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Induction and inheritance of a variegated leaf and an apical chlorosis mutant in chickpea (*Cicer arietinum* L.)

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Abstract

Several mutants were induced in the chickpea (*Cicer arietinum* L.) cultivar JG 315 following seed treatment with ethyl methane sulphonate (EMS). A variegated leaf mutant (named Jawahar Gram Mutant 3 or JGM 3) was isolated in the M_2 of 0.4% EMS treatment for 6 h. All leaflets of the variegated leaf mutant showed a large single white lesion on the foliage blade at both sides of the midrib. Each lesion was associated with a constriction at the leaflet margin. Another mutant with apical chlorosis (named JGM 6) was isolated in the M_2 of 0.6% EMS treatment for 6 h. In this mutant the young foliage was always yellow at the growing apex while lower leaves developed chlorophyll and turned light green as they grow older. Foliar spray of 0.5% $FeSO_4$ had no effect on the chlorotic foliage although it corrected soil-borne chlorosis in the accession ICC 4992. This shows that the chlorosis in JGM 6 was not a result of iron deficiency. Both mutations were found to be recessive. The genes controlling the variegated leaf and the apical chlorosis traits are assigned symbols *var* and *ach*, respectively. Variegated simple leaf segregants were recovered from the F_2 of the cross JGM 3 × ICC 10301 (simple leaf) which have ornamental appearance.

Key words: Chickpea, apical chlorosis, induced mutation, inheritance, leaf variegation, ornamental chickpea.

Introduction

Induced mutagenesis has played an important role in making available additional genetic variability for genetic improvement of chickpea (*Cicer arietinum* L.). Among various mutants induced, the agronomically useful mutants include those for high yield [1-4]; resistance to ascochyta blight [3-5], fusarium wilt and root rots [1, 3], nematodes [6], stunt [1, 3], and leaf miner [5]; high protein content [2, 10]; early maturity [1, 2, 4, 5, 10]; root nodulation [7]; erect plant type [1, 8, 10]; determinate growth [9]; and compact growth (PM Gaur, unpublished results). Some of these mutants have led to the development of commercial varieties, e.g. Pusa 408 (Ajay), Pusa 413 (Atul), Pusa 417 (Girnar), RS 11, RSG 2 (Kiran), and WSG 2 in India [10, 11]; CM 72, CM 88, CM 98, and CM 2000 in Pakistan [4, www.niab.org.pk]; and Hyprosola in Bangladesh [12].

In addition, there are many other induced mutants that may have specific uses and can be used in genetic studies. For example, Pundir and Reddy [13] reported a glabrous mutant that was highly susceptible to black aphid (*Aphis craccivora* Koch.) and may be used for raring of this insect. Further study of this mutant led to the identification of a linked gene for leaf necrosis [14] and mapping of these linked loci to a linkage group using isozyme markers [15].

The primary objective of the present study was to induce genetic variability in a well-adapted chickpea variety through chemical mutagenesis. Among several mutations for morphological traits identified, six were stable and their inheritance was studied. They include mutations for compact growth habit (Jawahar Gram Mutant 1 or JGM 1), stem fasciation (JGM 2), variegated leaf (JGM 3), broad-few-leaflets (JGM 4), outwardly curved wings (JGM 5) and apical chlorosis (JGM 6). Three of these mutants, JGM 2, JGM 4 and JGM 5, were described earlier [16, 17] and registered with the National Bureau of Plant Genetic Resources (NBPGR), New Delhi under the INGR numbers 03061, 03062 and 03063, respectively. This paper describes two mutants JGM 3 and JGM 6. The remaining mutant JGM 1 is being studied for allelic relationships with a similar spontaneous mutant known in chickpea.

Materials and methods

Seeds of the chickpea cultivar JG 315 were treated with ethyl methane sulphonate (EMS). JG 315 is a wilt-resistant high yielding cultivar of *desi* chickpea grown widely in central India. Seeds, presoaked in distilled water for 2 h, were treated with EMS at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 % concentrations for 6 and 8 h. The details of the experiment were reported earlier [16, 17].

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The mutations with variegated leaves (JGM 3) and apical chlorosis (JGM 6) were identified in the M_2 of 0.4% - 6 h and 0.6% - 6 h treatments, respectively. JGM 3 was crossed with ICC 4929 (double-pods, white flower with pink-veins), ICC 5325 (light- green foliage), ICC 10301 (simple leaf), ICC 12450 (blue flower), and ICC 15566 (glabrous shoot, leaf necrosis). The mutant JGM 6 was crossed with ICC 5316 (multipinnate leaves, white flower, pea-shaped seed, and light brown seed coat) to study the inheritance of leaf variegation and apical chlorosis and determine their linkage with other morphological genes. The F_1 and F_2 populations were raised in field with standard cultural practices. Observations were recorded visually on each individual F_2 plant. Inheritance and linkage analyses were carried out using the computer program LINKAGE-1 [18].

The effect of foliar spray of $FeSO_4$ was evaluated on the mutant JGM 6 and the germplasm line ICC 4992, which is known to be susceptible to iron deficiency leading to chlorosis. They were sown side-by-side in two-row plots each and irrigated frequently to induce iron deficiency symptoms in ICC 4992. One row in each genotype was sprayed with 0.5 % $FeSO_4$ 30 days after sowing and visual observation on foliage colour was recorded a week there after.

Results and discussion

Variegated leaf mutant (JGM 8): This mutant was characterized by the presence of a single large white lesion on the foliage blade at both sides of the midrib in all leaflets (Fig. 1). The white lesion was always accompanied by a constriction at the leaflet margin. The plants were partially sterile and matured about 10 days later than the parental variety.

The variegated mutant was crossed to five marker lines. In each of the five crosses the F_1s had normal leaves and the F_2 gave a good fit to a 3:1 ratio for normal and variegated plants (Table 1), suggesting that leaf variegation is caused by a single recessive gene. Gene symbol *var* is proposed for this gene. The joint segregation of the variegated leaf did not reveal linkage with double-podding (JGM 3 × ICC 4929), white flower with pink veins (JGM 3 × ICC 4929), light green foliage (JGM 3 × ICC 5325), simple leaf (JGM 3 × ICC 10301, ICC 10301 × JGM 3), blue flower (JGM 3 × ICC 12450), glabrous shoot (JGM 3 × ICC 15566), and leaf necrosis (JGM 3 × ICC 15566) traits. The constriction of leaflets always expressed in segregants with variegated leaf.

Many morphological mutations of chickpea drastically change the appearance of the chickpea plant. Two such mutations are with simple and multipinnate leaves. When the multipinnate leaf trait is combined with stem-fasciation, the chickpea plants look very attractive and may have ornamental value [16]. Another combination of two recessive traits, simple leaf and leaf variegation, with possible ornamental use was obtained in the F_2 of the cross JGM 3 × ICC 10301. The large area provided by the simple leaf phenotype enhanced the expression of leaf variegation (Fig. 2). Selection was made for high fertility and high level of expression of leaf-variegation in the succeeding generations. Several advanced breeding lines were established that produced higher number of pods per plant than both the parents (JGM 3 and ICC 10301).

Apical chlorosis mutant (JGM 6): The apical foliage of this mutant is always yellow and the foliage turns light green as it gets older (Fig. 3). The yellowing of foliage at the top was so prominent that the mutant line could be easily spotted from a distance (Fig. 4).

Reciprocal crosses of JGM 6 were made with ICC 5316, an accession with multipinnate leaves, white flowers, pea-shaped seed and light brown seed coat. Both the crosses gave a good fit to a 3 normal green: 1 chlorotic apical foliage plants in F_2 (Table 1), suggesting that the apical chlorosis trait is monogenic recessive. The gene symbol *ach* is assigned to this mutation. The gene for yellow apical foliage segregated independently of the loci for leaf type (pinnate vs. multipinnate), flower color (pink *vs.* white), seed shape (angular *vs.* pea), and seed coat (dark brown *vs.* light brown).

Apical (terminal) chlorosis often appears in chickpea under iron deficiency which is a frequent phenomenon on alkaline (pH 8.0) calcareous soils with high (20-30%) calcium carbonate content [19, 20] or in wet soil conditions [21, 22]. The symptoms of

Table 1. Goodness-of-fit $\chi 2$ test for 3:1 single-locus F_2 segregation of the variegated leaf (JGM 3) and the apical chlorosis (JGM 6) mutant traits

Cross	F_2 segregants		χ^2 (3:1)	P
	Normal leaf	Variegated leaf		
JGM 3 × ICC 4929	158	60	0.74	0.39
JGM 3 × ICC 5324	245	67	2.07	0.15
JGM 3 × ICC 10301	186	54	0.80	0.37
ICC 10301 × JGM 3	133	35	1.56	0.21
JGM 3 × ICC 12450	144	42	0.58	0.45
JGM 3 × ICC 15566	115	35	0.22	0.64
Pooled data	981	293	2.72	0.10
Heterogeneity			3.25	0.66
	Normal leaf	Apical chlorosis		
JGM 6 × ICC 5316	139	39	0.91	0.34
ICC 5316 × JGM 6	172	56	0.02	0.88
Pooled data	311	95	0.37	0.54
Heterogeneity			0.56	0.45

iron deficiency chlorosis include general yellowing of plants starting from the apical leaves and extending to necrosis in severe cases. The plants recover if the causal factor is eliminated at early stage of chlorosis or by foliar spray of $FeSO_4$ [20]. Differences in varietal response for chlorosis induced by iron deficiency have been observed and the resistance has been reported to be under the control of a single [21, 23] or two dominant genes [22]. In contrast to chlorosis observed in the genotypes sensitive to iron deficiency chlorosis, the induced mutation for apical chlorosis always showed yellowing of terminal shoots irrespective of soil conditions and never developed into necrosis. Spray of 0.5% $FeSO_4$ 30 days after sowing corrected the symptoms of iron deficiency chlorosis in the line ICC 4992 but it had no effect on apical chlorosis mutant. The fact that the chlorotic mutation does not lead to necrosis and its leaves develop chlorophyll as they grow in age indicates that the mutation in gene *ach* leads to age-dependent partial breakdown in the chlorophyll synthesis pathways, which shows some degree of recovery with advancing age. It is not related with iron uptake, as is the case in genotype ICC 4992, but the meatabolic defect is at some other point in chlorophyll synthesis.

The variegated leaf and the apical chlorosis mutants described here are a valuable addition to the pool of marker stocks and very convenient for genetic studies. These traits can be easily scored even at the seedling stage. The use of the variegated leaf mutant as an ornamental plant is another possibility.

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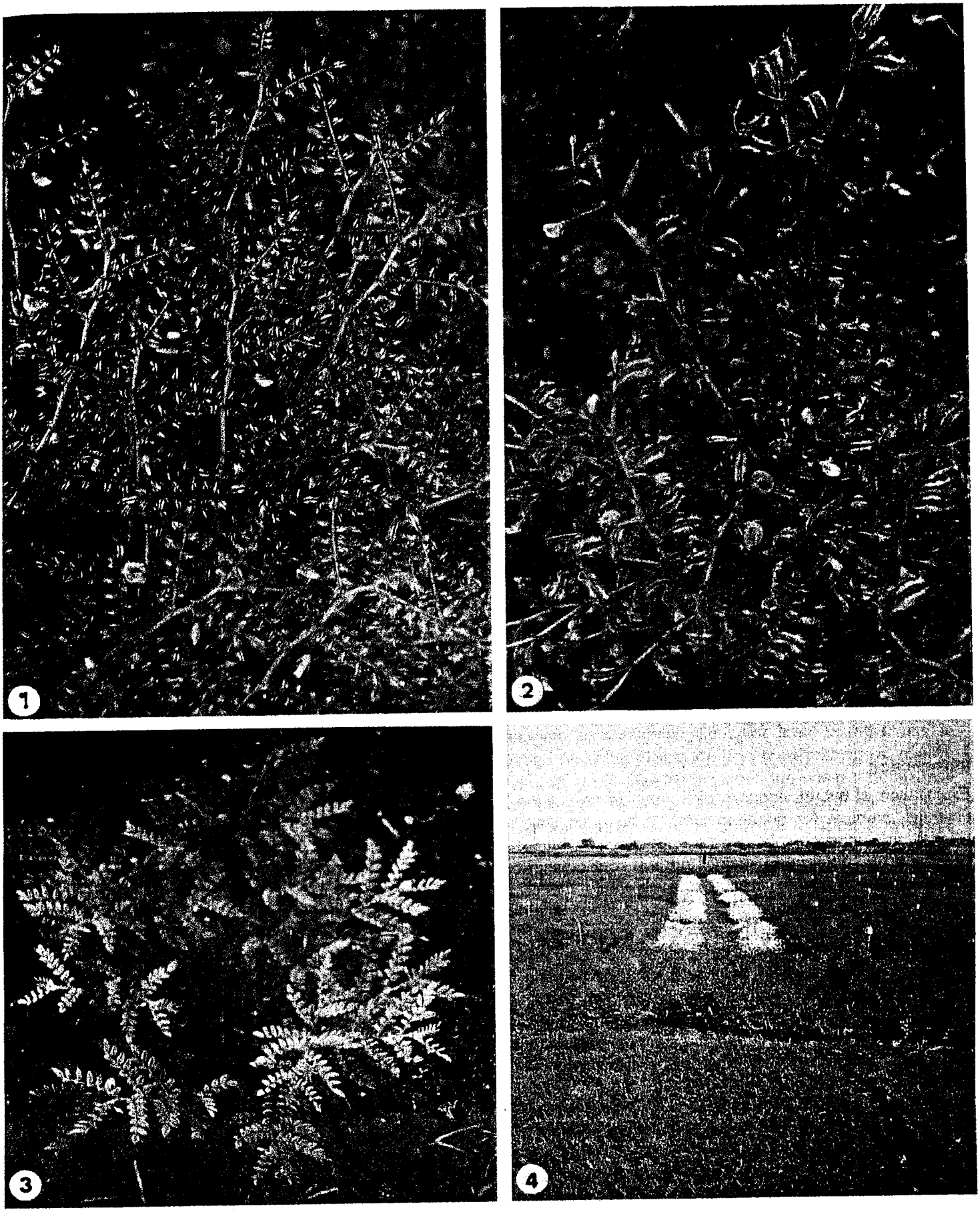

Fig. 1-4. (1) JGM 3 (Jawahar gram mutant 3), an induced leaf variegation mutant of chickpea cultivar JG 315; (2) Variegated simple leaf recombinant obtained from the cross JGM 3 x ICC 10301 (simple leaf). Expression of leaf variegation magnified in the simple leaf; (3) JGM 6, an apical chlorosis mutant induced in chickpea cultivar JG 315. Note chlorophyll development in leaves with age; (4) Field view of the apical chlorosis mutant JGM 6 (used as border rows to demarcate two experiments).