Published January, 1966

Identification of Super Cup Mutant in Cotton, *Gossypium hirsutum* L.[1]

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ABSTRACT

An aberrant plant with an extreme cup leaf expression was found in a population of true breeding cup leaf plants. The mutant was called super cup. Cytological analysis revealed that the super cup mutant was a trisomic plant. A crossing program with known translocations identified the replicated chromosome as chromosome 4 of the A genome. Genetic tests determined that the cup leaf locus is independent of the replicated chromosome; and the super cup phenotype is due to an interaction of the cup leaf locus with the replicated chromosome.

CUP LEAF was the name given to a simply inherited upland cotton mutant reported by Lewis[3] in 1954. Phenotypically this mutant is characterized by an upward rolling of the edges of the leaf blades, which gives the leaves a cupped shape. Although the mutant is considered a recessive, the heterozygote has an intermediate degree of the cup leaf expression. An aberrant plant was found with an extreme cup leaf expression in a population of true breeding cup leaf plants. This plant was called super cup, and seeds of the stock have been maintained at this station for several years as a potential genetic marker.

The purpose of this paper is to report the results of studies of the super cup mutant in an attempt to place the cup leaf locus on the linkage map and to identify the replicated chromosome.

PROCEDURE

In 1960, an observational planting of the super cup stock was grown to determine the feasibility of beginning a study of the inheritance of the mutant character. This population contained 14 plants; 6 of these were classified as cup leaf and 8 as super cup. The super cup plants lacked vigor and were sterile under field conditions. Therefore, they were ratooned and taken to the greenhouse where they were grown during the winter. They were more vigorous under greenhouse conditions and it was possible to make controlled crosses. We grew inbred progeny from the super cup plants, and the F_1 hybrids between super cup and cup leaf, and between super cup and a normal leaf inbred line. The super cup inbred progeny segregated 16 cup leaf to 21 super cup, and the F_1 (super cup $\times$ cup leaf) segregated 5 cup leaf to 5 super cup. Segregation in the inbred progeny of super cup, and the F_1 hybrid between super cup and cup leaf, suggested the possibility that super cup was a heterozygote and that the homozygote was lethal. Several mutant characters in cotton are inherited in this manner.[4] However, the 10 F_1 (super cup $\times$ normal) plants were all intermediate for the cup leaf expression, which failed to support the first hypothesis. The inability to fit the data for such discrete segregation to a simple genetic model suggested the possibility that super cup might be due to a cytological aberration. The super cup inbred progeny were analyzed cytologically, and it was established that super cup plants were trisomic while cup leaf plants were disomic.

[1] Contribution from the Crops Research Division, ARS, USDA, in cooperation with the Texas Agricultural Experiment Station. Received Aug. 27, 1965.

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[3] Lewis, C. F. 1954. The inheritance of cup leaf in cotton. J. Hered. 45:127-128.

[4] McNamara, H. C., and D. D. Porter. 1950. Heritable abnormalities in cotton and their segregation ratios. J. Hered. 41:310-315.

RESULTS

The association of the super cup phenotype with the trisomic cytotype suggested a dosage effect due to linkage of the cup leaf locus and the trisomic chromosome. Additional phenotypic evidence for placing the cup leaf locus on the trisomic chromosome came when two extreme super cup plants were recovered in a super cup inbred progeny and were assumed to be tetrasomic. These plants were maintained for several seasons in the greenhouse but were never used successfully in crosses or analyzed cytologically. However, a few inbred seeds were obtained.

Recovery of a tetrasome from a trisome would require pollen transmission of the extra chromosome. We had not expected the transmission of (n+1) gametes through the pollen. Theoretically, the expectation is a 1:1 ratio of (n) and (n+1) gametes. In cotton, a 1:1 gametic ratio is approximated in the eggs, but generally only (n) gametes function or are recovered as pollen as a result of competition between (n) and (n+1) gametes.

In the 10 F_1 (super cup $\times$ cup leaf) plants the ratio of (n) and (n+1) gametes transmitted in the eggs was 1:1. When the total of all inbred progenies of super cup were considered, 56% of the 64 plants were super cup. Even with perfect recovery of 1:1 gametic segregation in the eggs, the reduced vigor of the super cup plants would be expected to reduce the super cup class rather than to produce an excess. Undoubtedly, pollen transmission of (n+1) gametes contributed to the larger-than-expected numbers of plants in the super cup class. Proof of pollen transmission of (n+1) gametes was established later when cytological analysis revealed that a tetrasomic plant was recovered in the advanced generation of a cross of super cup with normal. The tetrasome was not homozygous for cup leaf genes and this facilitated the cytological analysis.

Genetic Tests of Linkage

The preliminary phenotypic data indicated that the cup leaf locus was located on the trisome. The 10 F_1 plants from the (super cup $\times$ normal) cross were grown before super cup was known to be a trisome; therefore the plants were not analyzed cytologically. Inbred seeds were available from 9 of the F_1 plants, and 9 F_2 populations were grown. These were scored for cup leaf segregation and analyzed cytologically. A total of 159 plants were grown and 61, or about 38%, were analyzed successfully. Three of the 9 F_2 populations segregated for trisomes or tetrasomes. In the 6 F_2 populations in which only disomes were detected, the cup leaf segregation was 30 normal, 61 intermediate cup leaf, and 28 cup leaf plants. This closely fit the expected 1:2:1 ratio ($p=0.95-0.90$). If there was linkage of the cup leaf locus and the trisomic chromosome, the F_1 trisome would have had one normal allele and two cup leaf alleles. In the 3 F_2 populations originating from trisomic F_1's, the segregation was 14

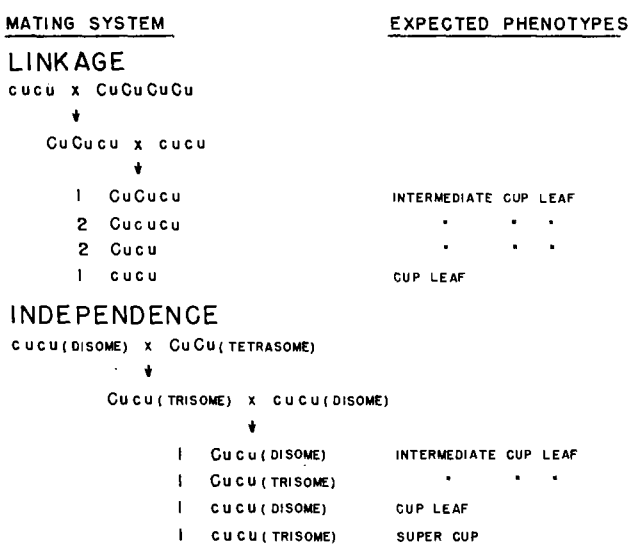

Figure 1. Test of association of cup leaf locus with replicated chromosome.

normal, 30 intermediate cup leaf, 4 cup leaf, and 2 super cup plants. Recovery of 14 plants with normal phenotype in a population of 50 was greater than expected with trisomic segregation. Also, trisomes and tetrasomes were recovered with the normal phenotype. Possibly the cup leaf genes were masked by normal alleles but progeny tests of the trisomes and tetrasomes all appeared normal. In a further progeny test the trisome was crossed to disome cup leaf. The entire population was heterozygous cup leaf, a fact which indicated that the trisome was homozygous normal. This evidence indicates that the cup leaf locus is not on the replicated chromosome.

We used the phenotypically normal tetrasome to carry out an additional test. From the extreme super cup "tetrasome" only "terasome" types were recovered, and the only four plants analyzed from the phenotypically normal tetrasome parent were tetrasomic. This indicated that the tetrasomic condition is stable and that only (n+1) gametes are formed. The phenotypically normal tetrasome was crossed as male to a disome cup leaf line. The resulting population should be all trisomes (cytological analyses verified that the 9 F_1's used in this test were all trisomes), with one dose of the cup leaf allele; any duplication will be of the normal allele. This trisome was crossed as female to disome cup leaf. The tests, mating system, and expected segregation are shown in Figure 1.

Assuming linkage, a phenotypic ratio of 5 intermediate cup leaf to 1 cup leaf was expected. Assuming independence, the cyto-genotype combinations were expected in equal frequencies; and phenotypically the expectations were for a ratio of 2 intermediate cup leaf, 1 cup leaf, and 1 super cup. The expectation of a super cup class associated with independence is the critical feature of this test.

From 9 F_1 trisomes, 93 testcross progeny were grown. The observed segregation was 41 intermediate cup leaf, 28 cup leaf, and 24 super cup in the ratio of 2:1:1 ($p=0.5-0.3$). These data firmly establish the independence of the cup leaf locus and the replicated chromosome. The phenotypic evidence, at first interpreted as dosage effects due to replication of the cup leaf locus, must be interpreted as a form of interallelic interaction between the cup leaf locus and the replicated chromosome.

Cytological Analyses

Upland cotton is an allotetraploid composed of 13A genome and 13D genome chromosomes. Cytologically a gross separation of the genomes can be made by size of the chromosomes. The A genome is made up of large chromosomes, and the D genome is made up of small chromosomes. The super cup trisome involved a large chrosomes and was therefore tentatively identified as an A chromosome.

A crossing program was begun to test the trisome with translocations involving known A chromosomes. The first cross made and analyzed was with a translocation between chromosomes 4 and 5. The A chromosomes are assigned the number 1-13. This test was positive and identified the trisome as either chromosome 4 or 5. The only other translocation involving either 4 or 5 that is available is an A-D translocation involving chromosome 4 and 15. This cross was made with the tetrasome as female and TT4-15 as male. The F_1 was analyzed cytologically, and the replicated chromosome was found to be associated with the translocation. Therefore, the replicated chromosome is chromosome 4 of A genome.

Frequency of Pollen Transmission of (n+1) Gametes

The frequency of pollen transmission of (n+1) gametes from the trisome was checked by crossing super cup as pollen parent to the normal disome. In the F_1, trisomic plants were expected to be more cupped than the disomes. One-hundred-eleven seedlings were grown and classified in the greenhouse. The phenotypic differences were small but a total of 20 plants were selected as possible trisomes. Cytological analyses were completed on 18 plants and 12 were trisomes and 6 were disomes. If this frequency was maintained in the remaining 2 plants, and if the 20 plants selected contained all the trisomes, 1/7 of the pollen was (n+1). If we assume that the excess of the super cup class was due to pollen transmission of (n+1) gametes, estimates from the inbred progeny of super cup showed that 1/8 of the pollen was (n+1). The lower estimate in the super cup inbred progeny probably reflected the reduced vigor of plants in the super cup classes. Throughout this study it has been noted that the last seeds to germinate yield a higher frequency of super cup plants than the remainder of the population. This slower rate of germination makes them more subject to the soil-borne seedling diseases.

ACKNOWLEDGMENT

The author wishes to acknowledge the contributions of Drs. J. E. Endrizzi and Meta S. Brown for cytological analyses performed in the course of this study.