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Semigametic Production of Haploids in Pima Cotton[1]

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ABSTRACT

Semigamy is a type of fertilization in which a sperm nucleus penetrates the egg cell but does not fuse with the egg nucleus. The egg and sperm nuclei divide independently, resulting in a heterogeneous embryo.

Haploids sectored for maternal and paternal tissue were obtained among F_1 progenies of crosses of a semigametic line of Pima cotton (*Gossypium barbadense* L.), as female, with recessive multiple-marked stocks. True-breeding lines of paternal origin were obtained by colchicine doubling of paternal sectors of certain chimeral haploids. Thus homozygous lines of selected parentage were produced.

Semigamy was controlled genetically and transmitted through both the egg and pollen but was functional only if the egg nucleus contained the factor or factors that condition it.

The development of a semigametic, marked stock widened the scope of material from which haploids can be produced effectively.

Additional index words: **Doubled haploids, Colchicine, Gamete selection, Variety improvement, Multiple marked-stocks, *Gossypium barbadense* L.**

HAPLOID sporophytes were reported in *Datura stramonium* L. by Blakeslee et al. (2) in 1922. Harland (4) noted an abnormal cotton plant in 1920 that was identified as a haploid in 1932 (5). Since 1932 the occurrence and characteristics of haploids in cotton have been reported by several workers including Meyer and Justus (10). Haploids have been recovered more frequently in *Gossypium barbadense* L. than in *G. hirsutum* L. Within either species they are found more frequently in some strains than in others (6).

We have reported a line, doubled haploid 57-4, of 'Pima' cotton (*G. barbadense*) that produces up to 65% haploids from single-embryo seed (12). Crosses involving this high-frequency haploid-producing line, as female, and various genetically marked stocks gave progenies with a few haploid plants that were chimeral for sectors of tissue of maternal and paternal origin. The haploid- and chimera-producing abilities of this line appeared to be associated, and we suggested that the chimeral plants were the result of semigamy (13), an abnormal type of fertilization (1). Semigamy occurs when a sperm nucleus penetrates the egg cell but does not fuse with the egg nucleus. The egg and sperm nuclei divide independently, resulting in a heterogeneous embryo with sectors of tissue of maternal or paternal origin.

The occurrence of haploid cotton plants that were chimeral for maternal and paternal tissue suggested the possibility of using the semigamy phenomenon to produce, at will, haploids of selected parentage. This study reports our progress toward producing haploids at will in cotton. Additional objectives were to determine the heritability of semigamy, and to develop a semigametic, marked stock.

Haploid Production

The female parent in this study was a plant of the high-frequency haploid-producing doubled haploid, 57-4, hereafter referred to as dbl. hap. 57-4-1. The male parent, PMM-2, was homozygous for the genes cream petal (y_1y_1), orange pollen ($P_1P_1p_2p_2$), Sea Island virescent (vv)[3], and *G. barbadense* cluster ($cl\ cl$)[3]. These genes are not linked. The hypocotyl, cotyledons, stem, and first and second leaves of 643 F_1 plants from the above cross were examined for sectors of virescent, paternal tissue. Six hundred and

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[3] Homology with mutants in *G. hirsutum* is unknown.

four plants were classified normal, and two virescent. Thirty-seven plants were sectored for green and virescent, and were considered chimeral for maternal and paternal tissue. At flowering, the two virescent and 37 chimeral plants were classified haploid or tetraploid. The two virescent plants did not shed pollen, and they were typically haploid in appearance (10). They were considered either androgenetic or chimeral haploids. If the latter, the maternal tissue could have been overgrown by paternal tissue at an early stage, so that only paternal tissue was evident when the plants were examined.

Of the 37 plants scored chimeral as seedlings, two were fertile and thus tetraploid, 34 plants were considered haploid on the basis of their appearance and lack of pollen shed, and one plant died before flowering and could not be classified. At flowering, 15 haploids showed maternal characteristics only, 16 showed paternal characteristics only, and three were chimeral for maternal and paternal tissue. The chimeral haploids with maternal characteristics were considered to have the same genotype as the female parent, dbl. hap. 57-4-1. The 16 haploids expressing paternal characteristics were of primary interest, since they would be from male gametes of the Pima multiple-recessive marked stock PMM-2. These plants show that it is possible to produce haploids of selected parentage, PMM-2 in this instance, through the use of the semigamy phenomenon. In *Nicotiana*, Nitsch and Nitsch (11) outlined a method for production of haploid plants at will by stimulation of cell division in immature pollen grains.

Thirty-three of the 34 haploids mentioned above were treated with colchicine (14). Doubled sectors were observed on five plants. The doubled portion of one plant showed maternal characteristics as evidenced by fertile flowers with green bracts, yellow petals, and yellow pollen. One seed was obtained on this plant and it resulted in a haploid plant with green leaves, yellow petals, and yellow anthers. This haploid probably can be explained on the basis that the doubled portion of the plant was maternal tissue, and the maternal parent normally produces, possibly via semigamy, up to 65% haploids from single-embryo seed.

The doubled sectors of the other four plants showed paternal characteristics only. The leaves were virescent, and fertile flowers had virescent bracts, cream petals, and orange pollen. These four plants produced seed which gave four, three, three, and two plants, respectively. The 12 plants were cluster and virescent, and had flowers with cream petals and orange pollen. Thus, they were doubled haploids from gametes of the male parent PMM-2.

Seven of the 12 plants were progeny tested, four from one doubled haploid and three from another. Virescent seedlings only were obtained, confirming that the plants tested were homozygous for the virescent gene from the male parent.

These results show that through the use of semigamy, it should be possible to obtain haploid plants and, by doubling, true breeding lines of any cotton stock. Harland (7) in cotton and Kimber and Riley (8) in several angiosperms discussed the development and uses of pure lines by doubling haploids. The latter authors pointed out that the major obstacle to the use of haploids is that "no technique exists by which forms with gametic chromosome constitutions can be freely produced when required." The use of the semigamy phenomenon overcomes this obstacle in cotton.

Heritability

The process of semigamy, as it operates in our material, apparently has a genetic basis as evidenced by chimeras obtained in F_1 plants and their F_2 progenies (13). Data in Table 1 show the transmission of the semigamy phenomenon from dbl. hap. 57-4-1 to its progeny. The transmission of semigamy is indicated by chimeral seedlings among the F_1 from crosses of 10 randomly selected plants grown from dbl. hap. 57-4-1, and crossed, as female, with multiple-marked stocks. The stocks used were PMM-2, a glandless strain of Pima cotton homozygous for the genes gl_2 and gl_3 (9), and PMM-5. The latter is homozygous recessive for the genes Sea Island virescent (*vv*) and glandless ($gl_2gl_2gl_3gl_3$). The three mutant genes of PMM-5 are not linked. Chimeral seedlings were obtained in 14 of 15 crosses. The female parent which did not produce chimeral seedlings with PMM-2 did produce chimeral seedlings with $gl_2gl_2gl_3gl_3$. One cross resulted in one chimeral and one virescent seedling. The virescent seedling when grown to maturity was classified haploid. It had the cream petal color, orange anther color, and virescent plant color of PMM-2, the male parent. This indicates that the haploid was androgenetic in origin. It possibly could have been a chimeral haploid in which the paternal tissue only was evident when the seedling was examined. The number of chimeral seedlings observed was higher in the progeny of crosses involving PMM-5 (*vv* $gl_2gl_2gl_3gl_3$) as the male parent than with the crosses involving PMM-2 and Pima glandless. Thus progeny derived from dbl. hap. 57-4-1 produced chimeral plants by semigamy when they were used as the female parent.

The transmission of the semigamy phenomenon through the pollen was studied. Three hundred and fifty-six F_1 plants from a cross of Sea Island virescent, as female, with dbl. hap. 57-4-1 were scored as normal. Five of these F_1 plants were progeny tested, and each had one or more chimeral seedlings in F_2. Twenty-eight F_1 plants from a cross of a Pima glandless strain, as female, with dbl. hap. 57-4-1 were scored as normal.

Table 1. Number of normal, recessive, and chimeral plants obtained in the F_1 of 15 crosses involving 10 plants derived from dbl. hap. 57-4-1, and three marked stocks of Pima cotton.

Cross		Number of plants		
Female*	Male†	Normal	Recessive	Chimeral
Dbl. hap. 57-4-1-1	× PMM-2	176	0	0
Dbl. hap. 57-4-1-1	× $\underline{gl_2gl_2gl_3gl_3}$	70	0	2
Dbl. hap. 57-4-1-2	× PMM-2	50	1	1
Dbl. hap. 57-4-1-2	× $\underline{gl_2gl_2gl_3gl_3}$	70	0	1
Dbl. hap. 57-4-1-3	× PMM-2	103	0	4
Dbl. hap. 57-4-1-3	× $\underline{gl_2gl_2gl_3gl_3}$	111	0	3
Dbl. hap. 57-4-1-4	× PPM-2	113	0	1
Dbl. hap. 57-4-1-4	× $\underline{gl_2gl_2gl_3gl_3}$	64	0	1
Dbl. hap. 57-4-1-5	× PMM-2	101	0	1
Dbl. hap. 57-4-1-5	× $\underline{gl_2gl_2gl_3gl_3}$	204	0	13
Dbl. hap. 57-4-1-6	× PMM-5	109	0	7
Dbl. hap. 57-4-1-7	× PMM-5	106	0	7
Dbl. hap. 57-4-1-8	× PMM-5	104	0	10
Dbl. hap. 57-4-1-9	× PMM-5	100	0	10
Dbl. hap. 57-4-1-10	× PMM-5	108	0	8

* Female-parent plants designated dbl. hap. 57-4-1-1 through dbl. hap. 57-4-1-10.
† Male-parent phenotypes: PMM-2 = cream petal, orange pollen, virescent plant, and cluster plant, $\underline{gl_2gl_2gl_3gl_3}$ = glandless, and PMM-5 = virescent plant and glandless.

Seven of these F_1 plants were progeny tested. Each contained one or more chimeral seedlings in F_2. Thus, the semigamy phenomenon expressed by dbl. hap. 57-4-1 is transmitted through the pollen. Dbl. hap. 57-4-1 probably is homozygous for the genetic factor or factors that condition semigamy since all the progeny derived from it were semigametic.

Chimeral F_1's were obtained when dbl. hap. 57-4-1 was used as the female parent. However, all F_1 plants were normal when dbl. hap. 57-4-1 was used as the male parent. This indicates that for semigamy to occur the egg nucleus must contain the factor or factors that condition semigamy. A sperm nucleus from a semigametic male parent appears not to induce semigamy if the egg nucleus is normal. In the parasitic wasp *Habrobracon juglandis* (Ashmead), Clark, Gould, and Potts (3) found that the production of several types of mosaics was associated with the ebony body color mutant. They determined that for mosaic and androgenetic progeny to be produced, the female had to be homozygous for ebony. The presence or absence of the ebony mutant in the sperm did not affect the production of mosaics; however, the sperm was involved as only mated females produced aberrant types. Data from Clark et al. provide additional evidence for genetic control of specific events before and after fertilization.

Semigametic, Marked Stocks

When using dbl. hap. 57-4-1, the production of paternal haploids via semigamy is limited to genetically marked material. It seemed desirable to develop marked, semigametic lines, so that normal material can be used as the male parent. Eleven virescent plants were selected at random in F_2 from a cross of dbl. hap. 57-4-1, as female, with Sea Island virescent. Progeny tests showed these 11 plants were homozygous virescent. The number of green, virescent, and chimeral seedlings produced by crossing each of 11 virescent F_2 plants, as female, with commercial 'Pima S-4' is shown in Table 2. Six progenies contained green seedlings only, three had green and chimeral, one had green, virescent and chimeral, and one had green and virescent. Larger populations from those crosses which produced green seedlings only might well contain virescent or chimeral seedlings. Green and chimeral seedlings were expected from these crosses, but the virescent seedlings were unexpected. The virescent seedlings were grown and examined at flowering. They were determined to be haploid. They were considered either maternal haploids, or chimeral haploids in which the maternal, virescent tissue overgrew and masked the paternal, green tissue at an early stage of development.

The presence of chimeral plants in progenies of four crosses indicates that the virescent-marked, female parent of these four crosses was semigametic. Thus, the phenomenon of semigamy has been recovered in a marked stock. This permits the production of haploids at will and their identification from unmarked stocks, thus widening the scope of material from which haploids can be produced effectively.

Table 2. Number of normal, virescent, and chimeral cotton plants obtained in the F_1 of crosses involving 11 virescent F_2 plants, derived from the cross dbl. hap. 57-4 × virescent, and Pima S-4.

Cross			Number of plants		
Female		Male	Normal	Virescent	Chimeral
Virescent-1	×	Pima S-4	97	0	2
Virescent-2	×	Pima S-4	72	1	0
Virescent-3	×	Pima S-4	90	0	2
Virescent-4	×	Pima S-4	76	0	0
Virescent-5	×	Pima S-4	90	1	1
Virescent-6	×	Pima S-4	29	0	0
Virescent-7	×	Pima S-4	41	0	1
Virescent-8	×	Pima S-4	55	0	0
Virescent-9	×	Pima S-4	70	0	0
Virescent-10	×	Pima S-4	72	0	0
Vireseent-11	×	Pima S-4	19	0	0

CONCLUSIONS

Semigamy can be employed to produce haploids at will, and, by doubling, pure lines of Pima cotton. The original semigametic line had normal characteristics, thus limiting haploid production via semigamy to marked stocks. The fact that semigamy is transmitted through the egg and pollen permits the development of semigametic, marked stocks. The use of a semigametic, marked stock, as female, allows the production of haploids and their identification within any cotton strain. In our work, emphasis will be placed on the production of haploids within early-generation, widely-segregating material. Doubling these haploids should aid in establishing new combinations of characters.

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