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Variegated Mutants in Cotton, *Gossypium hirsutum* L.[1]

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ABSTRACT

Variegated plants were observed as spontaneous mutations in the cotton nurseries, as an induced mutation in a line isolated from a seed-irradiated population, and as reoccurring mutations in a genetic marker line. Genetic analysis of this mutant-condition indicated that it is maternally inherited. Observations of the segregation patterns and development of this mutant led to the conclusion that mutant plants result from plastid mutations. The influence of the environment on the expression of this mutant phenotype and the possibility of nuclear control of the plastid mutation were discussed.

Additional index words: **cytoplasmic inheritance, plastid mutation.**

IN the spring and summer of 1962, a high frequency of variegated plants appeared in the cotton nurseries at College Station, Texas. More than 100 such plants were identified as variegated in the four-hectare breeding nursery. The high frequency of occurrence can be attributed to the common parentage of many of the lines, rather than to the occurrence of independent mutations. However, this fact would not explain why so many more variegated plants appeared in 1962 than in previous years when these same lines were grown.

The high frequency of occurrence of this mutant in the cotton nurseries and the occurrence of phenotypically identical mutants in an irradiated population and in a genetic marker line provided stimulus to initiate an inheritance study of the variegated mutations.

Occasional variegated plants had been seen in previous years, but attempts to study them had been unsuccessful. A literature reference (8) to the 1928 Texas Experiment Station Annual Report refers to a report of a cytoplasmically inherited variegated mutation. Annual reports for this period are not available, and no other published report of this study was found. However, a paper published in 1931 by researchers at the Texas Station (Horlacher and Killough, 9) included a report of the occurrence of variegated plants following the use of X rays. Cotton researchers have indicated (personal communication) that variegated mutants are frequently noted. However, the few attempts to study these plants have been unsuccessful.

Variegated plants (Fig. 1) used in the present study originated from three sources. The majority of the plants grown were the offspring of those discovered in the 1962 nursery; the remainder were from a line isolated from a seed-irradiated population and from a line carrying the genetic marker abnormal palisades (Kohel, 14) with the genotype $lp_1lp_1lp_2lp_2$.

PROCEDURE AND RESULTS

In 1962, open-pollinated seeds from variegated plants induced by irradiation were drill-planted in a progeny row. Approximately a fourth of the resulting seedlings were chlorophyll-

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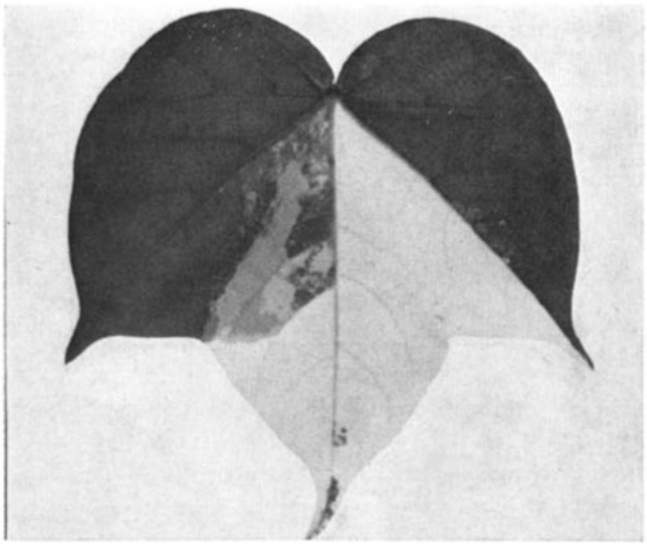

Fig. 1. A leaf from a variegated plant illustrating the mosaic development.

deficient (albino) and died. The remaining plants segregated for variegated and normal green, but because routine thinning could have biased the results, segregation frequencies were not recorded. This segregation pattern favored the hypothesis that variegated plants are heterozygous and albino plants are homozygous mutant forms. This attractive hypothesis could explain simultaneously how variegated plants would be maintained in a rogued population and why so many variegated plants could appear in a single year. Variegated plants generally flower in field plantings before they are detected. However, the occurrence of a cytoplasmically inherited variegated mutation must also be considered as an alternative hypothesis in explanation of the mode of inheritance of this mutation.

To test these alternative hypotheses, crosses were set up reciprocally between the variegated mutants (VM) and several Texas Marker lines (TM). The variegated plants lacked vigor and were unproductive as female parents. In 1963 and 1964, we used the TM lines as female parents; not until 1965 were the variegated plants used successfully as female parents (Table 1).

The recovery of only green plants in the F_1, when the TM lines were used as females, ruled out the possibility that a single dominant allele controlled the variegated expression. The occurrence of variegated and albino plants in the reciprocal F_1's, in which variegated plants were female parents, supported the hypothesis of cytoplasmic inheritance.

Spontaneously occurring variegated plants and those from the irradiation population were progeny tested; the progenies segregated in an erratic fashion. Open-pollinated seeds were harvested from 81 of the variegated plants found in the field in 1962. Progeny rows from all 81 plants were drill-planted in 1963, but only 26 progenies segregated for variegated or variegated and albino plants. The remaining 55 progenies contained only normal green plants. Since the progenies were drill-planted, an arrangement which did not allow close observation of individual seedlings and plants, remnant seeds were planted in individual-plant containers in the greenhouse in 1964. The seedings were transplanted to the field at the age of three weeks. Remnant seeds were available and progenies were grown from 58 of the original 81 variegated plants. Twenty-two progenies were from the 26 that had segregated in the drill-planted rows. Fourteen of these segregated and eight had all green plants. Thirty-six progenies were from the 55 that had been all green in 1963. Two of these progenies segregated and the other 34 were all green. In total, 16 of the 58 individual-planted progenies segregated and 42 had all green plants. Phenotypes in the 16 segregating progenies were distributed as follows: 18 albino, 44 variegated, and 480 green plants (1,780 plants total in the 58 progenies).

Table 1. Segregation of the F_1 generation of crosses between variegated mutant plants (VM) and Texas Marker lines (TM).

Year	Cross	Number of plants			
		Albino	Variegated	Green	Total
1963	TM-3 · VM-1			20	20
	TM-4 · VM-1			20	20
	TM-5 · VM-1			20	20
1964	TM-2 · VM-1			15	15
	TM-3 · VM-1			15	15
1965	TM-1 · VM-1			15	15
	VM-1 · TM-1		3	12	15
1966	TM-1 · VM-1			49	49
	TM-1 · VM-2			48	48
	VM-1 · TM-1	16	7	19	42
	VM-2 · TM-1	34	1	8	43
	VM-3 · TM-1	22	13	13	48

The segregation pattern did not suggest any clear-cut genetic ratio within the influence of the variegated type cytoplasm. It was further noted in the next generation that progenies from variegated plants segregated, but progenies from green plants originating in variegated progenies bred true for green plants.

In the 1964-65 greenhouse season, variegated plants ratooned from the field were grown and allowed to set fruit. The fruits were separated into two classes: *(1)* those that developed from extreme mosaic sectors and appeared to lack chlorophyll (white bolls); *(2)* those completely green or with small mosaic sectors (green bolls). Fruits were not set on albino branches. Mature seed was harvested from 11 bolls in the white-boll class and 47 bolls in the green-boll class. The seeds from each boll were planted and identified as a separate progeny.

Ten progenies from the white bolls segregated and one did not, while seven progenies from the green bolls segregated and 40 did not. In the segregating progenies, there appeared 98 albino, 36 variegated, and 43 green plants from the white-boll class and 65 albino, 35 variegated, and 66 green plants from the green-boll class. Progenies from the white-boll class had a higher frequency of segregating progenies and a higher frequency of mutant types in the segregating progenies, than those from the green-boll class. Mutant types from variegated plants apparently originated from the direct inclusion of a mutant plastid form in the maternal germ line.

Variegated plants are not able to compete with normal green plants; mosaic branches on plants are crowded out by normal green branches; and mosaic sectors in leaves succumb readily to moisture stress, insecticide damage, and other external stresses. Variegated plants develop vigorously only in the winter greenhouse; even under this environment, they are unproductive and do not set a normal fruit load. It can be noted that the above mentioned green-boll class had 24 seeds vs. 18 seeds per fruit for the white-boll class.

Interseasonal competition was observed also. Occasional variegated plants have been seen every year as random mutants, both in closely and distantly related material. However, in 1962 and 1963, unusually large numbers of variegated plants were noted. Spring temperatures were unusually cool in both years. Presumably, cooler temperatures allow greater develop-

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ment and expression of the mutations. Woods and du Bury (18) noted that variegated *Hosta* had more variegation and a higher proportion of mutant plastids in spring and autumn than in summer.

DISCUSSION

A recent review by Gibor and Granick (5) presented evidence for presence of DNA and existence of a heritable system in plastids. They proposed a model which ascribes both nuclear and self regulatory control of plastid functions. This model allows mutations in either nucleus or plastid that would result in mutant plastids.

Variegated mutations under cytoplasmic control have been reported in many plant species. Maize (Anderson, 1; Demerec, 4), tobacco (Burk and Grosso, 2; Burk et al., 3), *Primula* Gregory, 6), sorghum (Karper, 13), *Dianthus* and *Euphorbia* (Stewart, 17), and *Hosta* (Yasui, 19) have been demonstrated to have maternally inherited plastid mutations causing variegated plants. Another type of maternally inherited variegated mutation has been reported in which the plastid mutation is initially induced by nuclear genes. Such types have been reported in barley and rice (Imai, 10, 11), maize (Rhoades, 15), and *Arabidopsis* (Röbbelen, 16). In the case of *Arabidopsis,* plastids mutate under the influence of the nuclear genes *am am,* and the self-duplicating ability of the plastids allows maternal transmission and maintenance of mutant types even in non-*am am* nuclear environments.

Much attention has been given to detection of mixed cells (heteroplastid) that give rise to variegated tissue and segregating progeny. Some investigators have found mixed cells only in very young tissue and not in mature tissue (Burk et al., 3; Gregory, 6; and Stewart, 17). Other workers have been able to distinguish mixed cells in mature tissue (Gustafsson, 7; Imai, 12; Röbbelen, 16; Woods and du Bury, 18; and Yasui, 19). In mixed cells of some organisms, or perhaps some mutations of an organism, individual plastid genotypic differences are expressed phenotypically and in other cases an apparent threshold exists at which the phenotype of all plastids is determined by the preponderant genotype.

Observations of the segregation patterns and development of variegated cotton plants led to the conclusion that mutant plants result from a plastid mutation and that mutant plastids, under a favorable environment, can multiply and compete with normal plastids to form mutant cells and tissues. Progenies segregating for albino, variegated, and green plants must have arisen from germinal tissue containing a mixed population of mutant and normal plastids. Cells must be able to maintain stable mixed populations of mutant and normal plastids. The high frequency with which the mutant cells appear in variegated plants indicates that distribution of plastids must not be completely random.

The variegated cotton plants reported by Horlacher and Killough (3), and the irradiation-induced variegated reported here were both from seed irradiation. I have not recovered variegated plants from irradiated cotton pollen (unpublished data). This fact suggests that variegated cotton plants may have resulted directly from plastic mutations without influence of nuclear changes.

Table 2. Segregation of the variegated plants in crosses of Texas Marker line (TM) with the abnormal palisade (AP) genetic marker line.

Cross	Segregation		
	Variegated	Green	Total
AP · TM-1 F_2	1	98	99
TM-1 · AP F_2		98	98
(TM-6 · AP) AP		67	67
AP(TM-6 · AP)	3	107	110
TM-5 · AP F_2		186	186

The genetic marker line abnormal palisades, which regularly produces a low frequency of variegated plants (Table 2), may represent a case in which a nuclear mutation causes mutant plastids.

The evidence presented in this study suggests that the plastid mutations are not under nuclear control. However, the data do not completely rule out this possibility. The data suggest that, if a nuclear mutation is controlling the plastid mutations, induction of plastid mutations is so low that large populations are needed to detect nuclear segregation. If techniques for detecting mixed cells in cotton could be developed, further study of the abnormal palisade line with larger populations would be justified and a decisive answer to the question of nuclear control might be forthcoming.

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