

## Somatic instability for chlorophyll pigmentation in cotton (*Gossypium* spp.)

R. Sheshagiri and B. M. Khadi

Agricultural Research Station, Dharwad Farm, Dharwad 580 007, India

Since centuries, variegated plants have been identified and preserved by man as ornamentals. The phenomenon of variegation is also observed in crop plants. In the present study, chlorophyll mosaics were identified and scored in various cotton populations. All four cultivated *Gossypium* species and their hybrids showed variegation, but high frequency of mosaics were seen in cytoplasmic male sterile (CMS) lines. The variegation was either limited to a leaf or branch or expressed throughout the plant. Various patterns of yellowing were observed on leaves, bractioles and bolls. The possible mechanisms involved in creation of such somatic instability are discussed, which can have implications in cotton genetics, breeding and biotechnology.

AMONG the living organisms, the phenomenon of variegation; the unusually spotted, striped, or otherwise mosaic appearance of some individuals as opposed to their normally uniform counterparts, has long been a source of fascination. In plants, variegation, very often ascribed to somatic instability, is most easily recognized as irregularities in pigment patterns on leaves, flowers and seeds. Researchers hope that variegation may contribute to our understanding of an organism's capacity for change and provide a starting point for unraveling the complex mechanisms underlying its development<sup>1</sup>. Analysis of somatic chlorophyll mutants has been a useful tool for the study of either spontaneous<sup>2,3</sup> or induced<sup>4,5</sup> genetic instability-related phenomena in plants.

Cotton (*Gossypium* spp.) is an important fibre crop of the world. Among the four cultivated species of *Gossypium* grown in India, *G. arboreum* and *G. herbaceum* are diploids and known as Desi cottons. The other two species, viz. *G. hirsutum* and *G. barbadense* are tetraploids and known as New world cottons. In our breeding programmes, (though it failed earlier to catch our attention), in the past few years we often encountered one or two cotton plants exhibiting yellow and/or white spots/stripes on green leaves. Such chlorophyll mosaic plants were observed commonly in varietal/hybrid trials and in crossing blocks. To understand the pattern of variegation and to know their frequencies, all the available breeding populations sown during Kharif 1998 were persistently screened for occurrence of the mosaic plants, at various plant developmental stages.

In a broad sense, the material screened in the study consisted of (i) varieties of four cultivated species of

*Gossypium*; (ii) inter-specific hybrids among two diploids (*G. arboreum* × *G. herbaceum*) and two tetraploids (*G. hirsutum* × *G. barbadense*); (iii) intra-specific *hirsutum* hybrids; and (iv) male sterile (MS) lines involving both cytoplasmic (CMS) and genetic (GMS) male sterility systems. The number of genotypes screened under each category and the number of plants scored for each genotype are presented along with the frequencies of chlorophyll mosaic plants in Table 1. In general, around 50 or more genotypes were considered in each population except for *G. arboreum* varieties (24), inter-specific diploid hybrids (9) and GMS lines (4). One hundred plants were scored for each genotype except for *G. barbadense* varieties (10) and inter-specific diploid hybrids (50).

As is evident from Table 1, all the cotton populations studied invariably had few chlorophyll mosaic plants. Out of the 736 genotypes screened, 177 genotypes ( $24.05 \times 10^{-2}$ ) yielded 330 mosaic plants ( $0.50 \times 10^{-2}$ ). In general, the frequency of unstable genotypes was more in male sterile lines ( $65.57 \times 10^{-2}$ ) followed by intra *hirsutum* hybrids ( $31.23 \times 10^{-2}$ ), *Gossypium* varieties ( $13.7 \times 10^{-2}$ ) and the inter-specific hybrids ( $9.17 \times 10^{-2}$ ). Though relatively less numbers of genotypes were screened, *G. arboreum* exhibited highest instability ( $45.8 \times 10^{-2}$ ) among the four species, followed by *G. hirsutum* ( $27.2 \times 10^{-2}$ ). The low frequency ( $2.4 \times 10^{-2}$ ) observed in *G. barbadense* may be due to the less number of plants scored, which can be supported by the moderate frequency ( $0.24 \times 10^{-2}$ ) observed on a whole population basis. However, *G. herbaceum* was distinctly stable with only two genotypes ( $2.5 \times 10^{-2}$ ) showing mosaics. Among the inter-specific hybrids, both the diploids ( $11.10 \times 10^{-2}$ ) and tetraploids ( $9.00 \times 10^{-2}$ ) had almost similar frequencies of unstable lines. In the intra *hirsutum* hybrids, CGMS-based hybrids ( $56.30 \times 10^{-2}$ ) dominated for instability and interestingly the conventional intra *hirsutum* hybrids ( $22 \times 10^{-2}$ ) almost maintained the trend of *hirsutum* varieties ( $27.2 \times 10^{-2}$ ). Among the male sterile lines, though GMS lines showed higher frequencies ( $75 \times 10^{-2}$ ) of unstable lines, it may have less implications as only four GMS lines were screened. However, the CMS lines exhibited a very high level ( $64.9 \times 10^{-2}$ ) of instability for chlorophyll pigmentation which was the maximum among any population screened in the present study followed by the CGMS-based hybrids.

The population frequencies (Table 1) further confirmed the higher instability of the CMS lines and the CGMS-based hybrids as they recorded  $1.44 \times 10^{-2}$  and  $1.11 \times 10^{-2}$ , respectively at the population level. Infact, 179 (54%) of the 330 mosaic plants isolated in the present study were contributed only by these two populations. Among the four *Gossypium* species, the frequency of mosaic plants was maximum for *G. arboreum* varieties ( $1.08 \times 10^{-2}$ ) and minimum ( $0.02 \times 10^{-2}$ ) for *G. herbaceum*, indicating its inherent stability. All other populations maintained

moderate frequencies between  $0.2$  and  $0.4 \times 10^{-2}$  except the GMS-based hybrids ( $0.75 \times 10^{-2}$ ). Table 1 also indicates that among the genotypes, in general, the frequency of mosaic plants ranged up to  $7 \times 10^{-2}$  except for the varieties of *G. herbaceum* and *G. barbadense* ( $1 \times 10^{-2}$ ), GMS lines ( $1 \times 10^{-2}$ ) and the inter-specific diploid hybrids ( $2 \times 10^{-2}$ ). However, a very high level of instability was noticed in one conventional hirsutum hybrid (DHH-156) with a frequency of mosaics as high as  $12 \times 10^{-2}$ .

The relationship between CMS and high frequency of chlorophyll mosaics was further confirmed through another study. In a crossing block, 10 plants of 64 CMS lines (known as A-lines) were grown along with 10 plants of their corresponding B (male fertile) lines to be used as

pollen parents for the maintenance of A lines. When all the plants were scored, 1–2 mosaic plants were seen in 14 A lines, however there was a single mosaic plant in only one B line. To analyse this phenomenon in detail, 100 plants of 24 A lines (which are extensively used in crossing programmes) and their corresponding B lines were scored for the occurrence of chlorophyll mosaics (data not shown). Mosaics were found in 15 A lines (frequency,  $62 \times 10^{-2}$ ) at a population frequency  $1.4 \times 10^{-2}$ , whereas only 5 B lines ( $20.8 \times 10^{-2}$ ) showed mosaics in a frequency of  $0.21 \times 10^{-2}$  which is much lower compared to the A lines.

Closer examination of the mosaic plants revealed interesting details about the variegation. In few plants, a single leaf was chimeric, while in others variegation was

Table 1. Frequency of chlorophyll mosaic plants in various cotton populations

Sl. no.	Population	No. of genotypes screened	No. of plants scored in each genotype	No. and frequency of genotypes showing mosaics		No. of plants scored in each population	No. and frequency of mosaics in each population		Range of frequencies of mosaic plants among genotypes ( $\times 10^{-2}$ )
				Number	Frequency ( $\times 10^{-2}$ )		Number	Frequency ( $\times 10^{-2}$ )	
Vareities of different <i>Gossypium</i> species									
1.	<i>G. herbaceum</i>	81	100	2	2.5	8100	2	0.02	1
2.	<i>G. arboreum</i>	24	100	11	45.8	2400	26	1.08	1 to 5
3.	<i>G. hirsutum</i>	81	100	22	27.2	8100	34	0.42	1 to 7
4.	<i>G. barbadense</i>	84	10	2	2.4	840	2	0.24	1
	Sub total	270	—	37	13.7	19440	64	0.33	1 to 7
Inter-specific hybrids									
1.	<i>G. herb.</i> $\times$ <i>G. arbo.</i> (Diploids)	9	50	1	11.10	450	1	0.22	2
2.	<i>G. hirs.</i> $\times$ <i>G. barb.</i> (Tetraploids)	111	100	10	9.0	11100	13	0.23	2 to 6
	Sub total	120	—	11	9.17	11550	14	0.12	2 to 6
Intra-specific ( <i>hirsutum</i> $\times$ <i>hirsutum</i> ) hybrids									
1.	Conventional hybrids	150	100	33	22.00	15000	56	0.37	1 to 12
2.	GMS-based hybrids	48	100	7	14.60	4800	14	0.29	1 to 4
3.	CGMS-based hybrids	87	100	49	56.30	8700	97	1.11	1 to 6
	Sub total	285	—	89	31.23	28500	167	0.59	1 to 12
Male sterile lines									
1.	GMS lines	4	100	3	75.00	400	3	0.75	1
2.	CMS lines	57	100	37	64.90	5700	82	1.44	1 to 6
	Sub total	61	—	40	65.57	6100	85	1.39	1 to 6
	Grand total	736	—	177	24.05	65590	330	0.50	1 to 12

GMS – Genetic male sterility, governed by nuclear genes.

CMS – Cytoplasmic male sterility, governed by cytoplasmic genes.

CGMS – Cytoplasmic genic male sterility, wherein CMS could be restored by nuclear genes.

*G. herb.* – *G. herbaceum*; *G. arbo.* – *G. arboreum*;

*G. hirs.* – *G. hirsutum*; *G. barb.* – *G. barbadense*.



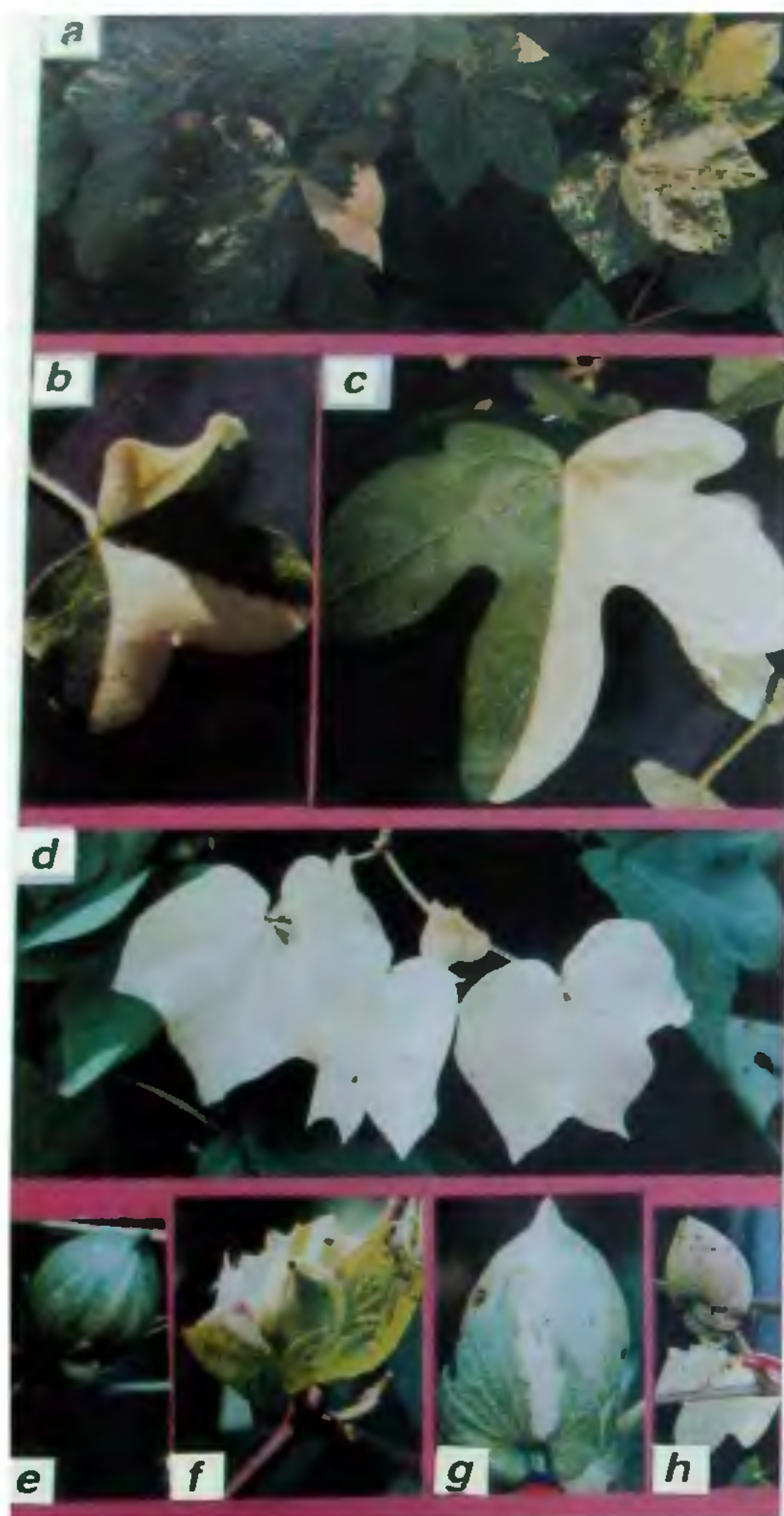
**Figure 1.** Distribution of variegation in various plant parts. *a*, Single leaf mosaic; *b*, Single monopodial branch mosaic; *c*, A lateral side (towards the viewer) of upper half mosaic; *d*, Upper half completely yellow; and *e*, Whole plant mosaic.

restricted to one monopodial branch. The lateral sides of the upper half of a few plants were chlorophyll-deficient but in others the upper half was completely yellow. There were also cases in which the variegation was distributed all over the plant (Figure 1). A wide spectrum of different patterns of variegation was also observed within individual leaves. The leaves had either white/yellow spots/stripes all over or had definite yellow sectors. Few leaves had exactly half yellow portions and some leaves were completely deficient in chlorophyll content (Figures 2 *a–d*). Further, variegation was not only restricted to leaves but extended on to the bractioles and bolls. Bractioles and bolls also exhibited various patterns of yellowing (Figures 2 *e–h*).

Genetic instability was first recorded in plants by Knight<sup>6</sup> and Emerson<sup>7</sup>. They noted that this instability can be associated with the behaviour of an unstable allele of an otherwise standard locus. McClintock<sup>8,9</sup> carefully studied the behaviour of a few maize (*Zea mays* L.) lines in generating variegated phenotypes and found that this characteristic was often associated with the activity of mobile DNAs termed controlling, or transposable elements (TEs). In the present study, insertion of such cryptic genetic elements into the genes involved in chlorophyll synthesis must have inactivated the host genes leading to chlorophyll-deficient yellow patches on leaves.

Further, their excision in future mitotic divisions resulted in reactivation (known as somatic reversion) of genes and synthesis of chlorophyll, finally exhibiting yellow spots or stripes in a green background. As shown in Figure 1, the distribution of variegation in a particular leaf, monopodial branch, lateral upper half, upper half or throughout the plant may be indicative of the temporal and spatial differences in the activation of the putative TEs. Similarly the various forms of variegation observed on leaves, bractioles and bolls (Figure 2) might denote the particular point of development of the respective tissue when mutations occurred. Variegation in the form of red stripes and sectors on leaves, flower and pericarp of the seed were noticed in candystripe genotype of sorghum, which was later confirmed to be due to activity of transposons<sup>10</sup>. Somatic instability has been proven to be an important tool for studying the fates of developing tissues by somatic sector analysis<sup>11</sup>.

Further, the present study also indicated a possible role of cytoplasm in creation of genetic instability. This conclusion was made based on two facts, viz. (i) the nuclear genomic constitution of A and B lines remains the same but differ only for their cytoplasm and hence the high frequency of mosaics observed in A lines as compared to B lines may be due to the unstable cytoplasmic genes; (ii) all the CGMS-based hybrids



**Figure 2.** *a–d*, Patterns of variegation on leaf. *a*, Yellow/white spots/strips on leaf; *b*, Yellow sectors; *c*, Half leaf yellow; *d*, Full leaf yellow. *e–h* Patterns of variegation on bractioles and/or bolls. *e*, Yellow spots/strips; *f*, Yellow sectors; *g*, Half boll yellow; *h*, Full boll yellow.

shown in Table 1 had one or the other 24 A lines described later as their female parents. The comparison of the 24 A lines ( $62 \times 10^{-2}$ ) and the CGMS-based hybrids ( $56.30 \times 10^{-2}$ ) for the frequency of mosaics exemplifies the possible maternal inheritance of the instability from CMS lines to CGMS hybrids. Existence of the TEs in chloroplast was first postulated by Sears<sup>12</sup> and the recent studies on chlorophyll-deficient mutants of barley<sup>13–15</sup> have confirmed chloroplast transposon activation under a particular nuclear genotype. McClintock<sup>16</sup> proposed that TEs in an organism get activated when the organism is subjected to genomic stress. In cotton, the CMS lines have been developed with the cytoplasm of a diploid species, *Gossypium herknessii* by transferring the nuclear genome of tetraploid species *G. hirsutum* which must have created such a stress to activate the putative cytoplasmic transposons. However, it needs confirmation through genetic and molecular analyses.

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