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Characteristics and Inheritance of Chlorophyll Mutations in *Phaseolus mungo*

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Abstract. Viable mutations affecting chlorophyll production in black gram (*Phaseolus mungo* L.) were recovered following treatments with X-rays and ethyl methanesulphonate singly or in combination. Of these the *viridis* mutant was characterized by viridine green colour of all the leaves and reduced plant size. In *chlorotica* the emerging leaves were initially yellowish green which gradually changed to dark green at flowering. The terminal 2—3 leaves always remained yellowish green in *chlorina-terminalis* whereas in *chlorina-virescence* the emerging leaves were yellowish green that changed to dark green at flowering. In *albo-virescence* the first 3—5 leaves at emergence were white in which small irregular green dots or patches often developed. The subsequent leaves were normal green. The emerging trifoliate leaves of *aureo-virescence* were light green which turned to turtle green within a week. It was established that *viridis*, *chlorotica*, *chlorina-terminalis*, *chlorina-virescence*, and *albo-virescence* mutants were conditioned by a single recessive gene, in each case. The *aureo-virescence* character was found to be inherited through the cytoplasm.

Chlorophyll deficient mutants provide genetic information and are often useful in exploring the metabolic functions and biochemical reactions in plants (MILLER 1968). Such mutants can be conveniently induced following treatments with various mutagenic agents. The present report includes information on different viable chlorophyll deficient mutants of black gram (*Phaseolus mungo* L.) not recovered earlier (JANA 1963, SUR 1969) with X-rays or chemicals.

Material and Methods

Dry seeds (11.4% moisture) of black gram were irradiated with 10, 20, 30 and 40 kR X-rays with a "Muller 150 kV X-ray tube" at a constant dose rate of 6 000 R per minute. Seeds were also treated with 0.1, 0.2, 0.3 and 0.4 per cent EMS solutions prepared in phosphate buffer of pH 7.0 for 12 hours at 20 °C. During combined treatments, seeds were treated with EMS immediately after X-irradiation. The treated seeds were allowed to germinate after thoroughly washing in distilled water. The culture of the plants, isolation of the mutants and hybridization techniques were same as described earlier (JANA and APPA RAO 1974). The colour of the mutants were described with reference to the Dictionary of Color (MAERZ and PAUL 1954).

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Results and Discussion

The viable mutants recovered after different mutagenic treatments are shown in Table 1. The description and mode of inheritance of the mutants (nomenclature according to BLIXT 1961) are as follows:

1. Viridis

The first pair of leaves were citron green (19K2) in colour. The trifoliate leaves were always cosse green (19L5) at emergence which turned viridine green (17I6) within a week and remained so till maturity. The size of the leaves was reduced though the leaf shape remained unaltered. The plants were slow in growth with reduced size and lower seed yield than normal plants (Table 2). The mutant was distinguishable because of its reduced height and its characteristic viridine green colour of the leaves.

From the reciprocal crosses between the normal and *viridis* mutant, the F_1 plants obtained were phenotypically normal indicating recessive nature of the mutant. In the F_2 generation, there was 3 : 1 segregation of normal and mutant types (Table 3). In F_3, out of 10 normal F_2 plants, 3 bred true, 7 segregated like F_1. All the mutant F_2 plants bred true as except for a recessive character. The gene symbol *Vim/vim* has been proposed for this allelic pair.

2. Chlorotica

The first pair of leaves were yellowish green (18K3). The emerging trifoliate leaves were also yellowish green (18K3) which slowly turned dark green (23E7) at flowering. The mutant was almost as vigorous as the normal plants.

Reciprocal crosses between the normal and *chlorotica* mutant produced normal F_1 hybrids. All the F_2 plants segregated into normal and mutant in 3 : 1 ratio (Table 3) revealing that the mutant was monogenic recessive to the normal which was further confirmed in F_3 generation. Out of 10 phenotypically normal F_2 plants, 4 bred true, 6 segregated into normal and mutant in 3 : 1 ratio. All the mutant F_2 plants bred true. The gene symbol *Chi/chi* has been proposed for this allelic pair.

3. Chlorina-terminalis

The first pair of leaves was yellowish green (18K3). The emerging leaves were also yellowish green (18K3) which turned rivage green (17I7) within 3 to 5 days. These leaves became darker with the approach of maturity, but never regained the normal green colour. The terminal 2 to 3 leaves, however, were always yellowish green at any stage of growth. The leaflets were narrow and lanceolate in shape instead of obovate in the normal. The different plant parts including yield of the mutant were very much reduced as compared to the normal (Table 2).

When reciprocal crosses were made between the normal and *chlorina-terminalis* mutant, the F_1 plants were phenotypically normal. In the F_2, there was segregation of normal and *chlorina-terminalis* plants in 3 : 1 ratio (Table 3). In F_3 out of 15 normal F_2 plants, 9 segregated like F_1, and 6 bred true. It is clear that the mutant character is monogenic recessive to the normal. The gene designation *Cht/cht* has been proposed for this allelic pair.

TABLE 1
ORIGIN AND CHARACTERISTICS OF VIABLE CHLOROPHYLL MUTANTS OF BLACK GRAM

Mutant	Origin	Leaf Colour				Plant size
		first pair	emerging trifoliate leaves before flowering	fully grown trifoliate leaves	emerging after flowering	
Viridis	40 kR X-rays + 0.2% EMS	citron green (19K2)	cosse green (19L5)	viridine green (17I6)	cosse green (19L5)	drastically reduced size, poor growth
Chlorotica	0.3% EMS	yellowish green (18K3)	yellowish green (18K3)	dark green (23E7)	dark green (23E7)	normal
Chlorina-terminalis	30 kR X-rays + 0.1% EMS	yellowish green (18K3)	yellowish green (18K3)	rivage green (17J7)	yellowish green (18K3)	drastically reduced size, poor growth
Chlorina-virescence	30 kR X-rays	yellowish green (18I5)	yellowish green (18I5)	dark green (23E7)	light green (18G7)	normal
Albo-virescence	30 kR X-rays + 0.2% EMS	white (10A1)	cream yellow (IH2)	dark green (23E7)	dark green (23E7)	poor growth
Aureo-virescence	20 kR X-rays + 0.2% EMS	citronelle (18L2)	light green (18G7)	turtle green (19G5)	light green (18G7)	normal
Normal	—	dark green (23E7)	green (21K7)	dark green (23E7)	dark green (23E7)	normal

TABLE 2

COMPARISON OF VIABLE CHLOROPHYLL MUTANTS OF BLACK GRAM WITH NORMAL FOR DIFFERENT MORPHOLOGICAL TRAITS

	Control	*Viridis*	*Chlorotica*	*Chlorina-terminalis*	*Chlorina-virescence*	*Albo-virescence*	*Aureo-virescence*
Plant height [cm]	28.6 ± 3.0	14.8 ± 1.9	21.6 ± 2.3	11.5 ± 1.8	18.8 ± 2.6	11.4 ± 1.2	18.6 ± 2.6
No. of branches	3.1 ± 0.4	2.1 ± 0.4	3.8 ± 0.4	1.7 ± 0.4	2.13 ± 0.4	1.4 ± 0.3	2.3 ± 0.4
No. of nodes [cm]	10.9 ± 1.2	8.8 ± 1.0	11.6 ± 1.4	10.6 ± 1.0	9.4 ± 1.0	8.6 ± 0.9	9.6 ± 1.0
Length of the leaf [cm]	8.9 ± 0.8	8.1 ± 1.0	8.6 ± 0.7	8.3 ± 1.5	8.3 ± 1.0	6.8 ± 0.6	8.4 ± 1.0
Breadth of the leaf [cm]	4.9 ± 0.5	3.5 ± 0.5	4.5 ± 0.4	2.1 ± 0.3	3.8 ± 0.5	3.5 ± 0.4	3.7 ± 0.5
No. of days to flower	40.0 ± 4.1	41.0 ± 6.1	43.2 ± 0.5	48.6 ± 0.6	44.6 ± 0.6	52.1 ± 0.7	42.6 ± 0.6
No. of peduncles	8.4 ± 1.2	5.3 ± 0.4	6.4 ± 0.8	4.8 ± 0.4	5.3 ± 0.6	2.4 ± 0.3	5.6 ± 0.6
Length of the peduncle [cm]	6.2 ± 0.8	4.2 ± 0.3	4.4 ± 0.4	4.1 ± 0.4	3.9 ± 0.4	3.8 ± 0.4	4.0 ± 0.4
Pollen sterility	2.8 ± 0.3	3.8 ± 0.5	3.6 ± 0.5	5.6 ± 0.8	7.6 ± 1.2	26.5 ± 3.5	8.2 ± 0.9
Ovule sterility	3.2 ± 0.3	6.2 ± 0.8	4.8 ± 0.6	8.3 ± 1.0	8.5 ± 0.9	18.7 ± 3.2	9.2 ± 1.2
Length of the pod [cm]	4.2 ± 0.4	3.3 ± 0.4	4.1 ± 0.4	3.4 ± 0.4	3.8 ± 0.5	2.8 ± 0.5	3.7 ± 0.5
No. of seeds per pod	5.9 ± 0.6	3.7 ± 0.5	4.2 ± 0.4	3.9 ± 0.5	4.2 ± 0.6	3.5 ± 0.5	4.1 ± 0.5
No. of pods per plant	16.3 ± 2.4	7.3 ± 0.8	13.5 ± 2.3	5.8 ± 0.7	11.2 ± 2.2	3.8 ± 0.5	9.2 ± 2.0

TABLE 3
INHERITANCE OF CHLOROPHYLL MUTATIONS OF BLACK GRAM

Parents	F_1	Segregation in F_2		χ^2 3:1	*P* value
		Normal	Mutant		
Normal × *viridis*	Normal	448	160	0.55	0.5—0.3
viridis × Normal	Normal	199	57	1.04	0.5—0.3
Normal × *chlorotica*	Normal	336	124	0.94	0.5—0.3
chlorotica × Normal	Normal	140	44	0.12	0.8—0.7
Normal × *chlorina terminalis*	Normal	222	46	0.44	0.7—0.5
chlorina terminalis × Normal	Normal	134	36	1.26	0.3—0.2
Normal × *Chlorina virescence*	Normal	356	110	0.48	0.5—0.3
Chlorina virescence × Normal	Normal	122	45	0.69	0.5—0.3
Normal × *albo virescence*	Normal	196	62	0.11	0.8—0.7
albo virescence × Normal	Normal	125	35	0.83	0.5—0.3
Normal × *aureo virescence*	Normal	364	—	—	—
aureo virescence × Normal	*Aureo virescence*	—	192	—	—

4. Chlorina-virescence

The first pair of leaves was yellowish green (18I5). The trifoliate leaves also after emergence were yellowish green (18I5) which gradually turned light green (18J7) within 3 to 5 days. As the plants grew, the leaves became progressively green, and at flowering all the leaves became normal green except the terminal 3 to 4 leaves which remained light green. The plants were less vigorous and all the plant parts were slightly reduced as compared to the normal (Table 2). The plant yield also was impaired.

The F_1 plants from the reciprocal crosses between normal and mutant were phenotypically normal. All the F_2 plants segregated into normal and mutant in 3 : 1 ratio (Table 3) suggesting that the mutant is single gene recessive in nature. This was further confirmed in F_3 as out of 10 normal F_2 plants, 6 segregated into normal and mutant in 3 : 1 ratio, whereas 4 bred true. All the mutant plants bred true. The gene symbol *Chves/chves* has been proposed for this allelic pair.

5. Albo-virescence

The first pair of leaves was small and white (10A1). The first trifoliate leaves were minute creamish white, (9H2) with varying shape and number of leaflets. As the leaves grew, green colour appeared on them in irregular dots and patches. After about 5 weeks the plants started producing normal green leaves by which time the earlier leaves also turned green. In general, the plants

were less vigorous with smaller leaves and reduced yield as compared to the normal plant (Table 2).

The F_1 plants obtained from the reciprocal crosses between the normal and *albo-virescence* mutant were normal. All the F_2 plants segregated into normal and *albo-virescence* in 3 : 1 ratio (Table 3), suggesting that a single recessive gene was responsible for the expression of the mutant character. In F_3, out of 10 phenotypically normal F_2 plants, 3 bred true, while the rest segregated like the F_1. All the mutant phenotypes bred true. The gene symbol *Alves/alves* has been proposed for this allelic pair.

6. Aureo-virescence

The first pair of leaves were citronelle (18L2) which gradually turned light green (18G7) within 2 to 3 days. The newly emerging trifoliate leaves were light green (18G7) but turned turtle green (19G5) within 5—6 days. When the plants were fully grown the leaves became darker, but never changed to normal green colour. The plant height, leaf size and other parts including yield were reduced as compared to the normal (Table 2).

When reciprocal crosses were made between the normal and *aureo-virescence* mutant the F_1 plants were different depending on the female parent. When normal plants were taken as female parent, the F_1 plants were normal, but when the female parent was *aureo-virescence* the F_1 plants were mutant type. The F_2 progeny of the cross normal × *aureo-virescence* produced normal plants, whereas the seeds from the reciprocal crosses produced only mutant type. The results indicate cytoplasmic inheritance of the mutant character, which may be due to plastid mutations.

All these chlorophyll mutant types recovered after different mutagenic treatments were fully viable but usually less vigorous than normal and gave lower seed yield. The *viridis* mutant was different from that recovered earlier in black gram (JANA 1964) after neutron irradiation which was completely sterile. In all these mutants chlorophyll deficiency was conspicuous in the first few leaves and became less intense as subsequent leaves were formed and in several cases turned normal green. However, there was considerable differences in such mutants concerning the time required for change in colour towards normal green. The plant type remained mostly same as the parent strain T_9, except the *chlorina-terminalis*, whose leaf shape was conspicuously different. The mutants were mostly single gene recessives except *aureo-virescence* which was due to plastid mutation. All these mutants of black gram could be useful in biochemical and genetic investigations, since each of the mutant phenotype was readily distinguishable and retained considerable fertility.

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Působením X-paprsků a etylmetansulfonátu — ať jednotlivém nebo kombinovaném — byly získány některé životné mutanty: Mutant *viridis* má všechny listy světle zelené a rostliny jsou menší. U mutanta *chlorotica* jsou vzcházející listy nejprve žlutozelené, postupně se měnící na tmavozelené v době květu. U m. *chlorina-terminalis* zůstávají terminální 2—3 listy vždy žlutozelené, zatímco u m. *chlorina-virescens* jsou nově vzcházející listy žlutozelené, měnící se na tmavozelené v době květu. U m. *albo-virescens* jsou první 3—5 listy bílé v době vzejití, s nepravidelnými zelenými skvrnami. Pozdější listy jsou normálně zelené. Mutant *aureo-virescens* má vzcházející listy velmi světle zelené, měnící se na žlutavě žlutozelenou během týdne. Mutant *aureo-virescens* je dědičný mimojaderně, ostatní jsou podmíněny každý jedním recesivním genem.