^{ph}ff$. Genotype $VV^{ph}ff$ would result in yellowish-brown background with brown stripes. This hypothesis accounts for (a) the absence of seeds having yellowish-brown background with black stripes, (b) the occurrence of beige seeds having either black or brown stripes, and (c) the absence of unstriped beige seeds.

Whether the mutated loci in mutants L22-1, L630-6, L252-15, and L742-2 are the same could not be determined. Studies are in progress to assess the allelic relationships among the induced mutants.

RESUMO

A herança da cor do tegumento de dez mutantes induzidos por EMS em *Phaseolus vulgaris* L. foi determinada na geração F_2 do cruzamento de cada um deles com a variedade original, de tegumento preto. Todos os mutantes comportaram-se como recessivos. Seis mutantes com tegumento uniformemente colorido foram herdados de forma monogênica. Há evidências de que

um deles seja resultado de deleção. A geração F₂ dos cruzamentos de dois outros mutantes uniformemente coloridos e dois mutantes variegados com a variedade original segregou na proporção 9 pretos:3 marrons-amarelados:4 beges, indicando dois locos mutados. São apresentadas evidências favorecendo a hipótese de que elementos transponíveis ativados pelo EMS, ainda não descritos em *Phaseolus*, são a causa das estrias nos tegumentos variegados.

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