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REPORT OF AN UNSTABLE GENE SYSTEM IN *BRASSICA JUNCEA* L. COSS & CZERN VAR. RLM 198

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ABSTRACT

An anthocyanin containing mutant was induced by 0.5% EMS treatment in *Brassica juncea* L. Coss & Czern. var. RLM 198 in M_1 generation. This mutant had purple coloured anthocyanin sectors on green leaves giving the plant a variegated phenotype. The mutant phenotype appears to be under the control of an unstable gene system or a transposable element system as evident by lack of consistent genetic segregation pattern in subsequent generations, somatic variegation, appearance of new allelic forms, stable green revertants and putative germinal revertants. Usefulness of these mutants in gene cloning strategies and in elucidating the role of anthocyanin pigment as U.V. protectants is discussed.

Keywords : *Brassica juncea*, unstable gene system, anthocyanin pigment, EMS induced mutant.

Since the landmark discovery of an unstable gene system in maize by McClintock [1] transposable element systems have been reported in several plants such as *Antirrhinum majus* [2] and *Arachis hypogea* [3]. Quite often the presence of somatic variegation which is a characteristic of unstable gene systems has led to their identification. It is now believed that transposable elements exist widely in nature in silent or cryptic form and can be activated by either genomic or environmental stress [4-5]. We here report the presence of an unstable gene system in *Brassica juncea* L. Coss & Czern. var. RLM 198 which was possibly activated by EMS treatment. These mutants had purple coloured anthocyanin sectors on green leaves. In some mutant lines leaves were almost completely covered by anthocyanin pigment. Such a system can be very useful in elucidating the physiological role of anthocyanin pigments as protectants against U.V. radiations [6-8]. Besides, these mutants can be potentially useful in gene cloning, as transposon tagging has emerged as a powerful tool in cloning plant genes that can not be cloned by traditional methods [9-10].

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MATERIALS AND METHODS

Brassica Juncea var. RLM 198 was used for this study. *EMS treatment* : Seeds were surface sterilized and soaked in water for 12 hours. 2000 seeds each were treated with 0.3%, 0.4% and 0.5% Ethyl methane sulfonate (EMS) for 6 hours [11]. The treated seeds were washed in running water and then planted in the fields of Water Technology Centre, Indian Agricultural Research Institute, New Delhi, India.

Screening for mutants : The plant population so obtained was screened for any visual mutation. One plant with purple coloured anthocyanin sectors on green leaves was obtained in M_1 generation. This variegated plant was selfed and the progeny was studied for the mutant phenotype as well as any new allelic form (s) appearing spontaneously. The frequency of plants showing variegated phenotype was determined in M_2, M_3 and M_4 generations.

Classification of variegated mutants : On the basis of the extent of individual leaf area containing anthocyanin pigment, plants were assigned a visual score of 0 to 5 (Table 1). The variegated mutants were visually classified further on the basis of intensity of anthocyanin pigment in the coloured sectors as normal, pale derivatives and pale mottled derivatives (Table 2). Pale derivatives and pale mottled derivatives were the two new allelic forms observed in the mutant population.

Table 1. Visual score of mutants on the basis of extent of individual leaf area covered with anthocyanin pigment.

Score	Phenotype	
0	Green leaves	
1	Only edges of leaf contain anthocyanin pigment	
2	Approx. 25% of individual leaf area contains anthocyanin pigment	
3	Approx. 50% of individual leaf area contains anthocyanin pigment	Plate 1
4	Approx. 75% of individual leaf area contains anthocyanin pigment	
5	More than 75% of individual leaf area contains anthocyanin pigment	

Table 2. Visual classification of the mutants on the basis of intensity of anthocyanin pigmentation in variegated leaves.

Visual Phenotype	Description	
Normal	Leaves had dark intense purple anthocyanin sectors	
Pale derivative	Leaves had pale coloured anthocyanin sectors	
Pale mottled derivative	Leaves had intense purple anthocyanin sectors superimposed on a pale coloured anthocyanin background	Plate 2

Genetics of variegated phenotype : Four variegated mutants in M_2 generation were taken as female parents and crossed with normal wild type taken as pollen parent. The crosses were designated as cross I, II, III and IV and were subsequently studied for variegated phenotype or any new allelic form arising spontaneously.

RESULTS AND DISCUSSION

0.5% EMS treatment induced an anthocyanin containing mutant in M_1 generation. This plant had purple colored anthocyanin sectors on green leaves (Figs. 1 & 2). The mutant was selfed and M_2, M_3 and M_4 generations were raised.

Fig. 1. Photograph shows anthocyanin containing mutant (Acy) with anthocyanin sectors in leaves.

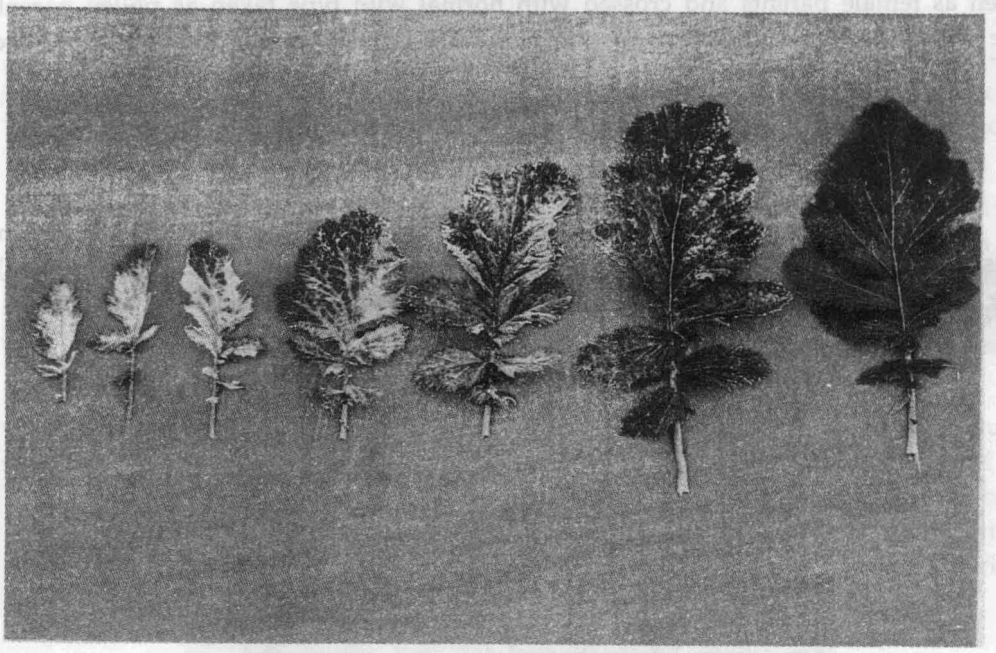

Fig. 2. Spontaneous new allelic forms in Acy mutants. Photographs shows pale and pale mottled derivatives alongwith control at extreme right.

Out of 110 plants obtained in M_2 generation, 47 had normal green leaves and 63 were variegated. This suggested a segregation ratio of 9 variegated : 7 green (χ^2 = 0.27 with 1 d.f.) and therefore involvement of two genes in complementary interaction. However, subsequently in M_3 and M_4 generations inconsistent genetic segregation pattern was observed.

When plants were classified on the basis of the extent of individual leaf area containing anthocyanin pigment, approximately 43% of plants had score 0, 1.8% had score 1, 19.1% had score 2, 17.3% had score 3, 14.6% had score 4 and 4.3% had score 5. When classified on the basis of the intensity of anthocyanin pigmentation out of 63 variegated plants 1 had pale phenotype while rest 62 had normal phenotype.

Out of 63 variegated mutants obtained in M_2 generation, only 8 M_3 progeny rows could be raised. The segregation for leaf phenotype in M_3 is shown in Table 3.

Table 3. M_3 families segregation for leaf phenotype in Acy mutants of *Brassica juncea* var. RLM 198

Families or lines	I	II	III	IV	V	VI	VII	VIII
Leaf phenotype	Frequency of plants							
Variegated	20	32	15	10	10	25	10	9
Green	15	30	25	30	10	13	16	20
Total	35	62	40	40	20	38	26	29

In M_4 generation, out of 192 plants, 190 had variegated mutant phenotype in line I and all 89 plants had variegated phenotype in line II. In line III, 84 out of 105 plants, in line IV, 42 out of 68 plants, in line V, 38 out of 100 plants, in line VI, 347 out of 557 plants, in line VII, 121 out of 151 plants and in line VIII, 299 out of 304 plants had variegated phenotype (Table 4).

Table 4. Extent of variegation in Acy mutants of *Brassica juncea* var. RLM 198

Phenotype	Score	Frequency of plants									
		M_2 generation	M_4 families								M_4 Total
			I	II	III	IV	V	VI	VII	VIII	
Green	0	27	2	-	21	26	62	210	30	5	356
Only edges contain anthocyanin	1	1	1	5	1	-	-	-	2	1	10
very light mottled	2	12	5	9	12	1	8	27	27	10	99
light mottled	3	11	13	29	47	6	8	67	42	49	261
medium mottled	4	9	118	41	24	22	11	196	44	164	620
heavy mottled	5	3	53	5	-	13	11	57	6	75	220
Total		63	192	89	105	68	100	557	151	304	1566

Germination is epigel in brassicas and the cotyledons become green and act as functional leaves until the emergence of true leaves. However, in the M_4 mutant lines, out of a total of 1566 plants, cotyledonary leaf itself had anthocyanin pigmentation in 30 young seedlings while 1180 young seedlings had mottled green cotyledons and remaining 356 had green cotyledons. The subsequently formed leaves however had variegated phenotype and anthocyanin was confined to sectors on green tissues. In the variegated mutants the extent of individual leaf area covered by anthocyanin pigment as well as the intensity of pigment was uniform in leaves at all nodes.

In each generation several plants with totally green leaves (same as wild phenotype) were obtained. These plants had stable green phenotype and did not revert to mutant phenotype in subsequent generations.

Genetics of variegated phenotype : In cross I plants having normal green leaves in F_1 generation showed stable green phenotype in F_2 also. In cross II, of the 279 plants scored 168 had variegated and 111 had green leaves giving a segregation ratio of 9 variegated : 7 green in F_2 (χ^2 = 1.88 with 1 d.f.) In cross III out of 121 F_2 plants, 70 plants had variegated phenotype and 51 had green leaves (χ^2 = 0.52 with 1 d.f.) In cross IV all 120 plants obtained in F_2 generation had variegated phenotype (Table 5).

Table 5. Extent of leaf variegation in F_2 progeny lines of cross Acy x Green leaf of *Brassica juncea* var. RLM 198

	Progeny lines	I	II	III	IV
Phenotypes	Score		Frequency of plants		
Green	0	200	111	51	-
Only edges contain anthocyanin	1	-	13	4	10
Very light mottled	2	-	71	21	23
light mottled	3	-	67	18	46
medium mottled	4	-	16	16	30
heavy mottled	5	-	1	11	11
Total		200	279	121	120

F_2 population was also visually scored for the extent and intensity of anthocyanin pigmentation and new allelic forms of leaf variegation. In cross II out of 279 plants scored, 1 was pale derivative. In cross III out of 121 plants scored 10 had pale and 1 had pale mottled phenotype.

These studies conducted over four generations of selfed mutants and F_1 and F_2 generations of crosses indicate the involvement of an unstable gene system. This hypothesis is further supported by following observations : (i) presence of somatic variegation (ii) variable frequency and pattern of anthocyanin sectors in population (iii) appearance of stable green revertants and (iv) new allelic forms. A silent or cryptic transposon can be activated by stress such as a mutagenic agent like ethyl methane sulfonate [4]. As long as the element remains inserted, leaves remain green.

The transposition of the elements away from the gene in either somatic or germinal tissues can restore its full activity [12] and anthycanin can be produced. The timing and exicision of the elements in somatic tissues during leaf development determines the extent of variegation or the spatial patterns observed [13]. Earlier the excision occurs during somatic development of leaf, larger will be coloured sector observed. In Brassica mutants with a score 4 or 5, the excision might have occurred much earlier during somatic development as compared to that of mutants with score 1 or 2. The quantitative variation in gene expression (such as intensity of anthocyanin pigment) has been reported to be generated due to imprecise excision of the transposable elements [8]. In *Antirrhinum majus* an unstable allele 'Pal 42' has irregularly distributed pale and dark spots of pigmentation. This mechanism may possibly be involved in generation of new allelic forms such as 'pale- mottled derivatives' with dark anthocyanin sectors superimposed on pale anthocyanian secotrs in Brassica also.

In about 2% of the M_4 mutant seedlings anthocyanin pigment was present on the cotyledonary leaves and (plate 3) subsequently formed true leaves were variegated in appearance. In rest of the seedlings cotyledonary leaves were green. Transient accumulation of flavonoid pigments has been reported in germinating seedlings in many plants [14], however the function of the pigment in seedlings remains unknown. The mutants with anthocyanin pigment in cotyledonary leaf can arise if exscision and subsequent loss of transposable elements occurred at the time of embryo development in the cell lines destined to form cotyledons (putative germinal revertants) or if more than one tissue specific regulatory gene is involved in anthycanin biosynthesis as in maize [15].

Further molecular proof awaits but phenotpic and genetic studies suggest the involvement of an unstable gene system or transposable element in *Brassica juncea* L. Coss & Czern. var. RLM 198. Since in some of the mutant lines leaves were completely covered with anthocyanin pigment such a system can be very useful in elucidating the role of anthocyanin pigment as protectants against U.V. radiation.

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