## Genetics and Cytology

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### I. Cytogenetic studies of the $Y_2$ ob marker genes and chromosome 18.

Yellow petal color of G. darwinii is determined by a single gene designated $Y_2$ which is a duplicate of the yellow petal gene $Y_1$ that occurs in many types of G. hirsutum. The original $Y_2$ stock, consisting of the second backcross generation of the hybrid of G. hirsutum and G. darwinii to G. hirsutum, was received from Dr. S.G. Stephens. Additional backcrosses to G. hirsutum were made to develop a G. hirsutum line with the $Y_2$ marker. A single plant of the third backcross generation was selected for selfing to produce a homozygous $Y_2Y_2$ line. Seed of an $F_2$ plant, which proved to be $Y_2Y_2$ by $F_3$ test, was the source for the genetic line designated AG205. This line was used in all genetic tests reported here. It was noted that line AG205 was homozygous not only for the $Y_2$ marker but also for the phenotype of "open-bud", a recessive character previously reported in G. hirsutum.

The $Y_2$ 'open-bud' marker line AG205 was crossed with the $y_2$ 'open-bud' (ob) G. hirsutum marker line AG209 (D:60:7:1976, Kohel-Texas) and the $F_1$s had the typical "open-bud" phenotype of both lines establishing that they are allelic. Our studies show that the ob locus of both lines and the $Y_2$ locus are located on chromosome 18. The ob mutant segregates as a simple recessive in G. hirsutum. The The 'open-bud' character did not exist in the G. darwinii stock or any of the G. hirsutum stocks used in the transference program.

The origin of ob in $Y_2$ marker line AG205 can be explained by assuming that G. hirsutum upland types contain the monomeric genotype ob ob (A genome) Ob Ob (D genome) and that G. darwinii contains the reciprocal genotype Ob Ob (A genome) ob ob (D genome) in which the latter locus (ob) is closely linked with $Y_2$. Therefore, in the course of transference, the D genome Ob $y_2$ genotype of G. hirsutum was substituted by the homoeologous D genome ob $Y_2$ genotype of G. darwinii.

Following the establishment that $Y_2$ ob loci are located on chromosome 18, studies were made to determine the map distance between $Y_2$ and ob and their arm location in chromosome 18. For the latter, two monotelodisomic stocks, telo 18L (long arm) and telo 18S (short arm) were used. These results are shown in Table 1 and described in the following.

Monotelodisomic 18L: Thirty-three $F_1$ plants were scored in the telo 18L Ob $y_2$ x ob $Y_2$ cross, and phenotypic results show that all monotelodisomic plants were ob $Y_2$ and all disomic sibs were Ob $Y_2$, showing that ob is located in the short arm. Two monotelodisomic $F_1$s were self-pollinated and their $F_2$ progeny had ob $Y_2$ phenotype, suggesting that $Y_2$ may be located in the short arm along with ob. $F_2$ progeny of a 2n $F_1$ was also studied for verification of the linkage observed in the monotelodisomic and as expected, the disomics segregated for both open-bud and yellow petal.

Monotelodisomic 18S: Fifteen $F_1$ plants were scored in the telo 18S x ob $Y_2$ cross, and the monotelodisomic $F_1$ plants, like their disomic sibs, had the Ob $Y_2$ phenotype, indicating that the ob locus is located in the short arm.

Testcross Results: The disomic testcross results given in Table 1 show that the two loci ob $Y_2$ are very closely linked with 1.24 $\pm$ 0.62% recombination occurring between the two.

In testcross of ob $y_2$ x telo 18S $F_1$, only the $Y_2$ allele of the $F_1$ was recovered in the progeny, indicating that (a) the $Y_2$ locus is located either in the short arm and tightly linked to the centromere or in the long arm and that (b) the telocentric chromosome is not transmitted through the male. The only recombinant type recovered was Ob $Y_2$, which shows that ob is distal to $Y_2$ and 1.25 cm from the centromere and perhaps from $Y_2$ also. The telo testcross data show a percent of recombination between ob $Y_2$ identical to that observed in disomic testcross progeny. Equivalence of the two map units shows rather unequivically that regardless of whether $Y_2$ is in the short or long arm, it must be tightly linked to the centromere. There was no evidence of male transmission of the telocentric chromosome since individual with the Ob $y_2$ genotype were not recovered; with the tight linkage any telocentric chromosome transmitted through the male would most likely carry the Ob - or Ob $y_2$ genotype. Additional tests will be conducted in an attempt to determine the exact arm position of the $Y_2$ locus.

Table 1. Cytogenetic analysis for the association of the ob $Y_2$ markers and chromosome 18 of cotton.

Monotelodisomic 18L

$F_1$ generation: telo 18L Ob $y_2$ x ob $Y_2$ → 18 $F_1$ monotelodisomic plants = ob $Y_2$
15 $F_1$ disomic plants = Ob $Y_2$

$F_2$ generation: telo 18L $F_1$ (Ob $y_2$/ob $Y_2$)S → 72 $F_2$ plants = ob $Y_2$

2n $F_1$ (Ob $y_2$ x ob $Y_2$)S →

| Ob $Y_2$ | Ob $y_2$ | ob $Y_2$ | ob $y_2$ |
|---|---|---|---|
| 10 | 11 | 10 | 0 |

Monotelodisomic 18S

$F_1$ generation: telo 18S Ob $y_2$ x ob $Y_2$ → 8 $F_1$ monotelodisomics = Ob $Y_2$
7 $F_1$ disomics = Ob $Y_2$

| Testcross | Genotypes NCO Ob $y_2$ | CO ob $y_2$ | CO Ob $Y_2$ | NCO ob $Y_2$ | Total |
|---|---|---|---|---|---|
| ob $y_2$ x 2n $F_1$ (Ob $y_2$ x ob $Y_2$) | 151 | 1 | 3 | 167 | 322 |
| % recombination ob $Y_2$ in disomic = 1.24 ± 0.62 | | | | | |
| ob $y_2$ x telo $F_1$ (telo 18S Ob $y_2$ x ob $Y_2$) | 0 | 0 | 4 | 317 | 321 |
| % recombination ob $Y_2$ in telosomic = 1.25 ± 0.62 | | | | | |
| % recombination $Y_2$ - centromere (c) = 0 | | | | | |
| % recombination ob - centromere = 1.25 ± 0.62 | | | | | |

Linkage = ob — $Y_2$ — c

$\bar{x}$ of two samples: % recombination ob $Y_2$ = 1.25 ± 0.62

II. Cytological tests for chromosome identification in G. hirsutum.

The program for establishing aneuploid stocks has the objectives of developing a complete series of monosomic (25 bivalents + univalent chromosome) lines and a complete series of monotelodisomic (25 bivalents + univalent and telocentric chromosome) lines for the 26 chromosomes of cotton. The primary use of these lines is for the locating genes for various plant characters on specific chromosomes. Following the isolation of a monosome or a monotelodisomic, tests are made with translocation tester lines to identify the chromosome involved in the deficiency. Translocation tester lines have been developed by Dr. Meta S. Brown at the Texas Station that mark 25 of the 26 chromosomes of cotton.

The results of our 1978 tests are given in Table 2 and they show that telo 3L is associated with translocation T3-5; results last year showed that telo 3L was associated with T2-3. These two combinations establish that the telocentric chromosome is indeed chromosome 3. Telo 7L is associated with T6-7, and in 1977 it was associated with T1-7, establishing that the telocentric involves chromosome 7. In 1977, telo 9L was associated with T9-17a and this year it was observed to be associated with T9-25, establishing that this telo is chromosome 9.

Telo 10L was isolated in the monosomic Mo34 line. This year Mo34 was identified as chromosome 10 by its association with T10-19 and by its plant morphological characters which are identical to Mo54, a monosome identified last year as chromosome 10. Telo 10L is associated with the T10-19 translocation indicating that the telocentric chromosome is chromosome 10; furthermore, the monotelodisomic plants are morphologically similar to mono-10 plants, which again indicates that the telocentric is chromosome 10. Telo 12L is independent of TX-11, but in the 1977 tests it was associated with T11-12; these data establish that the telocentric is chromosome 12. Telo 25L was isolated in the line monosomic for chromosome 25, therefore, it was assumed to be chromosome 25. The test with T9-25 was positive indicating that the telocentric is chromosome 25. To establish unequivically that telo 25 and telo 10L also, are correctly identified, a second test with the appropriate translocation lines will be made in the future. The telocentric D chromosome isolated the Mo52 line is independent of the T9-25 translocation showing that the D telo is not the D chromosome number 25. Additional tests with translocations carrying D chromosomes are underway to identify this telocentric chromosome. The tests that identified Mo34 as chromosome 10 was described above.

Over the past few years tests made with several translocation lines involving chromosomes 7 and 18

gave conflicting results indicating that the chromosomes in some of these translocation lines may have been incorrectly identified. The last two tests in Table 2 involving monosomes 12 (Mo54, H12) and 18 (M10, H18) were conducted to check the chromosome identity in those particular translocations to which they were crossed. These tests show that the 1052 translocation does not carry chromosome 12, but that 1043 translocation does. The 4659 translocation line involves chromosome 18 along with an unknown chromosome.

Table 2. 1978 Cytological test for chromosome identification.

+ = associated; - = independent; A chromosomes = 1-13 and D chromosomes = 14-26.

| | TRANSLOCATION TESTER LINES | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CYTOTYPE | 8-5Gb T3-5 | 1048 T6-7 | 2870 T9-25 | 1626 T10-19 | 1052 Tx-11 | 1043 T7-12 | 8-5Ga T15-16a | 1036 T9-17a | 4659 Tx-18 | 7-3F T20-21 | 2775a T5-23 |
| D7 telo 3L/H3 | + | | | | | | | | | | |
| D14 telo 7L | | + | | | | | | | | | |
| D18 telo 9L | | | + | | | | | | | | |
| E2 telo 10L/Mo34 | | | | + | | | | | | | |
| E6 telo 12L/H12 | | | | | - | | | | | | |
| F3 telo 25L/H25 | | | + | | | | | | | | |
| F2D telo L/Mo52 | | | - | | | | | | | | |
| F15D telo L/mo58 | | | | | | | - | - | | - | - |
| E3 Mo34 | | | | + | | | | | | | |
| E6 Mo54 H12 | | | | | - | + | | | | | |
| E18 M10 H18 | | | | | | | | | + | | |
| | | | | | | | | | | | |

III. Tests for the association of marker genes with monosomes and telosomes.

The G. barbadense orange pollen mutant ($P_1P_1$ $p_2p_2$) is independent of chromosomes 1, 2, 4, 6, 16, 17, 18 and 20, establishing that the orange pollen character is not located in these chromosomes. Chromosome 25 does not include the brown lint locus $Lc_4$ nor the duplicate brown lint loci Lcy Lcz of line G201. The smooth plant mutant $Sm_1$ and telo 25L segregated independently, indicating no association. The recessive naked seed mutant $n_2$ is located in the long arm of chromosome 26.

Summary

1. The mutants ob $Y_2$ are 1.24 recombination units apart in chromosome 18. The ob locus, and most probable $Y_2$ also, are located in the short arm of the chromosome.

2. Telocentric chromosomes for the long arm of chromosomes 3, 7, 9, 10 and 25 were identified in crosses with translocation tester stocks. Monosome 34 proved to be chromosome 10.