

Figures 1 and 2. 1. Metaphase I showing 11 II + 2 I ( $\times 2500$ ); 2. Diakinesis showing 8 IV + 2 III + 5 II ( $\times 1600$ ).

formation in premeiotic mitosis in some percentage of sporogenous cells. This leads to the formation of cells with two ploidy levels ( $2n, 4n$ ) which either undergo number of regular mitotic divisions prior to meiosis or directly act as pollen mother cells. The present report of spontaneous mixoploid in *capsicum* is of immense importance as it is capable of generating triploids and tetraploids in subsequent generations which in turn are valuable tools in cytogenetical research.

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## PLASTID MUTATIONS INDUCED IN RED PEPPER BY NITROSOMETHYL UREA

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EARLIER reports have shown that chlorophyll chimeras can readily be induced by the alkylating agent, ethylmethane sulphonate<sup>1-3</sup>. A high percentage of plastome mutations was obtained by Hosticka and Hanson<sup>4</sup> and Hagemann<sup>5</sup> using nitrosomethyl urea (NMU). Hagemann<sup>5-7</sup> described NMU as a potent mutagen for obtaining plastid mutations, an observation that is yet to receive much exploitation. This report describes the induction of a high frequency of chlorophyll chimeras in chilli by using mutagen NMU. Data are also presented on the inheritance of viable chlorina mutant.

The seeds of *Capsicum annum*, cv. Calwonder (CW) were soaked in distilled water for 24 h. Imbibed seed lots were incubated in 0.1 and 0.2% NMU (Sigma) several times (8, 16 and 24 h) or in distilled water as a control. Following the mutagen treatment, seeds were rinsed thoroughly in distilled water and sown in vermiculite in plastic pots which were subirrigated twice daily with a 1.2 g/l solution of Hyponex (7-6-19 by N:P:K: analysis, Hydroponics Chemical Co., Copley, OH) and grown in a controlled chamber under a 16-h-light/8-h-dark photoperiod (9:1 energy mixture of fluorescent and incandescent light at  $17.6 \text{ W. m}^{-2}$ ) at  $24^\circ\text{C}$ . Control and treated seeds were allowed to germinate in petri plates on moist filter paper underlined with cotton and germination counts were recorded on the 15th day. Numbers of chlorophyll chimeras and viable chlorina plants were determined after the first true leaves appeared.

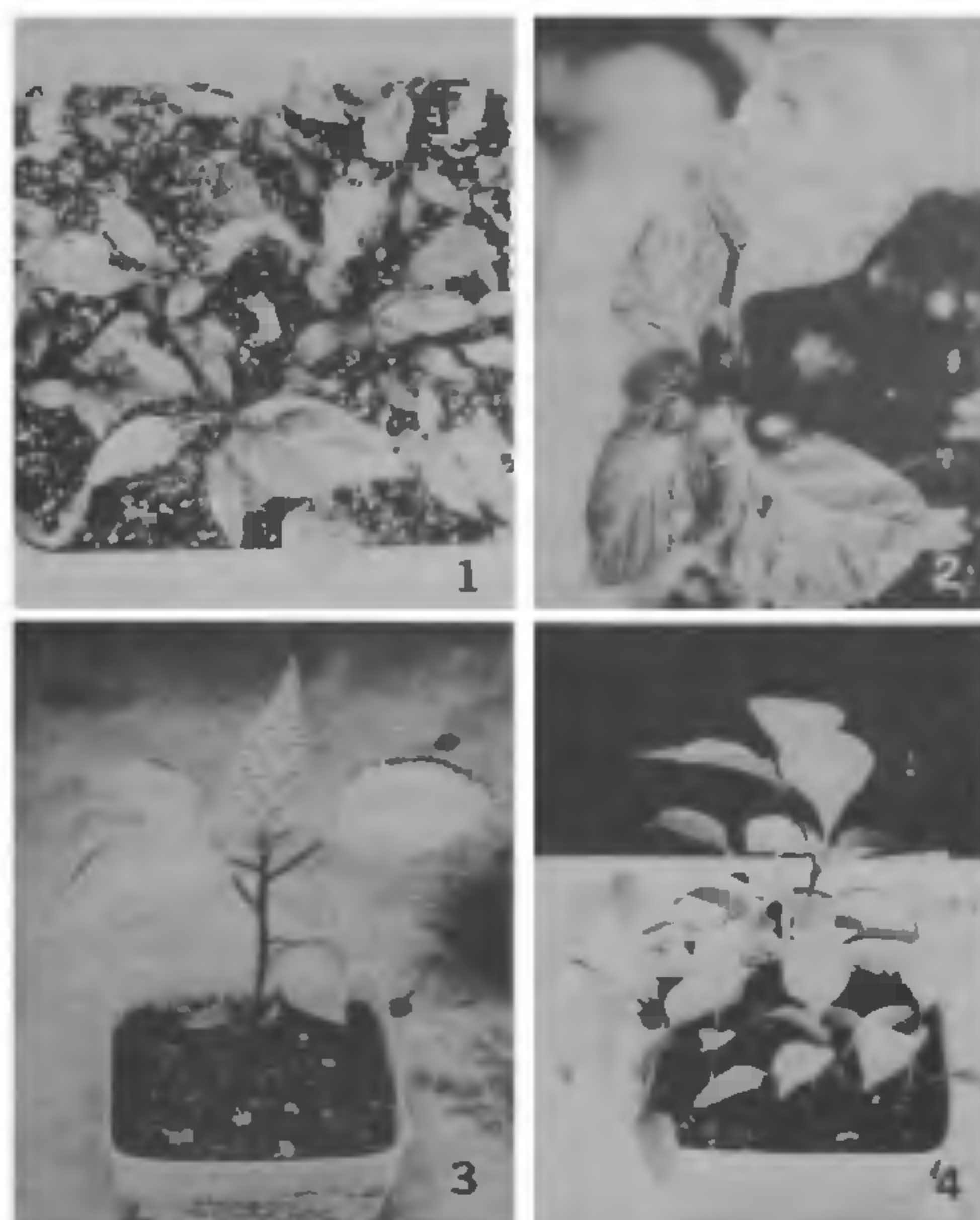
Preliminary experiments revealed that NMU treatment caused much effect on the induction of chlorophyll chimeras. Variegation rates as high as 81% were obtained when the seeds were treated with 0.1% NMU for 24 h. NMU treatment at 0.2% was found to be lethal. The per cent seed germina-

**Table 1** Phenotypes of plants in  $M_1$  generation grown from NMU-mutagenized *C. annuum*, cv. CW seed

NMU Conc. (%)	Characteristics	NMU treatment (h)			
		0	8	16	24
0.1	Germination (%)	90	72.10	59.80	32.50
	Variegated (%)	—	58.00	76.00	81.00
	Chlorina (%)	—	—	0.92	—

tion decreased as the duration of NMU treatment increased and it was delayed considerably. The growth was very slow and some of them did not grow at all. Some seedlings showed aberrations like bifurcation of main root and adventitious type of roots (2–5). It is interesting to note that 0.1% NMU induced a high percentage of chimeric plants in  $M_1$  generation and there was a concomitant increase in the percentage of chimeras as the length of treatment increased (table 1). Plants were scored as variegated if even one leaf exhibited the variegation pattern. Chimeric plants were of two types, white-green and yellow-green (most predominant) variegated. The pattern of variegation was so different from plant to plant and mostly the variegation was irregular in distribution (figures 1 and 2). In addition, the plants with variegated leaves also exhibited certain other anomalies like alteration of leaf shape (upturned and crinkled leaves) and texture (thick and leathery). A marked decrease in growth rate was also noticed. Chimeric plants were stunted and most of them (over 50%) did not set any fruits. It is surprising that no other types of chlorophyll mutations such as albina, xantha, viridis, striata, etc. were observed. Other leaf abnormalities also recovered in the  $M_1$  population, which includes unifoliate, tri- and tetrafoliate, adnate, palmate like, bifurcation and irregular leaf apices.

Besides, some viable chlorina plants (figure 3) were also screened in the  $M_1$  generation (table 1), which had light yellowish green leaves. These plants were grown to the flowering and set few fruits. Furthermore, these plants exhibited a greater reduction in height, number of branches, diameter of the fruit and yield. The growth rate was also slow. They bred true in further generation (figure 4). To further elucidate the breeding behaviour, these plants were crossed reciprocally, which indicated that the mutant phenotype was governed by a single recessive gene (the  $F_2$  progeny comprised of 70 normal and 20 chlorina mutants which closely fitted 3:1 ratio,  $\chi^2 = 0.371$ ;  $P = 0.70-0.50$ ).



**Figures 1–4.** 1. White and yellow sectorial chlorophyll chimeric seedlings obtained from 0.1% NMU treatment for 16 h; 2. Close-up of the chimeric seedling following transfer onto plastic pot; 3. Viable chlorina mutant, and 4. True breeding nature of the viable chlorina mutant in  $M_2$  generation.

NMU treatments of *C. annuum* seed greatly increase the number of variegated plants in  $M_1$  generation. Similar observations were also made in tomato<sup>4</sup>. NMU has also been reported to be highly mutagenic for plastid DNAs of *Helianthus*, *Saint-paulia* and *Antirrhinum*<sup>7</sup>. Information about NMU mutagenesis in other genera is scanty and merits a thorough investigation. The question as to why NMU is particularly effective for induction of  $M_1$  generation *Lycopersicon*<sup>4</sup> and *C. annuum* plastid mutations in comparison to other mutagens<sup>2,8-11</sup> is open to speculation.

The present results suggest that NMU is a useful mutagen in many species where chlorophyll mutations are required for physiological and genetic work<sup>1</sup> and also provide an effective method to produce agronomically useful mutants, particularly with alterations in photosynthetic properties, antibiotic resistance, or herbicide tolerance<sup>4</sup>.

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# INHERITANCE OF SMALL LEAF AND CRINKLED LEAF NATURE IN BLACKGRAM [*VIGNA MUNGO* (L.) HEPPEL]

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In blackgram cultivars, normally the trifoliate leaf consists of three leaflets varying from obovate to linear in shape and fairly large in size. In the germ-plasm of blackgram preserved at this School, two distinct mutant phenotypes are maintained, one characterized by crinkled leaflets instead of leaflets with normal appearance and the other is distinct with small size of leaflets. Both the genotypes are small in plant stature and are true breeding. Both the types have been isolated as mutants. In this study, it was designed to understand the nature of inheritance of the two distinct leaf phenotypes.

In Rabi 1984-85, direct and reciprocal crosses were made between crinkled leaf type with cultivar, TMV.1 blackgram. Utilizing small leaf type, three sets of direct and reciprocal crosses namely, TMV.1 × small leaf mutant; CO.5 × small leaf mutant and CO.3 × small leaf mutant were made. Their F<sub>2</sub>'s were studied during summer 1986 for segregation of leaf characters.

In all the crosses, the hybrids were normal in leaf shape and size. In F<sub>2</sub> generation, segregation was noticed for normal and small or crinkled leaf type.

**Crinkled leaf:** The segregation for normal and crinkled leaf types in F<sub>2</sub> of direct and reciprocal crosses involving crinkled leaf with cultivar TMV.1 is given in table 1.

**Small leaf:** In three sets of direct and reciprocal crosses TMV.1 × small leaf mutant, CO.5 × small leaf mutant and CO.3 × small leaf mutant, segregation was noticed for normal and small leaf in F<sub>2</sub> generation (table 2).

Table 1 Segregation for normal and crinkled leaf phenotypes

Parentage	Number of segregants for		Segregation ratio	$\chi^2$
	Normal leaf	Crinkled leaf		
Crinkled leaf × TMV.1	50	13	13:3	0.150 <sup>NS</sup>
TMV.1 × crinkled leaf	43	6	13:3	1.370 <sup>NS</sup>

NS-Non significant.

Table 2 Segregation for normal and small leaf phenotypes

Cross	Number of segregants for		Segregation ratio	$\chi^2$
	Normal leaf	Small leaf		
TMV.1 × small leaf	20	3	13:3	0.4830 <sup>NS</sup>
Small leaf × TMV.1	116	26	13:3	0.0167 <sup>NS</sup>
CO.5 × small leaf	83	29	13:3	3.7480 <sup>NS</sup>
Small leaf × CO.5	35	10	13:3	0.3748 <sup>NS</sup>
CO.3 × small leaf	137	55	3:1	1.3610 <sup>NS</sup>
Small leaf × CO.3	83	34	3:1	1.0280 <sup>NS</sup>

NS-Non significant.