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AN EFFORT TO PRODUCE A HOMOZYGOUS DOMINANT MALE-STERILE COTTON[1]

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ABSTRACT

Two techniques were used in an effort to develop double-haploid plants homozygous for dominant male-sterile genes. One approach was to find a haploid seedling that carried the dominant gene Ms_4 from a twin embryo seed and double the chromosomes with colchicine. One of the two seedlings from twin embryos is normally expected to be haploid. Four twin embryos were found in about 100,000 seed germinated from Ms_4 ms_4 male-sterile plants but no double-haploid plants were produced from these seedlings. Another approach for the production of a haploid was the utilization of crosses between *Gossypium hirsutum* L. male-steriles (Ms_4ms_4 and Ms_7ms_7) and double-haploid 57-4, Pima cotton *(G. barbadense* L.). Two haploid plants were observed in the first backcross progeny of (Ms_7ms_7 x DH 57-4) male-sterile x DH 57-4. Treatment of apical meristems of haploid plants with colchicine produced diploid tissue on one of the plants. The flowers of resulting double-haploids were fertile, indicating that the haploid plant did not carry the gene for dominant male-sterility.

Deficiency in the expected number of male-sterile plants was observed in the majority of the segregating families. Appearance of fewer male-sterile plants than the expected 50% for the dominant male-sterile genes in the present study might be due to some kind of genetic, embryonic or zygotic lethality.

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INTRODUCTION

Several genes conferring male-sterility have been identified in Upland cotton, *Gossypium hirsutum* L. These genes are ms_1 (3), ms_2 (7), ms_3 (4), Ms_4 (1), ms_5 ms_6 (12), Ms_7 (14) and Rhyne's male-sterile genes (9). Each of these has potential but somewhat limited usage as a means for utilizing hybrid vigor in cotton on a practical scale. One important consideration in the commercial production of hybrid cotton seed is large-scale production of male-sterile plants. Although ms_2, Ms_4, ms_5ms_6 and Ms_7 stocks are capable of producing complete male sterility, a 1:1 ratio of male-sterile to male-fertile plants is the maximum ratio capable of being produced in any given lot of seed. Weaver and Ashley (14) proposed a double-haploid technique for producing homozygous dominant male-sterile cotton plants. Crosses with homozygous dominant male-sterile plants would produce all male-sterile offspring.

Haploids (n=2x=26), from twin-embryo seed, occur at a very low frequency in cultivated American Upland cottons. Double-haploids are of interest to research workers because they provide an easy way of obtaining genetically pure lines. The production of large numbers of haploid plants in Pima cotton (*G. barbadense* L.) is now possible by the use of the semigamy phenomenon reported by Turcotte and Feaster (10,11).

The purpose of this paper is to report efforts to develop double haploids homozygous for dominant male-sterile genes.

MATERIALS AND METHODS

Two methods were tested for the production of haploid plants with a dominant male-sterile gene. The first method consisted of germinating seed collected from Ms_4 male-sterile plants. The germinated seedlings were classified for twin embryos. Approximately 100,000 seeds were germinated over a period of two years, 1970-72, in a germinator. Seeds with two embryos were placed in pots and all others were discarded.

In the second method the double haploid 57-4 (hereafter referred to as DH 57-4) of Pima cotton, obtained from Dr. Turcotte (Geneticist, ARS, USDA, University of Arizona Cotton Research Center, Phoenix, Arizona), was used as a pollen parent in the crosses because it produces a high number of haploids (65%) from single embryo seeds (10).

In the summer of 1970 crosses were made between dominant male-sterile plants, Ms_4ms_4 and Ms_7ms_7 and DH 57-4 at the Plant Sciences Farm, University of Georgia. F_1 hybrid seed was planted in a greenhouse during the winter of 1970-71 and the progeny was classified for sterility and ploidy. Sib-crosses were made between F_1 male-sterile and fertile plants. The F_1 male-sterile plants were also backcrossed to DH 57-4. Seeds were collected from the male-sterile plants and were planted in the field during the summer of 1971. Crosses involving F_1 male-sterile (Ms_7ms_7 x DH 57-4) backcrossed to DH 57-4 produced two haploid plants. The haploid plants were identified by their smaller plant parts and also by their inability to set seed on cross pollination.

The haploid plants were transferred to a greenhouse and maintained in pots. One percent colchicine was applied to the axillary buds of these haploid plants to double their chromosome number.

RESULTS AND DISCUSSION

Crosses of male-sterile Ms_4ms_4 plants with pollen from ms_4 ms_4 produced 35 male-sterile and 51 fertile plants during the summer of 1971. The χ^2 value for 1:1 ratio was 2.98, with a probability value of 0.05 - 0.10. A total of 127 male-sterile and 197 fertile plants was observed from crosses involving Ms_7ms_7 and fertile sibs. The percentage of male-sterile plants was 39.2 instead of the expected 50% for a single dominant gene. Chi-square for fit to a 1:1 ratio showed a significant deviation from the expected.

During the summer of 1972, two haploid plants were found in the first backcross progeny of male-sterile (Ms_7ms_7 x DH 57-4) and DH 57-4. From a total of 132 plants scored, 68 were fertile, 64 were male-sterile diploids and two were haploid plants. The haploid plants were both male and female-sterile, did not set seed when cross-pollinated in the field and had characters typical of haploid plants. Treatment of apical meristems of haploid plants with colchicine produced homozygous diploid tissue in one of these plants. The diploid tissue produced thicker stems and larger leaves with dark green color. The flowers of the doubled haploids were fertile and produced one self-pollinated and two cross-pollinated seeds. The seed, when planted in a greenhouse during the 1972-73 winter, produced three fertile plants, indicating that the original haploid plant did not carry the dominant gene for male-sterility.

During the summer of 1973, twenty-three plants of (Ms_7ms_7 x DH 57-4) x DH 57-4 BC_3 were grown in the field. Eleven of the plants were male-sterile but none were haploid. Twelve BC_4 plants were grown in the greenhouse during the winter of 1973-74 but none were haploid. Thus, it appears that the presence of the Ms_7 gene prevents or greatly reduces the occurrence of haploid plants. The DH 57-4 as a pure line produces approximately 50% haploid seedlings.

The deficiency in the expected number of male-sterile plants observed in this study follows the same pattern as previously reported for Ms_4 (13) and Ms_7 (14) dominant male-sterile genes. Weaver (12,13) reported the deficiencies of male-sterile plants for ms_2ms_2 and $ms_5ms_5ms_6ms_6$. Although the deficiency of male-sterile plants was observed in all the crosses involved in the present study, there were some families that segregated for sterility in the expected Mendelian ratio.

Weaver (13) indicated that there might be some unexplained factors associated with genetic male-sterility in cotton, causing deficiencies in the expected number of male-sterile plants in the majority of segregating ratios. Appearance of fewer male-streile plants than the expected 50% in the present study might be due to some kind of lethality or other associated factors. Lethal factors may cause death of the zygote or embryo of the affected individuals. The effect of lethal genes may vary, so that not all genotypically affected individuals are phenotypically lethal. Some lethal genes might have a high degree of expression, permitting little or no survival of affected genotypes. Others might permit larger proportions of affected genotypes to survive. Male-sterile genes, recessive or dominant, may be associated with lethal factors and their effect may be expressed in the appearance of a fewer number of male-sterile plants than expected. The present studies are inconclusive. Further research is needed to recognize the suspected lethal factors associated with male-sterile genes.

The frequency of haploids in Upland cotton is one in several thousands. Development of a hypothetical double haploid plant of Upland cotton with homozygous dominant male-sterile genes might be difficult. Association of suspected lethality with dominant genetic male-sterility and appearance of fewer male-sterile plants than expected might make it even more difficult to produce a haploid plant with a dominant male-sterile factor. The expression of the suspected lethality might be such that a haploid plant with a dominant male-sterile gene may not be able to survive beyond the embryonic stage.

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