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An unstable gene controlling developmental variegation in okra (*Abelmoschus esculentus* (L.) Moench)

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Summary. Genetic studies on radiation-induced chlorina and variegated mutants of okra (*Abelmoschus esculentus* (L.) Moench) revealed the existence of an unstable gene. The normal green color of the leaves is controlled by duplicate genes C_1 and C_2, either of which produces the green colour. The chlorina plants are $c_1c_1c_2c_2$. The allele c_1^Y is dominant to both c_1 and c_2 but is unstable. The homozygote $c_1^Yc_1^Yc_2c_2$ is a normal green while the heterozygote $c_1^Yc_1c_2c_2$ has a variegated phenotype as a result of the mutation of c_1^Y to c_1 during development. In green plants with a $c_1^Yc_1^Yc_2c_2$ genotype, the autonomous mutation of one of the c_1^Y alleles to c_1 may take place at the pre-meiotic stage. In the variegated genotype ($c_1^Yc_1c_2c_2$), the mutation of c_1 to c_1^Y may take place in early ontogeny, thus producing green plants. The allele C_1, when associated with c_1^Y in a heterozygous condition, mutates to c_1 at the pre-meiotic stage even in the presence of the allele C_2.

Key words: Okra – *Abelmoschus esculentus* – Developmental variegation – Unstable genes – Paramutation

Introduction

Paramutation, an interaction between alleles that leads to directed, heritable changes at the locus, was first demonstrated by Brink (1954, 1956, 1958) at the *R* locus in maize.

Similar behaviour was observed at the *B* locus in maize (Coe 1959, 1966) and the *sulf* locus in tomato (Hagemann 1958, 1969; Ecochard 1972). In each case particular alleles (paramutagenic) of a gene cause heritable alterations in a sensitive allele (paramutable) in the direction of reduced function.

This paper describes the behaviour of an unstable locus controlling chlorophyll expression in okra (*Abelmoschus esculentus* (L.) Moench).

Materials and methods

Origin of chlorina and variegated leaf mutations

The chlorina mutation has yellowish green foliage and was isolated in an M_2 plant progeny following gamma irradiation (40 kR dose of ^{60}Co) of seeds of the okra variety 'Vaishali Vadhu'. Stem and petiole of the mutant had normal green colouration. The segregation patterns of normal green plants in M_3 and M_4 generation progenies indicated monogenic dominance of normal green leaf colour over the chlorina phenotype (Jambhale and Nerkar 1979).

In one of the M_4 progenies raised from a normal green M_3 plant in a line segregating for chlorina, three phenotypes – green, chlorina and variegated – were observed. The variegation of the leaf consisted of chlorophyll deficient yellowish green sectors of irregular shape and size scattered within the green tissue of the leaf lamina. The yellowish green sector resembled the colour of the chlorina mutant. The size of these sectors varied from that of a pinhead to about three-quarters of the leaf lamina. Variegation appeared on most of the subsequent leaves. Sectors persisted throughout the growth of the plant. Variegated and chlorina plants were as viable as normal green plants. Variegated, chlorina and green plants were selfed from the M_4 to M_8 generations and segregation patterns were recorded for the progenies. Variegated and chlorina plants (in the M_8 generation) were crossed. These were also crossed to the normal green cultivars: 'Pusa Sawani', 'Vaishali Vadhu', 'Red okra' and 'Pishor local'. Observations were recorded on the F_1 and F_2 generations.

Results

Segregation pattern in the selfed progenies

Segregation patterns in the selfed progenies are presented in Table 1. The M_4 progeny consisted of 5 normal green, 16 variegated and 10 chlorina plants. The segregation did not fit into Mendelian ratios, but the chlorina phenotype accounted for about 25% of the progeny. In the M_5 generation, chlorina plants bred true while the variegated plants segregated for green,

Table 1. Breeding behaviour of variegated, green and chlorina plants upon selfing

Generation and leaf phenotype	Assigned genotype	Breeding behaviour upon selfing			X^2 3 (Green + Variegated): 1 Chlorina
		Green	Variegated	Chlorina	
Cv. 'Vaishali Vadhu': green	$C_1C_1c_2c_2$	391	–	–	–
M_1: green (mutated)	$C_1c_1c_2c_2$	42	–	14	0.00
M_2: 10 green	$C_1C_1c_2c_2$	212	–	–	–
32 green	$C_1c_2c_2c_2$	584	–	180	0.85
3 chlorina	$c_1c_1c_2c_2$	–	–	48	–
M_3: 48 green	$C_1C_1c_2c_2$	612	–	–	–
116 green	$C_1c_1c_2c_2$	2,241	–	894	1.93
1 green (mutation at gametogenesis)	$C_1c_2c_2c_2 \rightarrow c_1^vc_1c_2c_2$	5	16	10	0.87
M_4: 10 variegated	$c_1^vc_1c_2c_2$	18	50	30	1.65
2 chlorina	$c_1c_1c_2c_2$	–	–	22	–
[a]M_5: 5 green	$c_1^vc_1^vc_2c_2$	118	35	–	–
17 variegated	$c_1^vc_1c_2c_2$	88	467	210	2.45
4 chlorina	$c_1c_1c_2c_2$	–	–	32	–
[a]M_6: 5 green	$c_1^vc_1^vc_2c_2$	102	47	–	–
8 variegated	$c_1^vc_1c_2c_2$	–	124	36	0.53
7 chlorina	$c_1c_1c_2c_2$	–	–	48	–
[a]M_7: 6 green	$c_1^vc_1^vc_2c_2$	118	3	–	–
9 variegated	$c_1^vc_1c_2c_2$	6	261	94	0.21
5 chlorina	$c_1c_1c_2c_2$	–	–	40	

[a] Selfed progeny of variegated plants

Table 2. Segregation pattern in the crosses of chlorina with variegated and normal cultivars

Sr. no.	Cross	Generation	Phenotype			X^2	Genotypes of parents
			Green	Variegated	Chlorina		
1.	Variegated × chlorina	F_1	–	23	21	0.09 (1:1)	$c_1^vc_1c_2c_2 \times c_1c_1c_2c_2$
2.	'Pusa Sawani' × chlorina	F_1	45	–	–		$C_1C_1c_2c_2 \times c_1c_1c_2c_2$
		F_2	85	–	36	1.43 (3:1)	
3.	'Vaishali Vadhu' × chlorina	F_1	30	–	–		$C_1C_1c_2c_2 \times c_1c_1c_2c_2$
		F_2	102	–	36	0.08 (3:1)	
4.	'Red Okra' × chlorina	F_1	32	–	–		$C_1C_1C_2C_2 \times c_1c_1c_2c_2$
		F_2	276	–	24	1.57 (15:1)	
5.	'Pishor local' × chlorina	F_1	20	–	–		$C_1C_1C_2C_2 \times c_1c_1c_2c_2$
		F_2	403	–	30	0.38 (15:1)	

variegated and chlorina phenotypes. The chlorina plants also bred true in subsequent generations while the variegated plants segregated in every selfed generation for the three phenotypes, chlorina accounting for about 25% of the population. In advanced generations, there was a deficiency of green plants and an excess of variegated plants in the progeny of variegated plants. In the M_6 to M_8 generation progenies, the green plants segregated for green and variegated phenotypes, the segregation being unusual.

Segregation pattern in the crosses involving chlorina

When chlorina was crossed with variegated, the F_1 segregated in the ratio of 1 variegated : 1 chlorina (Table 2). The F_2 populations of the crosses of chlorina with normal green cultivars segregated in the ratio of either 3 green : 1 chlorina or 15 green : 1 chlorina (Table 2).

Segregation pattern in the crosses involving variegated

The reciprocal F_1 hybrids between variegated and normal green cultivars were all green (Table 3). In the F_2 generation, the crosses 'Pusa Sawani' × variegated and 'Vaishali Vadhu' × variegated segregated in the ratio of 3 (green + variegated) : 1 chlorina. F_2 populations of the crosses 'Red Okra' × variegated and 'Pishor' × variegated segregated in the ratio of 15 (green + variegated) : 1 chlorina. The proportion of green : varie-

Table 3. Segregation pattern in the crosses of variegated plants with normal green cultivars

Sr. no.	Cross	Generation	Phenotype			X^2	Genotypes of parents
			Green	Variegated	Chlorina		
1.	'Pusa Sawani × variegated	F_1	45	–	–	–	$C_1C_1c_2c_2 \times c_1^vc_1c_2c_2$
		F_2	251	43	92	47.30 (2:1:1) 0.28 (3:1)[a]	
2.	Variegated × 'Pusa Sawani'	F_1	86	–	–	–	$c_1^vc_1c_2c_2 \times C_1C_1c_2c_2$
		F_2	263	53	103	45.02 (2:1:1) 0.03 (3:1)[a]	
3.	'Vaishali Vadhu' × variegated	F_1	22	–	–	–	$C_1C_1c_2c_2 \times c_1^vc_1c_2c_2$
		F_2	102	19	40	16.96 (2:1:1) 0.01 (3:1)[a]	
4.	Variegated × 'Vaishali Vadhu'	F_1	35	–	–	–	$c_1^vc_1c_2c_2 \times C_1C_1c_2c_2$
		F_2	210	48	90	25.03 (2:1:1) 0.14 (3:1)[a]	
5.	'Red okra' × variegated	F_1	53	–	–	–	$C_1C_1C_2C_2 \times c_1^vc_1c_2c_2$
		F_2	238	2	15	13.24 (14:1:1) 0.06 (15:1)[b]	
6.	Variegated × 'Red okra'	F_1	63	–	–	–	$c_1^vc_1c_2c_2 \times C_1C_1C_2C_2$
		F_2	435	16	31	7.06 (14:1:1) 0.03 (15:1)[b]	
7.	'Pishor local' × variegated	F_1	72	–	–	–	$C_1C_1C_2C_2 \times c_1^vc_1c_2c_2$
		F_2	237	–	16	16.92 (14:1:1) 0.01 (15:1)[b]	
8.	Variegated × 'Pishor local'	F_1	58	–	–	–	$c_1^vc_1c_2c_2 \times C_1C_1C_2C_2$
		F_2	451	10	33	15.07 (14:1:1) 0.15 (15:1)[b]	

[a] 3 (green + variegated) : 1 chlorina
[b] 15 (green + variegated) : 1 chlorina

gated plants did not conform to a mendelian ratio in any of the F_2 populations.

Discussion

Genotype of the chlorina mutation

In our previous studies (Jambhale and Nerkar 1979), the chlorina phenotype was determined to be controlled by a recessive gene (*cl*). This was confirmed in the present studies from the segregation pattern in the selfed progenies of the variegated plants and true breeding nature of the chlorina plants (Table 1). In the crosses of chlorina with normal green cultivars, the chlorina phenotype appeared in the F_2 either as a monogenic recessive or a digenic recessive trait (Table 2). Hence, normal chlorophyll production in the cultivars 'Pusa Sawani' and 'Vaishali Vadhu' is assigned to the C_1 (or C_2) gene and in the cultivars 'Red okra' and 'Pishor local' to the duplicate genes C_1 and C_2, either of which can produce normal green plants. Thus, the genotype of the chlorina mutation could be $c_1c_1c_2c_2$.

Genotype of the variegated phenotype and somatic mutation

The variegated phenotype first appeared in the variety 'Vaishali Badhu' ($C_1C_1c_2c_2$) as a segregant in the M_4 generation progeny of a green plant. It is presumed that this green plant (in M_3) was heterozygous and genotypically $C_1c_1c_2c_2$. It is further presumed that just before gametogenesis the allele C_1 mutated to c_1^v. Thus, the genotype of this plant at gametogenesis was $c_1^vc_1c_2c_2$, forming two types of gametes: $c_1^vc_2$ and c_1c_2. In the selfed progeny three genotypes would be produced: $c_1^vc_1^vc_2c_2$, $c_1^vc_1c_2c_2$ and $c_1c_1c_2c_2$. The former and latter homozygotes produced normal green and chlorina phenotypes, respectively, while the heterozygote produced a variegated phenotype. Due to the dominance of the allele c_1^v over c_1 and c_2 the heterozygote also is normally green. However, it is postulated that during the course of development of this heterozygote plant, somatic mutation of the allele c_1^v to c_1 occurs. The cell lines having this mutation would be genotypically $c_1c_1c_2c_2$, producing a chlorina phenotype in the back ground of the normal green tissue ($c_1^vc_1c_2c_2$). Thus, the variegated or mosaic leaf is produced in the heterozygote. In subsequent selfed progenies studied up to the M_8 generation the variegated plants always segregated for the three phenotypes, indicating that the appearance of heterozygotes is similar to the phenomenon of paramutation reported by previous workers in maize and tomato (Brink 1958; Coe 1959; Hagemann 1958). The allele c_1 is paramutagenic while the allele c_1^y is paramutable in the present case.

The green plants appearing in the progeny of variegated plants are genotypically $c_1^y c_1^y c_2 c_2$. These plants are normally not expected to segregate upon selfing. However, these green plants segregated for green and variegated phenotypes in their selfed progenies. It is presumed that during an early ontogenic stage of some plants there would be somatic mutation of one of the c_1^y alleles to c_1, thus producing heterozygote ($c_1^y c_1 c_2 c_2$). Such plants would exhibit the variegated phenotypes. Thus, the allele c_1^y is unstable and can undergo an autonomous change from c_1^y to c_1 during a very early ontogenic period of a plant.

Genetic behaviour of variegated in crosses with normal green cultivars

The crosses 'Pusa Sawani'× variegated and 'Vaishali Vadhu'× variegated. The cultivars 'Pusa Sawani' and 'Vaishali Vadhu' have one dominant gene for normal chlorophyll production ($C_1 C_1$). Their F_1 hybrids with variegated plants are expected to have two genotypes – $C_1 c_1^y c_2 c_2$ and $C_1 c_1 c_2 c_2$. In the F_2 generation, green variegated and chlorina phenotypes appeared, the chlorina accounting for 25% of the population (Table 3). This sort of segregation can be explained if it is presumed that in the F_1 generation the genotype $C_1 c_1^y c_2 c_2$ is converted into $c_1 c_1^y c_2 c_2$ at the premeiotic stage as a result of the mutation of the dominant allele C_1 to c_1. The allele c_1^y acts as if paramutagenic to C_1. After this paramutation, the F_1 population with the genotypes $C_1 c_1 c_2 c_2$ and $c_1 c_1^y c_2 c_2$ in equal frequency is expected to segreagate in the F_2 in the proportion 2 green : 1 variegated : 1 chlorina. However, in the observed segregation there was a deficit of variegated plants (Table 3). This could happen if there is a somatic mutation of the allele c_1 to c_1^y in the early ontogony of the variegated ($c_1 c_1^y c_2 c_2$) plants. With mutation the plants would be $c_1^y c_1^y c_2 c_2$ and produce the green phenotype.

The cross 'Red Okra'× variegated and 'Pishor'× variegated. The cultivars 'Red okra' and 'Pishor' have two dominant genes for normal chlorophyll production ($C_1 C_1 C_2 C_2$). Their F_1 hybrids with the variegated plants will be of two genotypes – $C_1 c_1^y C_2 c_2$ and $C_1 c_1 C_2 c_2$. The F_2 population segregated for green, variegated and chlorina phenotypes, the chlorina accounting for 1/16 of the population. This segregation pattern can also be explained by the presumptions described above, that is, conversion of the F_1 genotype $C_1 c_1^y C_2 c_2$ into $c_1 c_1^y C_2 c_2$ at the meiotic stage. With this paramutation, the F_2 population is expected to segregate in the ratio of 14 green : 1 variegated : 1 chlorina. However, the observed frequency of the variegated phenotype was very low compared to the green plants (Table 3). This could be due to the mutation of the allele c_1 to c_1^y in the very early developmental stage of the variegated plants, as described earlier.

In conclusion, the findings of the present investigation can be summarised as follows:

1. The normal green colour of the okra leaf is controlled by the dominant duplicate alleles C_1 and C_2, either of which is capable of producing the normal phenotype.
2. The chlorina phenotype is produced by the recessive state at both loci: the chlorina condition is $c_1 c_1 c_2 c_2$.
3. The allele c_1^y is dominant to c_1 and c_2, but is unstable. The homozygote $c_1^y c_1^y c_2 c_2$ produces a normal green phenotype. The heterozygote $c_1 c_1^y c_2 c_2$ produces a variegated phenotype as a result of the mutation of c_1^y to c_1 during development. In the mosaic tissue the normal green colour is due to $c_1 c_1^y c_2 c_2$ and the chlorina colour is due to $c_1 c_1 c_2 c_2$ (c_1 paramutagenic to c_1^y).
4. In the green plants of the $c_1^y c_1^y c_2 c_2$ genotype, the autonomous mutation of one of the c_1^y alleles to c_1 occurs at the pre-meiotic stage.
5. In some of the variegated genotypes ($c_1 c_1^y c_2 c_2$), mutation of c_1 to c_1^y occurs in early ontogeny, thus producing a green plant.
6. The allele C_1, when associated with c_1^y in a heterozygous condition, is mutated to c_1 at the pre-meiotic stage even in the presence of the allele C_2.

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