



Utilization of Cotton Haploids in Basic and Applied Research

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ABSTRACT

Systematic cytological work in the genus *Gossypium* L. resulted in isolation of haploids ($2n=2x=26$) in tetraploid ($2n=4x=26$) *G. hirsutum*, *G. barbadense* and their F_2 interspecific crosses and monoploid ($n=x=13$) cultivated Asiatic ($2n=2x=26$) cotton. The haploids and monoploids were studied for differences in their morphological characters at haploid and diploid levels. On the basis of cytological observation in haploids and monoploids, a new concept of the basic chromosome number ($x=7$) in the genus and the polyploid nature of *Gossypium* spp. ($2n=2x=26$) is proposed. The haploids were found to be a shorter and easier means for securing desirable interspecific genetic combinations. Most interspecific hybridization reported in *Gossypium* so far are at the tetraploid and diploid levels. The success in transfer of characters in such crosses is limited by sterility in F_1 s. Development of fixed lines from these interspecific gene transfer becomes a long-term program with a low success probability. The use of *G. hirsutum* haploids ($2n=2x=26$), A_hD_h , for hybridization with different wild *Gossypium* species is a new and shorter approach in this regard.

Introduction

Hybridization between species is resorted to for securing genes or gene combinations that are not normally available within the limits of a species. Further improvement is also possible in certain characters through transgressive breeding. Most work along this line in *Gossypium* spp. has been on hybridization between tetraploid ($2n=4x=52$) x diploid ($2n=2x=26$) species. Such transfer is possible with difficulty and such breeding programs are comparatively lengthy because most F_1 s, being triploid, are sterile. Only after doubling of chromosome complements can these be backcrossed to cultivated tetraploids (*G. hirsutum* L., *G. barbadense* L.) to obtain plants with $2n=4x=52$ chromosomes having desirable character combinations (Sikka and Joshi, 1960).

Haploids have been reported in cotton via semigamy (Turcotte and Feaster, 1974), interspecific hybridization (Lee, 1970) and x-ray treatment (Mehetre and Thombre, 1977). Detailed cytological studies and morphological observations in x-ray induced haploids are reported in the present paper.

The utilization of haploids of tetraploid cultivated cotton species in interspecific hybridization with wild diploids offers scope for minimizing the period for interspecific transfer of a trait (Hougas *et al.*, 1958). Beasley (1942) and Harland (1955) discussed the potential of haploids in interspecific cotton breeding.

Material and Methods

Search for haploids was undertaken from natural populations irradiated with x-rays or isolated from F_2 populations of interspecific hybrids. The details of haploids obtained and studied are given in Table 1. The haploids were detected by critical study of their morphological characters as reported earlier by Mehetre and Thombre (1980, 1981). Cytological studies (Mehetre and Thombre, 1980), observations on stomata and chloroplast (Mehetre and Thombre, 1982), and analysis of differences among morphological characters were carried out as per Panse and Sukhatme (1957).

Results and Discussion

Use of haploids in interspecific hybridization

A new interspecific hybrid ($2n=26$) was obtained by crossing *G. hirsutum* haploid ($2n=2x=26$, A_hD_h) with *G. thurberi* Tod. ($2n=2x=26$, D_1D_1). The hybrid was found to be highly vigorous and fairly fertile. Characters in the F_1 like leaf shape, gossypol glands, boll bearing and shape, carpel wall thickness and earliness were typical of *G. thurberi* and are traits associated with bollworm tolerance. Other economic characters like plant habit, bract shape, flower colour and shape, anther arrangement, seed fuzz and presence of lint typical of *G. hirsutum* were found to be dominant. The F_1 , being partially fertile, is likely to give segregants with different character combinations on both glanded and glandless

background. The possibility of incorporating insect resistance in glandless lines has been indicated.

An interspecific hybrid between haploid *G. hirsutum* and *G. anomalum* Waw. and Peyr. was obtained and its morphological characters and meiosis studied. The hybrid had $2n=26$ chromosomes with an average of 7.2 II per PMC, the remaining chromosomes being univalents or, exceptionally, in trivalent associations. Second meiotic division was highly irregular giving rise to 1-8 sporads per mother cell and sterile pollen grains. An amphidiploid ($A_hD_hB_1B_1$) with $2n=52$ chromosomes from this combination is likely to be a good source of jassid and blackarm resistance and desirable fiber characters like strength and fineness.

Morphological observations: The data indicated that all morphological characters were reduced approximately one-half in size/number in haploids compared to the parents (Tables 2 and 3).

Cytological studies

Monoploids and haploids: The cytological studies of monoploid ($2n = x = 13$) chromosomes in *G. arboreum* and haploids of *G. hirsutum* and *G. barbadense*, and their interspecific F_2 haploids, indicated bivalent formation ranged from 0-6. Bivalent formation in monoploids indicated the presence of residual homology and the possible archaic polyploid nature of *G. arboreum* cotton with $2n=26$ chromosomes. It is likely that seven is the base chromosome number of *Gossypium* species with $2n=26$ chromosomes, and these species can now be considered as being derived from aneuploids followed by duplication of chromosomes. It is, thus, considered that the base chromosome number of the genus is $x=7$ rather than $n=x=13$ or $n=x=26$ as others have concluded from studies of somatic chromosomes (Sikka *et al.*, 1944) and interspecific crosses, (Krasichkov and Shevitchenko, 1972), respectively. This is also further confirmed from the chromosome pairing in haploids ($n=2x=26$) and in both *G. hirsutum* and *G. barbadense* cotton (Mehetre and Thombre, 1980, 1982). The pairing behavior in these plants indicated frequent occurrence of $6II + 1I$ in haploids (Mehetre and Thombre, 1977). These observations on chromosome pairing in haploids are different from those reported earlier and appear to be of significance in indicating the base chromosome number of the genus *Gossypium*.

Interspecific Hybrids involving haploids and wild species: It was possible to obtain and study meiosis in hybrids between *G. hirsutum* haploid ($1n=2x=26$, A_hD_h) x *G. thurberi* ($2n=2x=26$, D_1D_1) (Thombre and Mehetre, 1981) and *G. anomalum* ($2n=2x=26$, B_1B_1) (Mehetre and Thombre, 1982). Specific rather

than random chromosome pairing, and closer homology of D chromosomes to A than to B genome chromosomes, was also observed in these hybrids, as reported earlier (Skovsted, 1937 and Webber, 1938) on the basis of bridges and fragments (Gerstel, 1953; Gerstel and Sarvella, 1956). Previous workers have also reported 7-9 II PMC in haploids (Kimber and Riley, 1936). Thus, data on chromosome pairing in haploids indicated both homologous and nonhomologous pairing. Bivalents observed in monoploid *G. arboreum* might be due to residual intergenomic homologies and polyploid or to an aneuploid origin of the contemporary chromosome number. Certain chromosomes of the diploid lineage of monoploids might have partially or completely duplicated during the course of evolution. On the basis of trivalent formation the base chromosome number of this species might be seven and present day diploid *G. arboreum* could be considered as an allotetraploid. Secondary allotetraploid nature with $4b-2$ constitution arising by hybridization between species of 7 chromosomes is already discussed. More or less similar observations and $x=6$ as the base number of *Gossypium* also has been reported.

The polyploid nature of *G. arboreum* and base chromosome number have been confirmed from the meiotic studies in monoploids. Confirmatory evidence was also obtained from chromosome pairing in interspecific hybrids between *G. hirsutum* (haploid) x *G. thurberi* and *G. anomalum*. Thus, it is concluded that the present day *G. arboreum* is a polyploid species and the $2n=26$ number might have arisen by loss of one chromosome followed by duplication of the whole genome of $x = 7$.

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Table 1. Monoploids ($n=2x=13$) and haploids ($2n=2x=26$) recorded from different sources in *Gossypium* species and their chromosome behavior during meiosis.

| Source | Gossypium sp | Variety | Chromosome number | | No. of PMCs analysed |
|-----------------------------|---|----------------|-------------------|---------------|----------------------|
| | | | Parent | Haploid | |
| Natural (Spontaneous) | <i>G. arboreum</i> | LD 132 | $2n = 2x = 26$ | $n = x = 13$ | 30 |
| | <i>G. hirsutum</i> | AHH 468 | $2n = 4x = 52$ | $n = 2x = 21$ | 60 |
| | <i>G. barbadense</i> | Giza 45 | $2n = 4x = 52$ | $n = 2x = 26$ | 63 |
| X-ray induced Interspecific | <i>G. hirsutum</i> | HG 108 | $2n = 4x = 52$ | $n = 2x = 26$ | 30 |
| | <i>G. hirsutum</i> x <i>G. barbadense</i> | F2 of Varlaxmi | $2n = 4x = 52$ | $n = 2x = 26$ | 64 |

| Source | Gossypium sp | Variety | Average chromosome configuration | | | | | | X'ta/ PMC at M-I |
|-----------------------------|---|----------------|----------------------------------|------|------------|------|-------------|------|------------------|
| | | | Pachytene | | Diakinesis | | Metaphase-I | | |
| | | | I | II | I | II | I | II | |
| Natural (Spontaneous) | <i>G. arboreum</i> | LD 132 | 12.60 | 0.20 | 11.20 | 0.00 | 10.28 | 1.36 | 0.03 |
| | <i>G. hirsutum</i> | AHH 468 | 25.70 | 0.15 | 24.00 | 1.00 | 24.00 | 1.00 | 0.12 |
| | <i>G. barbadense</i> | Giza 45 | 24.68 | 0.66 | 23.56 | 1.22 | 23.00 | 1.50 | 0.09 |
| X-ray induced Interspecific | <i>G. hirsutum</i> | HG 108 | 21.48 | 1.86 | 17.72 | 4.14 | 15.44 | 5.46 | 0.30 |
| | <i>G. hirsutum</i> x <i>G. barbadense</i> | F2 of Varlaxmi | 20.25 | 2.87 | 21.60 | 2.20 | 19.85 | 3.32 | 0.15 |

Table 2. Mean and C.V. for eleven characteristics of monoploid ($2n=x=13$) and diploid ($2n=2x=26$) plants of *G. arboreum* LD132.

| Character | Diploid | | Monoploid | |
|-----------------------------------|---------|-------|-----------|-------|
| | Mean | C.V. | Mean | C.V. |
| Plant height (cm) | 91.80 | 16.80 | 41.50 | |
| No. of sympodia | 9.10 | 6.78 | 6.00 | |
| No. of monopodia | 3.00 | 6.96 | 2.00 | |
| Internode length (cm) | 4.98 | 8.16 | 2.78 | 12.16 |
| Petiole length (cm/petiole) | 9.87 | 8.26 | 4.98 | 26.12 |
| Leaf area (cm ² /leaf) | 98.58 | 21.96 | 3.22 | 26.12 |
| Stomata size (cm/stoma) | 32.48 | 16.69 | 21.83 | 21.96 |
| No. of chloroplast/stoma | 18.26 | 17.76 | 8.27 | 26.08 |
| No. of anthers/flower | 47.98 | 16.96 | 28.78 | 18.90 |
| Petal length (cm) | 5.12 | 4.76 | 3.98 | 6.78 |
| style length (cm) | 4.75 | 5.12 | 2.75 | 7.68 |