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A NEW LINKAGE RELATIONSHIP IN COTTON¹

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ABSTRACT

A chromosome, or a chromosomal segment, transferred from *Gossypium davidsonii* Kell., an American diploid taxon, to the glandless *G. barbadense* stock, 15-4, harbored the gland-determining allele, Gl_3^{dav} , and the complementary lethal factor, Le^{dav} . Linkage between the genes was estimated at 25.9 ± 3.0 recombination units.

Additional index words: *Gossypium barbadense* L., *Gossypium anomalum* Wawra ex Wawra & Peyr., *Gossypium davidsonii* Kell., *Gossypium klotz schianum* Anderss., *Gossypium hirsutum* L., Complementary lethality.

Viable offspring are rarely obtained from crosses of *Gossypium davidsonii* Kell., an American diploid taxon designated D_3 in the genomic nomenclatural system of Beasley (1), with the tetraploid cottons, *G. barbadense* L. and *G. hirsutum* L. (4, 6). A kind of complementary lethality acting during embryonic or seedling development has been suggested as the cause of failure (4, 5, 6). Lee and Smith (2) described 15-4, a glandless stock of *G. barbadense* that is exceptional in that it crosses readily with D_3 , giving vigorous triploid plants. These triploids are easily transformed into hexaploids by treating seedlings with colchicine.

Among a group of pentaploid plants produced by crossing 15-4 back to one such hexaploid were a few that displayed evidence of being duplex for a leaf-gland allele.

One of these pentaploids was selected for earliness of fruit set and backcrossed again to 15-4.

I experienced no difficulty following the phenotype of the allele in question through two additional backcrosses to 15-4, but when the *G. barbadense* stock, glandless Pima S-4, was substituted to make an additional backcross, most of the gland-bearing seedlings in the ensuing generation showed necrotic flecks by the 5th day after germination and died shortly thereafter. Necrosis and early death are the classic signs of D_3 complementary lethality in crosses between *G. davidsonii* and its near relative, *G. klotz schianum* Anderss., and many stocks of tetraploid cottons (4, 6). Pima S-4 bears factors that act in a complementary fashion to promote seedling lethality in crosses with D_3 .

Evidently a complementary lethal factor had accompanied the gland-determining allele from D_3 as the latter was transferred into the 15-4 background. Linkage was suspected. The objectives of the work reported herein were to relate the gland allele from D_3 to a suspected homeologue in *G. hirsutum*, and to estimate linkage between the gland allele and the complementary lethal factor that seemed to be accompanying it.

METHODS AND RESULTS

A plant apparently heterozygous for the gland allele in question was selected from the third backcross of the pentaploid with 15-4 and selfed to produce plants homozygous for the gland-determining allele. Fifteen of the homozygous plants thus recovered were crossed with the *G. hirsutum* stock, 17-B-1, a line monomeric for Gl_3 , the weaker of the two leaf-gland alleles in *G. hirsutum*. This stock, genotype $gl_2gl_2Gl_3Gl_3$, produces embryonic lethality in crosses with D_3 . The cotton has normally-glanded cotyledons and reduced numbers of glands on mature plant parts. Only 2 of the 15 selected plants produced viable seedlings when crossed with 17-B-1. Survivors from both plants were grown out and selfed. Neither group of populations segregated for gland expression on cotyledons, so I concluded that the gland allele derived from D_3 resided at the gl_3 locus and designated it Gl_3^{dav} .

One of the 13 plants that failed to produce viable seedlings when crossed with 17-B-1 was crossed with 15-4 to produce plants that were heterozygous for both Gl_3^{dav} and the accompanying D_3 complementary lethal. Five of the resulting F_1 plants were crossed with glandless Pima S-4 to test for linkage between the alleles in question. Each progeny contained the four classes expected. The proportions of each phenotypic class in each of the five progenies proved to be homogeneous ($p = 0.10$ to 0.25), so the data were pooled for a linkage estimate (Table 1). In the 15-4 background linkage between Gl_3^{dav} and the D_3 complementary lethal factor, designated Le^{dav} , was estimated at 25.9 ± 3.0 crossover units.

Table 1. Phenotypic ratios from the linkage test and linkage estimate.

Phenotypes	No. plants in class	Linkage estimate
Glanded lethal	75	25.9 ± 3.0
Glanded viable	32	
Glandless lethal	23	
Glandless viable	82	

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DISCUSSION

Phillips (3) induced a tetraploid from a cross of D_3 with the African diploid, *G. anomalum* Wawra ex Wawra & Peyr. The latter species, designated B_1 by Beasley (1), is one of the very few taxa of *Gossypium* that regularly produces viable hybrids in crosses with D_3 (5, 6). In a cross of the derived tetraploid, $2(B_1D_3)$, with *G. hirsutum*, Phillips recovered two viable hybrid plants. He reasoned that there had been intergenomic recombination in the newly-created tetraploid and that had led to the elimination of a complementary lethal factor from the D_3 genome. The possibility of mutation at such a locus was not ruled out, but even so, the exercise provided good evidence for the occurrence of a single complementary lethal factor in the D_3 genome. Transfer of a D_3 complementary lethal factor to *G. barbadense* and documentation of linkage of the gene with Gl_3^{dav} is further evidence supporting a single-factor hypothesis.

The finding of a single complementary lethal factor in D_3 fosters the expectation that the lethal complement of *G. barbadense* and *G. hirsutum* might be dependent upon one or perhaps two genes. Skovsted (5) considered such a possibility some 43 years ago. The evidence I have accumulated thus far suggests that no more than two factor pairs are involved, and that these act in an accumulative manner in determining the time of the onset of lethality in plant development. The more of these potentially lethal alleles present to be challenged by Le^{dav} , the earlier the onset of necrosis and death.

Is Le^{dav} allelic with any of the complementary factors in the tetraploid cottons? Such information does not appear to be obtainable by direct methods of testing, inasmuch as any combination of the alleles with Le^{dav} leads to the death of the plant harboring them. Testing for linkage between the "native" leaf-gland alleles of the tetraploids and the complementary lethal factors of these cottons could afford a clue. Before this objective can be accomplished, the complementary alleles of the tetraploids must be isolated and their expressions documented. The use of a tetraploid stock homozygous for Le^{dav} as a tester promises to facilitate such an effort.

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EFFECTS OF DIFFERENT TYPES OF SAND AND VERMICULITE GRADES ON THE TEST TUBE METHOD FOR EVALUATING PREINOCULATED SEED¹

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ABSTRACT

Combinations of two types of sand and two grades of vermiculite in test tubes (25 × 200 mm) were evaluated for growth of seedlings with root nodules at 21 days of age. *Rhizobium*-inoculated alfalfa seeds were planted two per test tube. Each type of sand was suitable for testing provided it was washed free of fine particles. Seedlings grown in substrate combinations which included the vermiculite having smaller (0.32 cm) particle sizes produced fewer nodules regardless of the sand type.

Additional index words: *Medicago sativa* L., Growing medium, Emergence, Nodulation.

THE Association of Official Seed Analysts Handbook Contribution No. 30³ describes three methods of testing preinoculated seeds utilizing evaluations of seedling growth and nodulation. Vermiculite and sand are recommended as a growing medium but precise specifications are not given. Particle size and/or textures of growing media are known to regulate the water holding capacity, moisture availability, and root aeration. The effects of two grades of vermiculite and two types of sand on emergence and nodulation of alfalfa were investigated

according to the general procedures described in the Handbook for testing preinoculated seed. Two seeds were planted in each test tube to conserve growth chamber space.

MATERIALS AND METHODS

Twenty cubic centimeters of vermiculite were added to the 25 × 200 mm test tubes. Thirteen milliliters of N-free nutrient solution were then pipetted into each tube at a slow rate to minimize disturbance of the vermiculite and prevent air pockets from forming. Five cubic centimeters of sand were added to form a level, smooth layer over the vermiculite. The tubes were covered with aluminum foil, autoclaved 30 min at 1.05 kg/cm² and cooled. Two *Rhizobium* inoculated alfalfa seeds were placed on the sand in each tube and covered with 2.5 to 3.0 cm³ of sand. An additional 2 ml of nutrient solution was pipetted on the wall of each tube. The tubes were covered with polyvinyl film and placed in a dark germinator at 18 to 20 C for 72 hours. Subsequently, the tubes with emerging seedlings were placed in a growth chamber with a 22 to 24 C range where they received 12 hours of light at approximately 1,000 lux intensity. The total duration of the growing period was 21 days from the time of planting. No additional moisture was added during the test.

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