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Somatic Crossing Over as a Cause of Intergenome Translocations in Haploid Cotton

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MOSAICISM or spotting in somatic tissues can result from several genetic phenomena. Genetic evidence of somatic crossing over to explain twin and single spots was provided by Stern[14] in *Drosophila* and by Jones[10] in maize. Vig[16] reported genetic evidence of somatic crossing over in soybeans. He also treated soybeans with chemicals known to increase somatic crossing over and observed an increase in the number of twin spots. Jones[11] also showed that twin spots could be a result of somatic crossing over between nonhomologous chromosomes (i.e., a translocation) in maize. Brown[7] presented an excellent review on somatic pairing and crossing over, and we[2] gave a more thorough review in a previous paper.

Barrow *et al.*[3] found twin spots in a strain of *Gossypium barbadense* L. homozygous recessive, v_7v_7, for a virescent foliage marker. The twin spots were represented as normal green and yellow on the virescent background. Turcotte and Feaster[15] established the phenotypic segregation ratio of the V_1 and V_7 loci of 5 green: 6 virescent: 5 yellow (white). Their findings established the genetic basis for the phenotypes observed in twin spots and showed that the genes responsible, V_1 and V_7, are inherited independently and behave as homoeoalleles.

Both cultivated New World cotton species, *G. hirsutum* L. (American Upland cotton) and *G. barbadense* (American Pima cotton) are amphidiploids ($2n = 4x = 52$). These species are composed of two subgenomes designated as A and D by Beasley[4]. The A genome chromosomes are larger than the D genome chromosomes. Cotton behaves as a diploid, forming 13 A and 13 D bivalents at metaphase I at meiosis.

Genetic homoeology between the two genomes has been demonstrated by the finding of duplicate linkage groups by Rhyne[12] and Holder *et al.*[9]. Chromosome homoeology is indicated by pairing between A and D chromosomes, which has been observed at early stages of the meiotic prophase of haploid plants ($n = 2x = 26$) by Barrow[1], Brown[6], and Endrizzi[8]. Brown[6] observed that these pairs fell apart as the chromosomes approached metaphase, and chiasmata were infrequent.

We[2] later found a $2n$ virescent *G. barbadense* plant in which a twin spot event occurred in an apical meristem of a lateral bud, resulting in a normal, green branch from which floral buds were used to analyze chromosomes at metaphase I in meiosis. The presence of multivalent chromosomes indicated somatic crossing between nonhomologous chromosomes, resulting in translocations. Sixteen of 52 chromosomes were involved, giving a more complex chromosome involvement than was expected. A virescent branch on the same plant had cells with 26 bivalents. In the summer of 1974, we found a virescent, haploid plant ($n = 2x = 26$) that also produced a green branch from the apical meristem of a lateral bud. This plant is the subject of this report. It would seem that translocations resulting from somatic crossing over would be simpler to analyze in haploid plants than in $2n$ plants.

It is appropriate to review the results of analysis of metaphase chromosomes of cotton haploids during meiosis. Webber[17] reported 0.2 chromosome associations per cell in a cotton haploid. Endrizzi[8] observed 0.16 bivalents per cell in a haploid plant from a twin seedling. Beasley[4] observed 0.8 associations and stated that in many cells it was impossible to detect chiasmata between these associations. Brown[6] found seven to nine associations during the pachytene stage between A and D chromosomes in a haploid of *G. hirsutum*. These chromosomes separated without chiasmata as meiosis progressed. Barrow[1] found 0.005 chiasmata per cell after analyzing over 1,000 metaphase cells in a haploid of *G. barbadense*. He also noted a large number of associations of A and D chromosomes at pachytene, which usually separated as meiosis progressed. Therefore, the predominant chromosome configuration during metaphase I and anaphase I of cotton haploids is 26 univalent chromosomes. The association of A and D chromosomes diminishes as meiosis progresses, indicating some affinity between these chromosomes, even though chiasmata are rarely formed. The fact that chiasmata are observed does establish that

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crossing over between homoeologous chromosomes does occur during meiotic prophase. With genetic markers, this crossing over can be detected phenotypically.

In the above paragraph, the terms "association" and "bivalent" have been used indiscriminately. We propose to use the term "association" as a close relationship without observed chiasma and "bivalent" where chiasma was observed.

The purpose of this investigation was to analyze chromosome configurations of metaphase I of a normal green branch of the haploid plant described above. A comparison of this analysis with the chromosome analysis of a virescent branch on the same haploid plant is presented. These analyses show the frequency of bivalent pairing between A and D genome chromosomes and are considered as evidence of somatic crossing over resulting in a translocation.

Materials and Methods

Buds at the metaphase I stage of meiosis were collected, killed, and fixed, and slides were prepared as previously described[1]. Over 400 cells with 26 chromosomes from the green and virescent branches were analyzed. Only AD associations were counted for reasons to be discussed later. Of the AD associations, the data were analyzed two ways: first, only AD bivalents with definite chiasmata vs 26 univalents; second, AD associations vs 26 univalents.

The data were analyzed by a contingency chi-square table described by Steel and Torrie[13]. The treatment consisted of the green and virescent branches, whereas AD associations vs. nonassociations constituted the columns. The chi-square was calculated to check the lack of difference between the green and virescent branches. A significant increase in AD bivalents in the green tissue versus the virescent tissue would provide evidence of an intergenome translocation by somatic crossing over as a cause of the green tissue.

Results and Discussion

Four hundred and seventeen cells were counted from buds collected from the green branch. Of these, 23 cells had AD bivalents with chiasmata. From the virescent branch, two cells had AD bivalents attached by chiasmata in 425 cells analyzed (Table I) The chi-square value of 18.595 with one degree of freedom exceeded the value of 7.88 for $P = 0.005$. On the basis of increased AD bivalents in the green tissue, we reject the null hypothesis and conclude that the AD bivalents resulted from somatic crossing over. Table II lists the total AD associations, including those with chiasmata for the green branch and virescent branch. The chi-square value of 29.69 indicated that AD associations were significantly greater in the green branch.

Figure 1 diagrams our proposed explanation of somatic crossing over, followed by mitotic segregation and subsequent production of AD bivalents as a result of translocation. We arbitrarily assigned the recessive v_7 gene to the A genome chromosome and the dominant V_1 gene to the D genome homoeologue. The haploid A-v_7 and D-V_1 would be virescent according to the gene dosage ratio described by Turcotte and Feaster[15]. In the F_2, three to four dominant genes (zero or one recessive) would give green color; two dominant genes (two recessives) would give the virescent color; and one or no dominant genes (three to four recessives) would give yellow color. The alternate situation with V_1 on the A genome and v_7 on the D genome is also possible and would give the same results. We further assume that a single crossover oc-

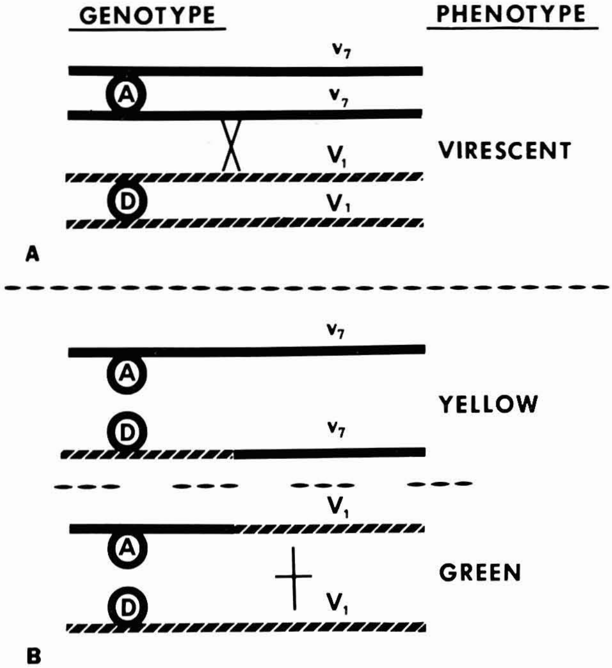

FIGURE 1—*A* shows somatic pairing of two A genome chromatids and two D genome chromatids of cotton with chiasma formation at the "X." *B*—daughter cells of cotton after the appropriate mitotic segregation of non-crossover and crossover chromatids. In our material, tissues derived from a V_1V_1 green cell were studied. The "+" indicates the homologous regions on the A and D chromosomes, where meiotic synapsis and chiasma formation may occur to form an A-D bivalent. The circles also represent the centromeres.

Table I. A–D bivalents at metaphase I from a green and a virescent branch of a haploid, chimeral cotton plant resulting from somatic crossing over

	Cells with A–D bivalents	Cells without bivalents	Total
Green branch	23	394	417
Virescent branch	2	423	425
$\chi^2 = 18.595$*; 1 df			

* Calculated from a contingency χ^2 table after Steel and Torrie[13] p. 376–377

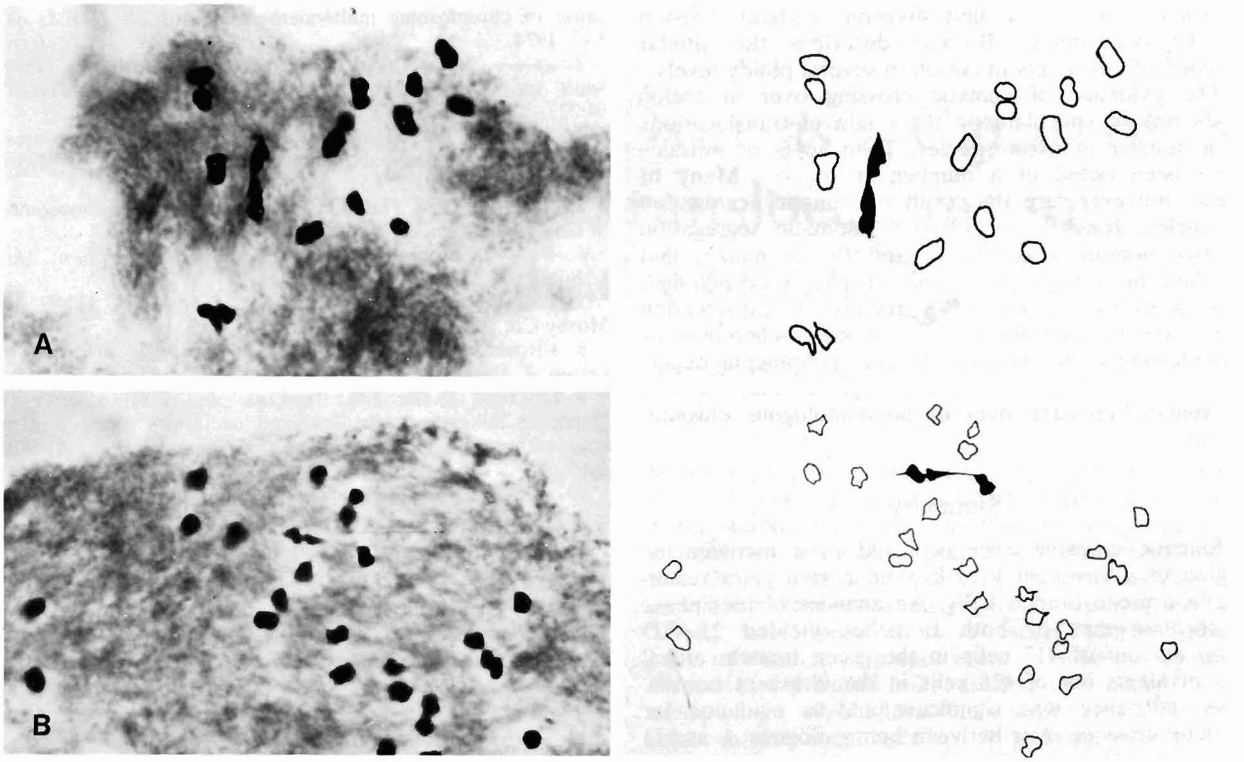

FIGURE 2—*A* and *B* shows photographs and drawings of A-D chromosome bivalents in meiotic cells of green chimeral tissue from a virescent haploid ($n = 2x = 26$) plant of *G. barbadense*. The bivalents are drawn in black. The univalent chromosomes in some cells were spread to the extent that it was not possible to include them all in the photograph. The cells had 24 univalents and the one A-D bivalent.

curs between the centromere and the *V* locus of the homoeologous A and D chromosomes in a meristematic cell. With appropriate mitotic chromatid segregation, an A-v_7 and a translocated A-D-v_7 go to one daughter cell giving a yellow phenotype. The translocated A-D-V_1 and D-V_1 chromatids go to the other daughter cell, giving the green phenotype. This V_1V_1 cell is ancestral to the gametophyte tissue undergoing meiosis in the green branch. In these cells, the D chromosome has a segment homologous to the A chromosome with the V_1 locus. The origin of this A–D chromosome would be a translocation between an A chromatid and a D chromatid during somatic crossing over. Pairing and crossing over in this homologous segment would result in an AD bivalent attached by chiasmata.

Table II. A–D associations at metaphase I from a green and a virescent branch of a haploid, chimeral cotton plant resulting from somatic crossing over

	Cells with A–D associations	Cells without associations	Total
Green branch	52	365	417
Virescent branch	11	414	425
$\chi^2 = 29.69$; 1 df			

In this study, the frequency of crossing over in this segment is 0.055. This may be indicative of the relative size of the translocated segment, or there may be reduced pairing of small homologous segments in a haploid.

Figure 2 illustrates the association of the large A and small D chromosomes. The AD associations are the result of homoeologous crossing over in somatic cells. Further evidence to substantiate this would involve duplicate genetic markers linked to v_1 and v_7. These linkage groups are not yet available.

The chiasmata and AD associations observed in the virescent branch indicate the occurrence of occasional meiotic crossing over between homoeologous chromosomes. The number of AD associations may vary between haploid plants due to genetic differences, the stage of observation (i.e., early or late metaphase), and the observation of chiasmata. By comparing virescent and green branches on the same plant, we were able to detect the actual increased frequency of AD associations due to somatic crossing over.

We noted two possible DD associations in the virescent branch. Later we observed similar configurations in three cells that had the apparent DD association, except that these cells in addition had 25 univalents. Therefore, it was suspected that the apparent DD association was an A chromosome attempting to divide transversally (i.e., one arm going to each pole) at the first division, without division of the centromere. Brown[5] describes the similar division of univalents in cotton in several ploidy levels.

The evidence of somatic crossing over in cotton leads one to speculate on the origin of translocations in a number of plant species. Twin spots or mosaics have been noted in a number of species. Many of these, however, are the result of somatic segregation of alleles. Jones[11] also observed somatic segregation of two nonlinked genes, *Su* and *Pr*, in maize, that resulted in a twin spot. This result, he concluded, was probably caused by a reciprocal translocation (i.e., somatic crossing over between nonhomologous chromosomes). We believe that the spontaneous occurrence of translocations in many plants may be due to somatic crossing over of nonhomologous chromosomes.

Summary

Somatic crossing over occurred in a meristematic region of a virescent V_1v_7 haploid cotton plant resulting in a green branch V_1V_1. An analysis of metaphase I chromosomes of both branches yielded 23 AD bivalents out of 417 cells in the green branch, and 2 AD bivalents out of 425 cells in the virescent branch. This difference was significant and is evidence for somatic crossing over between homoeologous A and D chromosomes.

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