

# A Genetical Scheme for Isolating Cotton Cultivars<sup>1</sup>

Joshua A. Lee<sup>2</sup>

## ABSTRACT

When the diploid cotton (*Gossypium davidsonii* Kell.) species, is crossed with cultivars of the tetraploid species, *G. hirsutum* L. and *G. barbadense* L., the hybrid embryos abort, or the seedlings become necrotized and moribund shortly after germination. A complementary lethality interreaction is indicated, the cultivated cottons contributing the factors  $Le_1$  and  $Le_2$ , whereas *G. davidsonii* contributes at least one factor,  $Le^{dav}$ .

A rare genotype in *G. barbadense* is  $le_1le_1le_2le_2$ . Crosses between plants of this genotype and *G. davidsonii* yield viable triploids. Using the technique of hexaploid bridging, I transferred  $Le^{dav}$  from *G. davidsonii* to the cross-compatible stock, 15-4. The resulting stock—genotype  $le_1le_1le_2le_2Le^{dav}Le^{dav}$ —is not cross-compatible with a series of cultivars of *G. hirsutum* and *G. barbadense*.

Utilizing the  $le$  and  $Le^{dav}$  alleles, I produced stocks of *G. hirsutum* that proved to be genetically isolated from a series of *G. hirsutum* cultivars under both greenhouse and field conditions. The use of the  $le$ - $Le^{dav}$  genetical system for isolating cotton cultivars grown for special purposes, such as gossypol-free seeds, is discussed.

**Additional index words:** *Gossypium barbadense* L., *Gossypium davidsonii* Kell., *Gossypium hirsutum* L., Complementary lethality, Glandless cotton, Hybrid cotton, Species hybrids.

GROWERS producing crops for special purposes often run the risk of having their product contaminated through outcrossing with cultivars of the same, or closely related, species grown in adjacent fields. If such crops could be isolated, great savings would be realized in maintaining seed purity and commercial acceptance. Glandless cotton appears to be a case in point. This phenotype of *Gossypium hirsutum* L. has gained a moderate degree of acceptance in Texas because of the superior value of products from the gossypol-free seeds. However, the hectareage planted to glandless cotton is, at present, relatively small when compared with that of normally-glanded cultivars. Maintenance of gland-free seed in glandless cultivars thus remains a problem even under conditions where the population of pollen vectors is sparse. In this report, I describe a system of cultivar isolation that employs complementary lethal factors, the alleles involved having been derived from the tetraploid species, *G. barbadense* L., and the diploid species, *G. davidsonii* Kell.

<sup>1</sup> Contribution from AR-SEA-USDA and the North Carolina Agric. Res. Ser., North Carolina State Univ. J. Ser. Paper No. 6508. Received 2 Sept. 1980.

<sup>2</sup> Geneticist, AR-SEA-USDA, and professor of crop science, North Carolina State Univ., Raleigh, NC 27650.

## MATERIALS AND METHODS

The genetics of complementary lethality between *G. davidsonii* and the cultivated tetraploid cottons have been presented elsewhere (2, 3). To review briefly, the cultivated cottons examined proved to be homozygous for the factors  $Le_1$  and  $Le_2$ , whereas *G. davidsonii* harbored the factor,  $Le^{dav}$ . In crosses between the diploid and tetraploid species,  $Le^{dav}$  interacts with  $Le_1$  and  $Le_2$  to condition either embryonic or seedling lethality.

An experimental line of *G. barbadense*, glandless 15-4, proved to be of the genotype  $le_1le_1le_2le_2$ . This cotton is cross-compatible with *G. davidsonii*, producing vigorous triploid plants from crosses with the diploid species (4). Through the technique of hexaploid bridging, I transferred  $Le^{dav}$  from *G. davidsonii* to 15-4, producing a cotton of the genotype,  $le_1le_1le_2le_2Le^{dav}Le^{dav}$ . Utilizing the  $le$  and  $Le^{dav}$  factors, I subsequently bred lines of both *G. hirsutum* and *G. barbadense* that are cross-compatible *inter-se* but are not cross-compatible with cultivars harboring  $Le_1$  and  $Le_2$ .

The experience of transferring the  $le$  and  $Le^{dav}$  alleles among tetraploid stocks provided convincing evidence that the barrier to gene flow is absolute when stocks harboring various combinations of  $Le_1$  and  $Le_2$  are challenged with the  $Le^{dav}$  allele. However, more formal experiments were required for purposes of documentation, so the following stocks and procedures were selected to attempt to demonstrate the effectiveness of the lethality barrier. NC-150-Compatible and NC-150-Incompatible are glandless lines of *G. hirsutum* stemming from a single  $F_2$  population. The former is of the genotype  $le_1le_1le_2le_2$  and is thus compatible with stocks harboring  $Le_1$ ,  $Le_2$ , and  $Le^{dav}$ . I have no data on the allelic relationships, if any, between the  $le$  and  $Le^{dav}$  alleles, so I shall write genotypes as if the genes were non-allelic. Furthermore, I have no evidence that *G. hirsutum* harbors an  $le^{dav}$  locus, so I shall omit such from the genotype of NC-150-Compatible. The genotype  $le_1le_1le_2le_2Le^{dav}Le^{dav}$  was assigned to NC-150-Incompatible. The stock should thus be cross-compatible with NC-150-Compatible but not with cultivars harboring  $Le_1$  and  $Le_2$ .

Two kinds of experiments were performed. In the first, both entries were grown in the greenhouse, and flowers were emasculated and pollinated with 'Coker 310,' a *G. hirsutum* cultivar proven to be homozygous for  $Le_1$  and  $Le_2$  (2). Ten cross-pollinated bolls per entry were harvested at maturity, the seed germinated, and the phenotypes of the seedlings scored. Cross and Richmond (1) advocated the use of glandless-seeded cottons in hybridization studies, and the phenotype proved useful in these studies. Gland on seedlings were taken as evidence of successful hybridization.

The second experiment consisted of planting the two entries in a cotton breeding nursery at the Central Crops Research Station, Clayton, NC. Five 17 m plots of each entry were distributed at random about the nursery which was about 0.5 ha in area. The surrounding cottons were all glanded and most were based upon germplasm of locally adapted cultivars. A random sample of open-pollinated seed was taken from each plot, 100 seeds from each sample germinated, and the seedlings scored for phenotype. The presence of glands was taken as evidence for cross-pollination.

**Table 1. Phenotypes of seedlings from pollinating two stocks of cotton with Coker 310 under greenhouse growth.**

Phenotype	Glanded	Glandless
NC-150-Compatible	240	1
NC-150-Incompatible	0	3

## RESULTS AND DISCUSSION

In the greenhouse experiment, NC-150-Incompatible produced only three viable seedlings in 10 pollinations, and these proved to be glandless (Table 1). The stigmas of emasculated flowers were not treated with dilute ethanol, as is usually routine with greenhouse crossing programs at Raleigh, therefore I attributed the recovery of the three seedlings to self-pollination. One of the three plants was grown out, and it proved to be homozygous for  $Le^{dav}$ . NC-150-Compatible produced 241 viable seedlings from 10 pollinations, and all but one bore glands. These results were anticipated and suggest that the barrier between Coker 310 and NC-150-Incompatible is absolute, whereas germplasm exchange between Coker 310 and NC-150-Compatible is potentially free.

The results from the field studies were likewise according to expectation (Table 2). NC-150-Incompatible produced no glanded seedlings, whereas NC-150-Compatible produced about 15% glanded seedlings. There was the scant possibility that NC-150-Incompatible rejected cross-pollination for reasons other than the occurrence of complementary lethality. A sample of 12 freshly-cracked bolls yielded 25 necrotized embryos, a result proving that some cross-pollination was accomplished on NC-150-Incompatible.

There are at least three questions that must be considered before attempts to use the  $le - Le^{dav}$  system in practical cotton production. Do the alleles contribute to problems of plant growth and production? Are the alleles easily manipulated in a breeding program? Is the barrier to gene flow as absolute as it appears to be from the current report?

Plants harboring nonlethal combinations of the complementary factors do not seem to be restricted in growth. However, the question of plant productivity remains moot. The compatibility alleles,  $le_1$  and  $le_2$ , were derived from a wild accession of *G. barbadense* (4), and might thus be expected to be accompanied by germplasm blocks capable of imparting inferior agronomic performance in intensely selected cultivars. Recent efforts to transfer the alleles into the *G. hirsutum* cultivars, 'Gregg 35-W' and 'Paymaster 464,' suggest that such a difficulty, if indeed any exists, can be overcome.  $Le^{dav}$  derives from the diploid species, *G. davidsonii*, and might, likewise, be expected to be accompanied by poor agronomic potential. The *G. hirsutum* lines homozygous for  $Le^{dav}$  currently at hand are not as advanced along the lines of agronomic fitness as are the cross-compatible accessions of Gregg and Paymaster.

The process of breeding for isolation promises to be straightforward but tedious. I have used continual backcrossing methods in transferring the critical alleles. Progeny testing is required at all stages of the process. There are two steps, as follows.

A stock of the genotype  $le_1le_1le_2le_2$  is crossed with the

**Table 2. Phenotypes of seedlings from open pollination of two stocks of cotton under field growth. Data are from 100 seedlings from each of five plots.**

Phenotype	Glanded	Glandless
NC-150-Compatible	76	424
NC-150-Incompatible	0	500

recipient cultivar, most likely of the genotype  $Le_1Le_1Le_2Le_2$ . The  $F_1$  is then backcrossed to the recurrent parent to produce the four expected genotypes in approximately equal proportions. The doubly-heterozygous plants are identified by crossing all plants in the  $BC_1$  with a stock homozygous for  $Le^{dav}$ . In addition, each plant should be crossed with the recurrent cultivar to provide seed for the next cycle. After the required number of backcrosses has been completed, the plants are tested and selfed seed taken from the doubly-heterozygous individuals. One-sixteenth of these should prove to be of the genotype  $le_1le_1le_2le_2$ .

The second stage involves the introduction of the  $Le^{dav}$  allele. The selected plants from first stage of breeding are crossed with a donor stock and the  $F_1$  crossed with the cross-compatible recurrent stock. Any cotton of the genotype  $Le_1Le_1Le_2Le_2$  can be used as a tester. About one-half of the plants in each backcross generation should bear the  $Le^{dav}$  allele.

Is the  $le - Le^{dav}$  system an absolute barrier to germplasm flow? The data at hand indicate that it is if the stock under challenge is homozygous for either  $Le_1$ , or  $Le_2$ , or both. If a stock is homozygous for  $le_1$  and  $le_2$ , or doubly-heterozygous for both alleles, at least some hybrid seedlings will survive. All *G. hirsutum* cultivars tested thus far have displayed evidence of being homozygous for  $Le_1$  and  $Le_2$ . The group includes Gregg 35-W, Paymaster 464, Coker 310, 'Empire,' 'McNair 220,' and 'Tancot SP-37' (2). Webber (6) found no strains of either *G. hirsutum* or *G. barbadense* that showed evidence of being cross-compatible with *G. davidsonii*. The testing of a given cultivar for the presence of  $Le_1$  and  $Le_2$  involves only the crossing of the stock with a cotton homozygous for  $Le^{dav}$  and subsequent examination of seed or seedling development.

Are the  $Le$  and  $Le^{dav}$  alleles stable over a variety of backgrounds? The  $Le$  alleles are doubtless mutable, although probably at a very low rate. I have no documented example of breakdown of the  $Le^{dav}$  allele in tetraploid background; admittedly the data are few and none derive from experiments designed to test such a possibility. Large plantings of a glandless cotton homozygous for  $Le^{dav}$  adjacent to a cultivar could be serviceable for studying mutation rates of the alleles involved, should the need and the interest arise.

A further question concerns the loss of seed from outcrossing between incompatible cultivars. Such losses would relate to the activity of pollen vectors and the size of the plots involved. Judging from a report by Simpson and Duncan (5), such losses could soar as high as 60%. However, these workers used small plots interplanted with a marked pollen donor. In large blocks, losses would be expected in field margins where pollen vectors are most likely to expend pollen loads transported from adjacent blocks.

Two different cultivars homozygous for  $Le^{dav}$  could, of course, hybridize freely. Therefore, a given region of cotton production should reserve the use of the  $le - Le^{dav}$  system for a particular genotype. For example, in the High Plains of Texas glandless cottons would be the logical candidates for isolation, whereas in the San Joaquin Valley of California cultivars especially tolerant to verticillium wilt might be profitably isolated. With interest in hybrid cotton mounting, the  $le - Le^{dav}$  system might be used to isolate A, B, and R lines into interbreeding groups.

#### ACKNOWLEDGMENTS

I thank the many reviewers of this paper, both known and anonymous. I especially thank Edgar Turcotte for calling my attention to the possibility that the  $le - Le^{dav}$  system might be useful in isolating the lines needed in the production of hybrid cotton.

#### REFERENCES

1. Cross, J. E., and T. R. Richmond. 1959. The use of glandless seed to determine the amount of natural crossing in *Gossypium hirsutum*. Agron. J. 51:511-512.
2. Lee, J. A. 1981. The genetics of D<sub>3</sub> complementary lethality in *Gossypium hirsutum* L. and *Gossypium barbadense* L. J. Hered. 72: (In press).
3. ———. 1981. A new linkage relationship in cotton. Crop Sci. 21:346-347.
4. ———, and F. H. Smith. 1970. Some chemical properties of hybrid seed from a cross between *Gossypium barbadense* L. and *Gossypium davidsonii* Kell. Crop Sci. 10:379-380.
5. Simpson, D. M., and E. N. Duncan. 1956. Varietal response to natural crossing in cotton. Agron. J. 48:74-75.
6. Webber, J. B. 1939. Relationships in the genus *Gossypium* as indicated by cytological data. J. Agric. Res. 58:237-261.