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An Induced Mutant for Blue Flowers in Common Bean That Is Not Allelic to *V* or *Sal* and Is Linked to *Fin*

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Abstract. **A mutation for blue (methyl-violet) flower color was induced by gamma irradiation of dry seed in common bean *(Phaseolus vulgaris* L.). Inheritance and allelism tests demonstrated that the mutation is controlled by a single recessive gene that is not allelic with *Sal* or *V*. The gene symbol *blu* is proposed for this mutant. Linkage was detected between *blu* and *Fin,* which controls the change from indeterminate to determinate plant habit. Three linkage estimates ranged from 19 to 35 cM, but the large variability (homogeneity $x^2 = 45.32$) precludes making a conclusive combined estimate of linkage. The tentative combined estimate of linkage is 27 cM.**

The most recent comprehensive review of flower color genetics in common bean is that of Yarnell (1965). A more recent but less comprehensive review was made by Leakey (1988), which has helpful illustrations [after Prakken (1972)] of color patterns on the wing and banner petals. In this paper, all color names and numeric designations are taken from the 1938 edition of the Horticultural Colour Charts of the Royal Horticultural Society.

In common bean a recessive allele at either *P* or *T* will produce an all-white flower. In the presence of *P* and *T,* the *V* locus can express a series of colors (wing petal only): *V* gives bishops-violet, *V/v* gives cobalt-violet, v^{lae} gives rose-purple, and *v* gives white. In the presence of *P* and *T* with *v* or v, rk^{d} gives red veins in the wing petals (Prakken, 1972). The *Sal* gene from *Phaseolus coccineus* gives camellia-rose with *v* and china-rose with *V,* which are distinctive red flower colors (Bassett et al., 1990).

The purpose of the this paper is to present evidence for the inheritance of a flower color mutant that gives a blue (methyl-violet) color relative to the wild-type color of common bean, bishops-violet.

Materials and Methods

Development of lines. The flower color names and numeric designations used are taken from the Horticultural Colour Charts (Royal Horticultural Society, 1938).

In 1979 dry seed of Florida breeding line 7-1404 was treated with 10 or 20 kR (1 rad = 0.01 Gy) of gamma radiation, using a Gammator M with a cesium-137 source (Radiation Machinery Corp., Parsippany, N.J.). The Ml was planted in the field in July 1979 and seed was harvested in bulk from all M_1 plants. The M_2 was planted in the field in Apr. 1980, and a selection was made for a lanceolate leaf mutation that was subsequently given the symbol *sl* (Nagata and Bassett, 1984). During progeny testing of this mutant in the greenhouse, it segregated for another mutation, blue flower color (methyl-violet 39/2). Line 4-67 is an M_5 derivative from the original M_3 selection for *s1* and blue flower.

Line B-351 is a dry bean breeding line released by the Tropical Agricultural Research Station, Mayaguez, Puerto Rico. It has semivine habit, bishops-violet (34/2) flowers, and small black shiny seeds. Florida breeding line 5-593 is closely related to B-351, but 5-593 has compact determinate habit. Other parental lines used below are described in Table 1.

Inheritance tests. The inheritance of the blue flower color was studied in two crosses. Line 4-67 (*sl* blue flower) was crossed with line 0-181 *(Sl* bishops-violet flower), and numerous F_2 plots (each from a different F_1 plant) were planted in the field in 1986. Breeding line 5-593 (bishops-violet flower) was crossed with line 7-364 (blue flower), and the F_2 was planted in the field in 1988. Flower color was classified in all the above segregating progenies.

Tests for allelism were made between the salmon flower color locus and the blue flower color locus in three crosses. Line 4-67 *(sl sal* blue flower) was crossed to line 4-72 *(Sl Sal* red flower), and the F_2 was planted in the field in 1985. This cross was repeated in 1986. Line 7-364 *(sal* blue flower) was crossed with line 7-34 *(Sal* red flower), and the F_2 was planted in the field in 1988. Flower color was classified in the F_1 and F_2 progenies from the above three crosses.

Tests for allelism were made between the *V* locus and the

Table 1. Descriptive list of parental lines used in crosses made to study the inheritance of the blue-flower mutant.

	Flower		Plant
Line no.	Color	Genotype[z]	habit
4-67	Blue	*blu V*	*Fin*
7-364	Blue	*blu V*	*fin*
4-72	Red	*Blue Sal V*	*fin*
7-34	Red	*Blu Sal V*	*fin*
0-181	BV[y]	*Blu V*	*Fin*
B-351	BV[y]	*Blu V*	*Fin*
5-593	BV[y]	*Blu V*	*fin*
3-5	White	*Blu v*[x]	*Fin*
7-518	White	*Blu v*	*fin*

[z] The genetic hypothesis of a *blu* locus is supported by experimental data in succeeding tables.
[y] BV = bishops-violet.
[x] This *v* allele is an induced mutant at *V*.

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Table 2. Segregation for flower color in F_2 populations from two crosses: #1 (4-67 *sl* blue flower x 0-181 *Sl* bishops-violet flower) and #2 (5-593 bishops-violet flower x 7-364 blue flower).

Cross	Phenotypic classes[z]		χ^2	
	Bishops-violet	Blue	3:1	*P*
#1	632	202	0.27	0.60
#2	138	41	0.42	0.52

[z] Genetic hypothesis: *V Blu/Blu* and *V Blu/blu* produce bishops-violet and *V blu* produces blue (methyl-violet) flower.

blue flower color locus in two crosses. Line 7-518 *v* white flower was crossed to 7-364 blue flower, and the F_2 was planted in “the field in 1988. The F_3 progenies from 16 randomly selected F_2 plants from the above cross were grown in the field in 1990. Line 7-364 blue flower was also crossed to 3-5 (mutant *v*, white flower), and the F_2 was planted in the field in 1988. Flower color was classified in the F_1 and F_2 for both crosses and the F_3 progeny tests from the cross with 7-518 *(v* white flower).

Tests for linkage between the blue-flower mutant and the *Fin* locus, which controls indeterminate vs. determinate habit, were achieved by the following crosses. The blue-flower mutant, which occurred in a plant with semivine (indeterminate) habit, was crossed to a determinate breeding line 5-593 as part of a large-scale program to convert marker genes to a common genetic background. The cross was 4-67 *Fin* blue flower x 5-593 *fin* bishops-violet flower, and the F_2 was planted in the field in 1987. Two other crosses of this type were made: 7-364 *fin* blue flower x B-351 *Fin* and 7-364 *fin* blue flower x 3-5 *Fin.* These latter two crosses were planted in the field in 1988. Flower color was classified in the F_2 populations from all three crosses, and linkage estimates were calculated by the maximum likelihood method (Allard, 1956).

Results and Discussion

Inheritance tests. Crosses between lines with bishops-violet 34/2 flower color (wild type) and the blue-flower mutant resulted in F_1 plants with bishops-violet flowers (data not shown). The F_2 progenies from these crosses segregated for bishops-violet and blue-flower plants in a 3:1 ratio, respectively (Table 2). These results are consistent with the hypothesis that blue flower color is controlled by a single recessive gene. The gene symbol *blu is* proposed for the mutant locus based on the inheritance and allelism test results that are presented below.

Crosses were made between lines carrying the Sal gene for red flower color (china rose 024/1 due to *Sal V)* and the blue-flower mutant to test for allelism between *Sal* and the mutant locus. The F_1 plants had red flowers (mallow-purple 630/0, data not shown), but the F_2 segregated for three flower classes, including the nonparental class bishops-violet (Table 3). My hypothesis is that the F_2 is segregating in a 12:3:1 ratio for red (china-rose and mallow-purple are combined), bishops-violet, and blue (methyl violet), respectively, but the obtained values are disturbed by a gametophyte factor locus linked to *Sal* (Bassett et al., 1990). This hypothesis has not been verified for these data, but it is clear that the locus of the blue flower mutation is not allelic with *Sal.*

Crosses were made between a line carrying the blue-flower mutant and two lines with white flower due to v. The F_1 plants had cobalt-violet 634/1 flowers, but the F_2 segregated for four flower color classes (Table 4). Clearly, the mutant blue flower locus is not allelic with *V.* In one of the crosses (#2), the bishops-violet and cobalt-violet classes were “combined, giving a good fit to a 9:3:4 ratio for bishops-violet, blue, and white, respectively. For cross #1, a good fit to a 3:6:3:4 ratio was obtained, where bishops-violet and cobalt-violet plants were successfully classified (Table 4).

The genetic hypothesis for the four classes is based on the fact that *V/v* gives cobalt-violet and on the inferences that *blu/blu is* epistatic to *V/–* and *v/v* is epistatic to *blu/blu* (Table 5). If the nine genotypes in the F_2 model are selfed, they should give rise to seven patterns of segregation in F_3 (Table 5). The genetic model for the F_2 was tested by analysis of F_3 progenies from 16 randomly selected F_2 plants. Six of the expected seven segregation patterns were observed, and they were derived from the F_2 plants with the appropriate phenotypes and hypothesized genotypes (Table 6). Taking the segregation patterns in the order in which they appear in Table 6 (top to bottom), the goodness-of-fit of the observed frequency of these patterns to the expected in the F_3 data was calculated as x^2(1:2:4:2:4:1:2) = 2.25, P= 0.895. The only missing segregation pattern was a progeny that is true-breeding for bishops-violet flower color. This result is not surprising because such a progeny should occur only once in 16 F_3 progenies and only 16 F_3 progenies were classified. The observed results support the model in Table 5, and they are not consistent with any alternative model.

Tests for linkage were made using crosses of three types (Table 7). Orthogonal contrasts were used to separately detect disturbance of segregation at the two segregating loci and distinguish these effects from linkage disturbance of the expected 9:3:3:1 ratio (Mather, 1957). In all three crosses, there was significant disturbance of segregation at either *Fin* or *blu*, or both. For example, there was a drastic disturbance in segregation at *Fin* in cross #1, resulting in a large shortage of semivine

Table 3. Segregation for flower color in F_2 allelism test[z] (*Sal* vs. *blu*) populations from three crosses: #1 (4-67 *sl blu sal* x 4-72 *Sl Blu Sal*), #2 (7-364 *sl blue sal* x 4-72 *Sl Blu Sal*), and #3 (7-364 *blu sal* x 7-34 *Blu Sal*).

Cross	Phenotypic classes			χ^2	
	China-rose[y]	Bishops-violet[x]	Blue	12:3:1	*P*
#1	136	77	36	60.318	<0.001
#2	182	95	18	35.455	<0.001
#3	429	163	58	56.12	<0.001

[z] Null hypothesis states that the blue (methyl-violet) flower mutant is allelic with the salmon (*Sal*) red flower color locus.

[y] Two color classes combined, china-rose *Sal/Sal V/V* and mallow-purple *Sal/sal V/V*.

Table 4. Segregation for flower color in F_2 allelism test[z] (*v* vs. *blu*) populations from two crosses: #1 (7-518 *Blu v* white flower x 7-364 F_3 *blu V* blue[z] flower) and #2 (7-364 *blu V* x 3-5 mutant *v*, white flower).

Cross	Phenotypic classes[y] Bishops-violet	Cobalt-violet	Blue	White	χ^2 ratio tested	χ^2 value	*P*
#1	73	130	58	76	3:6:3:4	2.861	0.41
#2	191[x]		50	81	9:3:4	2.32	0.31

[z]Null hypothesis states that the blue (methyl-violet) flower mutant is allelic with the *v* locus.

[y]Flower color genotypes: *V Blu*/− bishops-violet, *V*/*v Blu*/− cobalt-violet, *V*/− *blu* blue (methyl-violet), and *v Blu*/− and *v blu* white.

[x]Two classes combined: bishops-violet and cobalt-violet.

Table 5. Genetic model for flower color segregation in F_2 progeny from crosses between *Blu v* and *blu V* and the expected segregation in F_3 progeny tests of each genotype in the F_2.

F_2 genotypes	Frequency[z]	F_2 phenotypes	Expected segregation in F_3
Blu/*Blu V*/*V*	1	Bishops-violet (BV)	True breeding for BV
Blu/*Blu V*/*v*	2	Cobalt-violet (CV)	1 BV : 2 CV : 1 white
Blu/*Blu v*/*v*	1	White	True-breeding for white
Blu/*blu V*/*V*	2	Bishops-violet (BV)	3 BV : 1 blue
Blu/*blu V*/*v*	4	Cobalt-violet (CV)	3 BV : 6 CV : 3 blue : 4 white
Blue/*blu v*/*v*	2	White	True-breeding for white
blu/*blu V*/*V*	1	Blue	True-breeding for blue
blu/*blu V*/*v*	2	Blue	3 blue : 1 white
blu/*blu v*/*v*	1	White	True-breeding for white

[z]Each value is the numerator (n) of the expected fraction (n/16) in the F_2, in which the expected ratio of phenotypic classes is 3 BV : 6 CV : 3 blue : 4 white.

Table 6. Segregation for flower color in F_3 progeny tests of 16 random plants from the F_2 of the cross 7-518 (*v* white flower) x 7-364 (*blu* blue flower or methyl-violet).

F_2 parent phenotype	Phenotypic classes in F_3 Bishops-violet	Cobalt-violet	Blue	White	χ^2 ratio tested	χ^2 value	*P*
Bishops-violet	110		44		3:1	1.05	0.31
	56		8		3:1	5.33	0.02
Cobalt-violet	41	69	50	51	3:6:3:4	4.16	0.25
	29	55		26	1:2:1	0.16	0.92
	15	27	8	8	3:6:3:4	6.51	0.09
	26	51	29	33	3:6:3:4	0.44	0.93
	29	46	26	32	3:6:3:4	1.06	0.79
	10	30	4	13	3:6:3:4	7.86	0.05
Blue			51				
			70	17	3:1	1.38	0.24
			57	22	3:1	0.09	0.34
			31	6	3:1	1.52	0.22
White				50			
				47			
				48			
				49			

Table 7. Segregation for plant habit and flower color in F_2 populations from three crosses: #1 (4-67 *Fin blu sl* x 5-593 *fin Blu Sl*), #2 (7-364 *fin blu* x B-351 *Fin Blu*), and #3 (7-364 *fin blu* x 3-5 *Fin Blu*).

Cross no.	Phenotypic classes (habit/flower color)[z] *Fin*/BV	*Fin*/blue	*fin*/BV	*fin*/blue	Orthogonal contrast χ^2 values[y] *Fin*/*fin*	*Blu*/*blu*	Linkage	Linkage cM
#1	807	634	916	57	301.64	16.92	1531.19	19.00±1.31
#2	1017	120	243	87	4.91	92.78	38.29	35.14±1.52
#3	191	28	20	22	11.05	4.75	25.55	23.9 ±2.1

[z]*Fin* = semivine, *fin* = determinate, BV = bishops-violet.

[y]Each of the χ^2 values has 1 d.f. and is significant, i.e., $P = 0.05$ at $\chi^2 = 3.84$.

plants. In cross #2, there was a large disturbance in segregation at *blu*, resulting in a large shortage of blue-flower plants. Nevertheless, there is a significant linkage chi-square for all three crosses (Table 7). The linkage estimates ranged from 19 to 35 cM, and the chi-square for the homogeneity was significantly large, 45.32 (P = 0.05 when x^2 = 5.99 and d.f. = 2). The combined estimate is 27.2 ± 1.06 cM. Although linkage is indicated from the data, it is inappropriate to regard the combined estimate of linkage as conclusive when the data have such high variability.

The original line, 4-67, in which the blue-flower mutant occurred, had apparently normal flowering, fertility, and yield. When the *blu* allele was combined with *fin*, most of the determinate segregants had little or no flowering and were completely barren. A single plant among the 57 *blu fin* F_2 segregants had normal fertility, (Table 7, cross #1). Line 7-364 *fin blu* was developed from this selection, and this line flowers more than 1 week later than 5-593. With breeding and selection, there is now a *blu* BC_2 5-593 line that flowers at the same time as 5-593. Seed of this material is available from M..T.B. on request.

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