Twin Spots on Leaves of Homozygous Cotton Plants

JERRY R. BARROW, HEMENDRA CHAUDHARI, AND MAX P. DUNFORD

MOSAIC patterns caused by various types of genetic changes in somatic tissues have been observed in the form of single and twin spots in a number of organisms. Stern\textsuperscript{15} observed single yellow (yg) spots and singed (sn) spots and twin yellow-singed spots on the wild-type body of a heterozygous female \(\frac{+ sn}{yg}\) in Drosophila melanogaster. He also observed three types of single spots on the bodies of \(\frac{ysn}{++}\) females. The expected yellow and singed spots, and the yellow and the singed single spots occurred from the separation of the linked y and sn genes by somatic crossing over in the four-strand stage.

Jones\textsuperscript{15} reported paired and unpaired spots involving several genes determining color and texture differences in the triploid aleurone layer of the maize endosperm. The aleurone tissue was heterozygous Aaa or AAs, depending on the genotype of the pollen and seed parents for each gene considered. Nondisjunction, deletion, incomplete fertilization, and mutations were given as possible explanations for the occurrence of single spots. A hypothesis of somatic crossing over appeared to fit many of the observations on twin spots, but Jones held some reservations on the acceptance of that explanation.

Vig and Paddock\textsuperscript{22} observed apparent somatic recombination at the \(Y_1\) locus in soybeans. Complete dominance was expressed by \(Y_1\), in that \(Y_{11}Y_{11}\) was normal green, \(Y_{11}y_{11}\) was greenish yellow. Unpaired and paired spots of normal green \(Y_{11}Y_{11}\) and greenish yellow \(Y_{11}y_{11}\) were observed on the two simple leaves and the first compound leaf of a heterozygous \(Y_{11}Y_{11}\) light-green plant. Treatment with mitomycin C, a chemical known to increase somatic crossing over in a number of organisms, increased both single and twin spots but dispropor-

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sister chromatid with $P^r-Mp$ and $tr-Mp$ at the same loci on the sister chromatid after segregation, would give the light variegated phenotype in the other daughter cell. Further division of the two daughter cells would give rise to tissues that were either red or light variegated.

Imai and Kanna\textsuperscript{11} found that three phenotypes of \textit{Portulaca grandiflora} L., namely, orange, striped yellow, and striped cream were homozygous dominant (CC), heterozygous (Cc) and homozygous recessive (cc), respectively. Within the striped yellow (Ce) plants, orange (CC) sectors frequently occurred. In selfed homozygous striped cream (cc) plants a low percentage of orange (CC) plants (1.5 percent) and 13.5 percent striped yellow (Ce) plants were observed in the progenies. Sectors of striped yellow and orange often were observed in striped cream (cc) plants. The orange plants did not revert to the striped yellow or striped cream phenotypes. These observations were explained by a high mutation frequency of $c$ to $C$.

Brown\textsuperscript{4}, in a review of the topic, states that somatic pairing of homologous chromosomes is apparently a more common phenomenon than was formerly thought. It has been observed in insects, fungi, and higher plants. In many cases genetic recombination of marker genes has been detected, as stated above. It has also been determined that in some plants of allopolyploid origin, homoeologous chromosomes may pair meiotically in the absence or mutation of specific genetic control of pairing\textsuperscript{10}. Somatic association of chromosomes in mitotic cells has also been shown in \textit{Triticum aestivum}\textsuperscript{11} and in \textit{Avena}\textsuperscript{16}. Endrizzi\textsuperscript{9} and Barrow\textsuperscript{1} detected association of chromosomes of the A and D genomes in haploids of cotton. Brown\textsuperscript{2} observed an average of seven to nine paired chromosomes per cell during pachytene in a haploid of \textit{Gossypium hirsutum}. However, as meiosis progressed, the associated chromosomes separated without chiasmata. It was assumed that the associated chromosomes were homoeologues from the A and D genomes.

Materials and Observations

The expression of virecent leaf color in cultivated cotton \textit{Gossypium} sp. is inherited as a recessive characteristic. The normal leaf color is determined by dominant genes. A yellow-green virecent \textit{(v$_r$)} strain of \textit{G. barbadense} L., that produces a high frequency (about 50 percent) of haploid \textit{(n=2x=26)} plants was obtained from E. L. Turcotte of Phoenix, Arizona, as an F$_4$ plant selection. The F$_1$ was grown in the greenhouse and selfed and plants of the F$_2$ were grown in the field and greenhouse during 1971. Of the surviving plants in the field, 261 were diploid and 120 were haploid. Only diploid plants were selected for greenhouse culture. As expected, all diploid plants were virecent, indicating complete homozygosity \textit{(v$_r$)} and all haploid plants were virecent \textit{(v$_r$)}.

During the growing season, three types of leaf spots were observed. Green spots darker than the virecent tissue, yellow spots lighter than the virecent, and green and yellow twin spots or adjacent areas of the green and yellow colors (see Figure 1). The diploid F$_1$ plants in the greenhouse were also examined and found to have spots.

Table I gives the number of plants and spots observed in the field and greenhouse. Table II gives the frequency of spots per leaf based only on leaves with spots. There were approximately five times as many spots per leaf in the greenhouse as were observed in the field. A possible cause of more spots in the greenhouse was that greenhouse summer temperatures averaged about 10°C warmer than the field. Also the more intense sunlight in the field may have washed out some spots, particularly yellows, before they were recorded. The increase of each type of spot in the greenhouse appeared to be approximately equal in magnitude.

![FIGURE 1—Spots on virecent r.r: leaf tissue. Left —green spot darker than virecent; note the yellowing effect of sunlight. Center—yellow spot lighter than virecent. Right—yellow-green twin spot.](image-url)
A close observation of haploid plants revealed all three types of spots present, with a lower proportion of twin spots and a higher proportion of single yellow spots (Table III). There was a lower percentage of haploid plants showing spots than diploid plants.

Cytological examination of PMC's of nine diploid plants with spots revealed no irregularities in meiosis. Examination of PMC's in meta-anaphase of two haploid plants with twin spots showed 26 univalents lacking any apparent bivalent formation.

Discussion

The reports of spotting and variegation in *Drosophila*, maize, tomato, soybeans, and apples, involved heterozygous material and the twin spots were thought to be the result of somatic crossing over and/or segregation of homologous chromosomes. That is, the twin spots were interpreted as homozygous sectors derived from heterozygous cells. In the above cases, except for *Drosophila*, incomplete dominance was noted in each instance and it was necessary to distinguish between the three genotypes AA, Aa, and aa in the sectors.

The spotting described in this report was in a strain of cotton that responded as if it were heterozygous. Apparently heterozygous sectors were derived from homozygous cells. If one considers somatic crossing over in the v1v1 cotton plants as the cause of spots, then some unknown type of heterozygosity that would have no apparent phenotypic effect must exist in the v1v1 plants. One possibility would be a pseudo-allelic condition where somatic crossing over within the allele between two minute mutations could give the segregation observed. Another possibility would be a pericentric inversion with the centromere lying between two hypothetical color-influencing genes located on each arm of the chromosome beyond the inversion. A crossover in the inverted region would give duplications and deficiencies of the two color genes that may be expressed as twin spots. In the above cases, the heterozygous tissue would be phenotypically indistinguishable from the homozygous tissue and any phenotypic change would be the result of somatic crossing over. Inversion and somatic crossing over between homologues are unlikely; however, for two reasons: 1) The expected frequency of heterozygotes would be reduced by self-fertilization from 50 percent in the F2 to 6 percent in the F3. It was observed that among 261 diploid plants, 236 (90 percent) expressed spots in the F2 generation of selfing. 2) All three types of spots were found in haploid plants, which rules out crossing over or unequal crossing over between homologous chromosomes.

The high frequency of spotting and the general low frequency of mutation makes an unlikely hypothesis that two different mutations (to green and yellow) occurred simultaneously in adjacent cells. However, an unstable v1 locus functioning similarly to the p' locus in maize with a transposable modulator as described by Greenblatt and Brink may provide a workable model. We also observed twin spots in a virescent v1v1 strain of *G. hirsutum* L., indicating the general instability of the virescent loci.

The best explanation for the spotting is based on information obtained by Turcotte and Feaster, combined with the results of somatic crossing over of homoeologous chromosomes. They found that in two different cotton strains the genes for virescent behaved as simple recessives. In a test for allelism they noted that the F1 of the two strains was virescent and the F2 segregated 5 green : 6 virescent : 5 yellow, a modified dihybrid ratio. Ap-

### Table II. The frequency of spots per leaf (counting only leaves with spots) in diploid plants in the field and greenhouse

<table>
<thead>
<tr>
<th></th>
<th>Green spots</th>
<th>Yellow spots</th>
<th>Twin spots</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field</td>
<td>0.51</td>
<td>0.30</td>
<td>0.33</td>
<td>1.14</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>2.85</td>
<td>1.12</td>
<td>2.08</td>
<td>6.05</td>
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<tr>
<td>Ratio: Spot</td>
<td>1:5.58</td>
<td>1:3.73</td>
<td>1:6.30</td>
<td>1:5.30</td>
</tr>
<tr>
<td>frequency</td>
<td></td>
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<tr>
<td>in field</td>
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<tr>
<td>to</td>
<td></td>
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<td></td>
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<tr>
<td>greenhouse</td>
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</table>

### Table III. Haploid v1 plants with single green, single yellow, and twin spots observed in the field

<table>
<thead>
<tr>
<th></th>
<th>No. plants with spots</th>
<th>No. leaves with spots</th>
<th>Green spots</th>
<th>Yellow spots</th>
<th>Twin spots</th>
<th>Total spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plants</td>
<td></td>
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<td>120</td>
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<tr>
<td>Field</td>
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<td>261</td>
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<td>824</td>
<td>421</td>
<td>252</td>
<td>270</td>
<td>943</td>
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<td>75</td>
<td>59</td>
<td>213</td>
<td>607</td>
<td>239</td>
<td>443</td>
<td>1289</td>
</tr>
</tbody>
</table>
FIGURE 2—A model of somatic pairing and crossing over between homoeologous chromosomes of the A and D genomes of haploid and diploid cotton plants. It is assumed that the V1 and V7 loci are on homoeologous chromosomes; however, it is not known in which genome the recessive gene resides. The segregation of the genes v1 and V1 leading to the formation of yellow and green chimeral spots derived from virescent tissue is shown. The chromosomes of the respective genomes are indicated by the letters within the circles representing the centromeres. The “x” represents an exchange between the chromatids of the homoeologous chromosomes.

Coiling is the reason for the diploid-like behavior of allotetraploid cottons.

The above evidence of homology between genomes and the reported duplicate linkage groups supports the hypothesis that the V1 and V7 loci are on homoeologous chromosomes. Somatic pairing of the homoeologues, with crossing over between the centromere and the V locus would explain the single and twin spots observed in both the haploid and diploid plants.

Figure 2 illustrates a model of crossing over between homoeologous A and D chromosomes in haploid and diploid plants assuming virescent genotypes are V1V1V7V7 and V1V1V1V1V7V7, and that the loci are on homoeologous A and D genome chromosomes. Which gene is in which genome is unknown, although we have, for purposes of illustration, arbitrarily placed the v7 gene in the A genome. In the haploid and diploid plants an A-D pair crosses over and in the diploid plants the other A-D pair goes in meiotic division. The daughter cells after somatic crossing over and mitotic segregation in the haploid plants would be v1v1V7V1V7V1 green. In the diploid plants daughter cells would be v1v1V1V1V1V1V1V1V1V1V1V1V1V1V1 green as a result of A-D crossing over. Single yellow or green spots would result from the same mechanism as twin spots by failure of one or the other of the daughter cells to divide, after somatic crossing over occurs.

Cytogenetically, somatic crossing over between homoeologous chromosomes will produce a structural change identical to a reciprocal translocation. If chromosome pairing and crossing over of this type occurred in floral meristems, some sterility
may be explained. It would also be expected that multivalents might be observed at meiosis.

Another explanation for the occurrence of twin spots and single spots may be mitotic nondisjunction in either of the homoeologous chromosomes. A color change in mitotic daughter cells would be expected due to changes in the genotype resulting from nondisjunction of chromatids of either an A genome chromosome or a D genome chromosome. For example, Figure 3 illustrates nondisjunction in an A genome chromosome. In haploids nondisjunction would produce $n+1$ and $n-1$ cells, with the possibility of lower viability for the latter. Figure 3 is drawn to show the $n+1$ cell as yellow and the $n-1$ cell as green. Lower viability of the $n-1$ cells may account for the observed higher frequency of yellow spots in haploids (Table III). Nondisjunction in diploid cells would result in $2n+1$ and $2n-1$ daughter cells, and no decrease in viability would be expected. The reason for the higher frequency of green spots in the diploids is unknown (Table I).

The authors, on the basis of more recent information, favor the somatic crossing over hypothesis for the cause of twin spots in cotton.

Summary

Single yellow, single green, and twin yellow-green spots were observed in apparently homozygous virescent cotton plants. The spots observed in both haploid and diploid plants were best explained by somatic crossing over between homoeologous A and D genome chromosomes, or mitotic nondisjunction of either an A or D genome chromosome, plus a dosage effect of independently segregating duplicate genes in the two genomes.

Literature Cited