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Maternal inheritance of plant variegation in cowpea, *Vigna unguiculata* (L.) Walp.

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Abstract A chimeric plant was observed in the F_2 generation of a cross between a mutant cultivar, Ife BPC, and a germplasm line, TVu 2, in cowpea, *Vigna unguiculata* (L.) Walp. The chimeric plant had four lateral branches, one of which was intensely variegated, while the others were mostly green with few white sectors. F_3 progeny from the intensely variegated branch of this plant were all variegated, while seed derived from the mostly green branches produced only green progeny. In subsequent generations, the descendants of the variegated branch bred true for the variegated trait, while those of the mostly green branches were also true-breeding for green colour. No pure-green or pure-white shoots were observed in any of the variegated plants examined in this study. Consequently, no pure-green or pure-white seedlings were produced from seeds harvested from the variegated plants. The results of reciprocal crosses between variegated and normal green plants indicate that variegation is inherited in a strictly uniparental maternal fashion. This is the first report of a cytoplasmically inherited mutation affecting foliage colour in cowpea.

Keywords Chlorophyll · Maternal inheritance · Cowpea · *Vigna unguiculata* (L.) Walp.

Introduction

Variegation in the vegetative parts of plants is often caused by variation in chlorophyll content within the same plant tissue. Such a variation may result from mutations of nuclear genes or those of the chloroplast genome itself (Kirk and Tilney-Basset 1978). Variegation resulting from mutations of nuclear genes may be distinguished from that caused by mutations in the chloroplasts by the inheritance pattern of the trait and the behaviour of the chloroplasts themselves during cell division. Chloroplast mutations normally show non-Mendelian inheritance and exhibit maternal inheritance after reciprocal crosses (Michaelis 1966; Kirk and Tilney-Basset 1978; Palmer and Mascia 1980; Hatfield et al. 1985; Shoemaker et al. 1985; Cianzio and Palmer 1992). Kirk and Tilney-Basset (1978) described many examples of variegation caused by mutations in the plastid genomes of a large number of plant species. In legumes, changes in foliage colour resulting from chloroplast mutations have been reported in several species, including *Glycine max* (Palmer and Mascia 1980; Cianzio and Palmer 1992) and *Phaseolus vulgaris* (Lee and Sears 1987). Plastid inheritance in these species is generally characterized by uniparental maternal inheritance. However, Smith (1988) listed other leguminous species which exhibit regular biparental inheritance of plastids.

Fery (1985) and Fery and Singh (1997) in their reviews of the world literature on cowpea genetics listed many mutations affecting nuclear genes and their inheritance patterns, but none involving the genetic material of cytoplasmic organelles was included. In our cowpea breeding populations at the University of Ibadan, Nigeria, a single plant with a highly variegated stem branch was found in the F_2 generation of a cross. Seeds harvested from the mostly green branches produced only green progeny while plants raised from seeds of the highly variegated branch were all variegated. In this paper, I describe the characteristics of the mutant plant and its progeny, and report on the inheritance of the variegated trait.

Materials and methods

Origin and description of the chimeric variant

During the early planting season of 1985, I observed a chimeric plant with green/white variegation on the vegetative parts in the F_2 generation of the cowpea cross Ife PBC × TVu 2. Ife BPC is a mutant from a local cultivar, Ife Brown (Fawole and Afolabi, 1983), and has been released as a cultivar (Fawole et al. 1985). TVu 2 is a

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Table 1 Phenotypic proportions in the F_3, F_4 and F_5 generations obtained by self-pollination of the chimeric plant

Parent	F_3 generation	F_4 generation		F_5 generation	
	No. of plants and phenotype	No. of F_3 plants	No. of plants and phenotype	No. of F_3 plants	No. of plants and phenotype
F_2 chimeric plant, highly variegated branch	26 (variegated)	22	418 (variegated)	48	912 (variegated)
F_2 chimeric plant mostly green branches	42 (green)	31	651 (green)	46	1054 (green)

high yielding, upright, bunch-type breeding line (Ojomo 1973). The variegated F_2 plant had four branches, one of which had highly variegated leaves while the other branches were mostly green with few white sectors. The plant was transplanted into a plastic pot filled with sterilized garden soil and transferred to a glasshouse. Both the variegated and the mostly green branches had fertile flowers and produced pods normally. At maturity, pods from variegated and green branches were harvested separately. Harvested seeds from both types of branch were grown in pots in the glasshouse for observation. All F_3 plants obtained from seeds of the mostly green branches were all green while those that developed from seeds of the highly variegated branch were all variegated. Individual F_3 plants from both variegated and green progenies were harvested separately and were advanced to the F_4 generation. Selected variegated and green F_4 plants were also advanced to the F_5 generation. Observations were made on the phenotypic expression of the variegated mutant and the normal green trait. The variegated line was selfed for at least 14 generations before being used for the inheritance study.

Inheritance study

The variegated line was crossed reciprocally with three green (non-variegated) lines. The normal lines had leaf traits that are under the control of nuclear genes and different from those of the variegated line. This permitted the observation of the genetic behaviour of the marker genes and the variegated variant. The green lines used were IBS 2497, a non-branching, non-petiolate line, IBCR-1, a dark-green crinkled-leaf plant and IBS 4474, a seven-leaflet variant. The non-petiolate trait, the crinkled leaf and the multi-foliolate leaf are each controlled by single major genes (Fawole 1988, 1997, and unpublished data). In addition, IBS 2497 and IBCR-1 had purple pigmented vegetative parts, flowers and pods.

Reciprocal F_1 seeds were grown in the glasshouse to produce F_2 seeds. Backcrosses were also made reciprocally between the parental lines and the F_1 generation. The crosses were planted on the Teaching and Research Farm of the University of Ibadan during the planting seasons of 1993 and 1994. Observations were made on the general performance of the plants and, at 6 weeks after planting, individual plants in each cross were classified as either green or variegated. The segregation patterns of marker genes were also noted. At maturity, 90–100 plants from each F_2 generation and its reciprocal were randomly selected and harvested separately. These were advanced to the F_3 generation in 1994 and 1995 and were scored as either green or variegated.

The data obtained from the experiments were statistically analysed and the segregation patterns of marker genes were tested for a monohybrid genetic ratio by the chi-square test.

Results

The chimeric plant produced two types of progeny. Forty two plants derived from seeds harvested from the mostly green branches were all green while 26 plants that developed from seeds obtained from the variegated branch were all variegated (Table 1). Variegated plants were characterized by irregular patches of white sectors on the normally green vegetative plant parts (Fig. 1). The very young leaves had bright yellow patches which gradually turned white as the leaves developed and expanded. All the vegetative parts of the plants, the stems, leaves and peduncles, were variegated. The intensity of sectoring on plant parts varied within and between plants. The dorsal side of the standard petal and the pods also showed the

Fig. 1 A typical variegated cowpea, *Vigna unguiculata* (L) Walp., plant

Table 2 Phenotypes observed in the F_1 and backcross generations from reciprocal crosses of variegated and green lines

Plants and phenotypes[a]		F_1 generation		Backcross generation Parents and variegated green phenotypes			
Female	Male	Variegated	green	Female	Male	Variegated	green
Cross 1							
IBVAR-3 (variegated)	IBS2497 (green)	88	–	VAR F_1[b]	IBS2497	203	–
IBS2497	IBVAR-3	–	103	IBS2497	VARF_1	–	153
Cross 2							
IBVAR-3 (variegated)	IBCR-1 (green)	45	–	VAR F_1	IBCR-1	150	–
IBCR-1	IBVAR-3	–	86	IBCR-1	VARF_1	–	119
Cross 3							
IBVAR-3 (variegated)	IBS4474 (green)	53	–	VARF_1	IBS4474	279	–
IBS4474	IBVAR-3	–	83	IBS4474	VARF_1	–	145

[a] The green parents. IBS2497, IBCR-1 and IBS4474, carry the non-petiolate, crinkled leaf and multi-foliolate marker genes respectively; [b] Variegated F_1 generation

Table 3 Observed numbers of progeny in the F_2 and F_3 generations of crosses between the variegated and green cowpea lines

Parents and phenotype		F_2 generation				Total	F_3 generation					Total
		Variegated		Green			Number of F_2 plants	Variegated		Green		
Female	Male	Normal	Marker[a]	Normal	Marker			Normal	Marker	Normal	Marker	
Cross 1												
IBVAR-3 (variegated)	IBS2497 (green)	584	224	–	–	808	100	1401	427	–	–	1828
IBS2497	IBVAR-3	–	–	564	210	774	92	–	–	1402	442	1844
Cross 2												
IBVAR-3 (variegated)	IBCR-1 (green)	499	143	–	–	642	90	1037	313	–	–	1350
IBCR-1	IBVAR-3	–	–	674	204	878	96	–	–	1238	394	1632
Cross 3												
IBVCAR-3 (variegated)	IBS4474	669	219	–	–	888	98	1188	380	–	–	1568
IBS4474	IBVAR-3	–	–	628	227	855	94	–	–	1287	406	1693

[a] The leaf traits used as markers are the petiolate/non-petiolate leaf in cross 1, the normal/crinkled leaf in cross 2 and tri-foliolate/multi-foliolate leaf in cross 3

green/white variegation. The stigmatic surface of variegated plants was yellow instead of the normal light-green colour. Variegated plants were slower in growth than normal green plants. They flowered later, with a mean number of days from planting to first flower of 53.8 ± 2.22 days compared to a mean of 43.0 ± 1.51 days for the green plants, but many of them developed into vigorous plants and set pods abundantly.

In the F_4 and F_5 generations all progenies derived from variegated F_3 and F_4 plants were true-breeding for the variegated trait while those derived from green F_3 and F_4 plants were also true-breeding for green colour (Table 1).

Data on the inheritance of green/white variegation in three crosses are presented in Tables 2 and 3. All plants in the F_1, F_2 and backcross generations of crosses between the variegated plant as female parent and the green lines as male parent were all variegated. However, single-gene leaf markers such as the non-petiolate leaf, multi-foliate leaf and crinkled leaf which were introduced through the pollen segregated in the appropriate genetic ratios, thus confirming that the cross-pollinations were successful. In the reciprocal crosses between normal green lines as female parents and the variegated mutant plant as male, all plants of the F_1, F_2 and backcross generations had the green-foliage phenotype. The single-gene leaf markers also segregated in a monohybrid ratio, thus indicating successful cross-pollinations. These results suggest that cytoplasmic factors in the variegated parent are responsible for the mutant variegated phenotype.

In the F_3 generation, all progenies from selected F_2 plants of crosses involving the variegated line as female parent and normal green lines as male parents were true breeding for the variegated phenotype, while those involving the green lines as female parents and the variegated line as male parent bred true for green plant colour (Table 3). The F_3 data thus support the maternal inheritance of variegation in the crosses studied.

Discussion

The variegated mutant was obtained from a chimeric plant that arose spontaneously from the F_2 generation of the cowpea cross Ife BPC × TVu 2. The origin of the variegated trait seemed to be a plastid mutation early in the development of the F_2 plant that eventually produced a highly variegated branch while other branches remained mostly green. The mutation encompassed the reproductive tissue of the highly variegated branch thus permitting transmission of the trait to the progeny, whereas progeny obtained from seeds of the mostly green branches did not transmit the variegated trait and were all green in colour. The mutant is characterized phenotypically by green/white variegation on the vegetative and reproductive parts of the plant. Green/white variegation in plants is often caused by defective plastids lacking the ability to produce chlorophyll (Kirk and Tilney-Basset 1978).

A basic criterion for the proof of cytoplasmic inheritance is the occurrence of maternal inheritance after a reciprocal cross (Michaelis 1966; Kirk and Tilney-Basset 1978). The results of reciprocal crosses in this study demonstrated strictly uniparental-maternal inheritance of the variegated trait. In the crosses of variegated by normal green lines, single-gene markers segregated with a monohybrid genetic ratio. Both variegated and green F_1 plants were also used in the reciprocal backcrosses. Backcross progeny of the variegated F_1 as female parent and the green parental line as the pollen source were variegated, while only green progeny were produced in backcrosses involving green plants as the female parent and variegated plants as the male parents. In all of these crosses, the pollen source, either variegated or green plants, had no influence on the expression of the variegated phenotype.

Kirk and Tilmey-Basket (1978) listed many examples of variegation that are caused by mutations of plastid genes which exhibit distinct all-green, all-white and variegated sectors on the same plant. In such plants, flowers, born on completely green shoots produce all green progeny, those on white or yellow shoots produce only white or yellow progeny, while flowers borne on variegated branches produce green, white or yellow and variegated progenies. In contrast, no pure-green and pure-white shoots were produced in any of the variegated plants examined in this study. Consequently, no completely green and completely white progeny were produced by the variegated plants. However, variegated plants showed a wide variation in phenotypic expression from slight to intense variegation of the different plant parts.

Plants with green/white or green/yellow variegation occur fairly frequently in crosses involving Ife BPC as the female parent but, in most cases, the trait failed to be transmitted to the progeny. The variegated trait reported in this study encompassed the reproductive structures of the plant and was therefore transmitted to the progeny in successive generations. In the original cross from which the variegated mutant was found, Ife BPC was the maternal parent. This line is a mutant from the cultivar Ife Brown, a widely grown cultivar in South Western Nigeria, and has itself been released as a cultivar (Fawole and Afolabi 1983; Fawole et al. 1986). Ife BPC has been shown to facilitate the occurrence of high-frequency mutations at some nuclear loci when used as the female parent in some crosses (Fawole 1988, 1997). The present study indicates that mutations in cytoplasmic organelles may also occur in the descendants of crosses involving Ife BPC as the female parent.

Mutations that cause variegation in the green colour of plants are rare in cowpea. Fawole (1994) obtained two variegated mutants in the M_2 generation of a cowpea line after irradiation with gamma rays. Preliminary data indicate that the mutations are controlled by nuclear genes (I. Fawole, unpublished data). The variegated mutant reported in this study is probably the first plastid mutant to be identified and characterized genetically in cowpea.

Cytoplasmic mutants are valuable materials for the study of organelle genome functions and nuclear cytoplasmic interactions (Kirk and Tilney-Basset 1978; Krishna Rao and Koduru 1978; Mackenzie 1995). This cowpea cytoplasmic mutant may serve as material for a preliminary study of the cowpea chloroplast genome.

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