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# Identification of the $Gl_2$ $gl_2$ $Gl_3$ $gl_3$ Genotype in Breeding for Glandless Cottonseed[1]

Robert J. Miravalle and Angus H. Hyer[2]

DEVELOPMENT of glandless-seeded genetic stocks by McMichael in the early 1950's and discovery of the inheritance of this character by McMichael (1) and Roux (4) have afforded cotton breeders an opportunity to rid cottonseed of the pigments contained in these glands. Notable among these pigments is gossypol, a phenolic compound, which produces an undesirable color in cottonseed oil and has toxic properties that limit the usefulness of cottonseed meal as an animal feed.

As soon as McMichael had selected a genetic stock with seeds free of glands, work was begun to transfer glandless seed to commercial cottons. McMichael's fundamental work and the breeding effort that quickly followed were done at the U. S. Cotton Field Station in Shafter, Calif. Cotton breeders in the U. S. Cotton Belt and in some foreign countries have subsequently engaged in similar breeding programs.

The identification of the $Gl_2$ $gl_2$ $Gl_3$ $gl_3$ genotype (double heterozygote) is important in breeding for glandless cottonseed. It has been shown (2) that plants of the doubly heterozygous genotype produce bolls with reduced gland content, so plants of this genotype could be identified by the gland content of their bolls. The glands are embedded in the surface of the carpel walls of the cotton fruit or boll. This method of identification has been further clarified by recent investigations. These investigations have led to a more accurate means of identification which will be presented here.

McMichael (1) and Roux (4), working independently, found that the glandless seed trait in cotton is primarily controlled by two independent genes which act in a complementary-type fashion. Both investigators suggested that other genes with minor effects may also be involved. McMichael reported that the 2 major alleles act as recessives, while Roux published that these alleles act as partial dominants. The senior author has reviewed the work of both Roux and McMichael and found that the data presented by each support the hypothesis of recessive gene action (3).

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[2] Geneticist and Research Agronomist, U. S. Cotton Field Station, Shafter, Calif.

A backcross breeding technique is employed by the authors in transferring the glandless-seed genes, $gl_2$ and $gl_3$, into commercial breeding stocks of cotton. Doubly heterozygous plants are used as donor parents. Theoretically, a backcross progeny in this program consists of four equally frequent genotypes—$Gl_2$ $Gl_2$ $Gl_3$ $Gl_3$; $Gl_2$ $gl_2$ $Gl_3$ $Gl_3$; $Gl_2$ $Gl_2$ $Gl_3$ $gl_3$; and the doubly heterozygous genotype $Gl_2$ $gl_2$ $Gl_3$ $gl_3$. The success of the technique depends in great measure on the breeder's ability to identify the young doubly heterozygous plants. Otherwise the breeder would have to turn to a more complicated and slower procedure, such as growing an $F_2$ generation following each backcross, to obtain homozygous doubly recessive plants ($gl_2$ $gl_2$ $gl_3$ $gl_3$) to use as donor parents in the next cycle of backcrossing.

## MATERIALS AND METHODS

A close examination of $F_1$ plants produced by crossing a glandless-seeded plant ($gl_2$ $gl_2$ $gl_3$ $gl_3$) with a normally glanded plant ($Gl_2$ $Gl_2$ $Gl_3$ $Gl_3$) led to the belief that the $Gl_2$ $gl_2$ $Gl_3$ $gl_3$ genotype could be recognized in a backcross progeny. The $F_1$ seeds are very difficult to distinguish from normally glanded seeds with any accuracy. However, the $F_1$ doubly heterozygous plants grown from such hybrid seed have very few or no glands on their bolls. All other parts of the $F_1$ plant, except the mature leaves, are fully glanded. Mature leaves on $F_1$ doubly heterozygous plants which have been observed at midseason and later have very few glands. The immature and mature leaves observed prior to first bloom on plants of $F_1$ progenies have had about the same number of glands as leaves of similar age on normally glanded plants. It therefore seemed appropriate to attempt identification of doubly heterozygous plants in backcross progenies on the basis of their having essentially gland-free bolls. This method of identification was first used in 1959, but no plants were found which had bolls with as few glands as the bolls of the $F_1$ doubly heterozygous plants. A wide range in number and distribution of glands on the bolls of plants in backcross progenies has since been observed. The gland content of a boll was determined as early as the third day after flowering. The following is a description of some of the gland conditions found in very young bolls of backcross progeny plants:

Boll Type 1: Glands densely cover carpel walls.

Boll Type 2: Glands dense near the carpel wall sutures and near the base of the boll; sparse near top of the boll and nearly absent in the middle of the carpel wall of each locule.

Boll Type 3: Glands more sparse than in Boll Type 2 and situated close to the carpel wall sutures and to the base of the boll; absent near the top of the boll and in the middle of the carpel wall of each locule.

Boll Type 4: Glands very sparse and situated adjacent to the carpel well sutures near the base of the boll and at the base of the boll; absent elsewhere.

Boll Type 5: Glands absent.

Progeny tests were made by growing progenies of seedlings from self-pollinated seeds produced on plants identified in the field as double heterozygotes. Seeds were germinated in flats of sand in the greenhouse. Relatively little space is required, and the tests are completed in about 10 days. Only those plants whose test progenies contain seedlings with glandless cotyledons in the expected proportion of 1 in 16 are judged to be double heterozygotes.

The material used consisted of backcross progenies involving Upland-type cottons under development at the U. S. Cotton Field Station, Shafter, Calif. These progenies range in number of backcrosses from 1 to 6. Several Western Acalas and Western Acala × (Triple Hybrid × Eastern) derivatives were used as recurrent parents. The recurrent parent strains were Acala 4–42 families 77, 615, and 135 and AxTE families 1, 11, 22, and 25. Three sources of the glandless seed genes were used as original donor parents. These were McMichael's genetic stocks 37–105, 41–4, and 41–63.

## RESULTS

In 1959, plants in progenies of the 1st, 2nd, 3rd, and 4th backcross generations were identified as double heterozygotes on the basis of reduced number of boll glands. Only plants which had bolls with very few glands, most likely type 3 and 4 bolls, were selected for backcrossing. Of the 226 plants so identified and selected, all but two, or 99%, were proved by progeny test to be double heterozygotes.

In 1960, plants in the progenies of the 1st, 2nd, 3rd, 4th, and 5th backcross generations were again identified as double heterozygotes on the basis of reduced number of boll glands. Plants with boll types 2 through 5 were selected for backcrossing. Of the 348 plants so identified and selected, all but 14, or 96%, were proved by progeny test to be double heterozygotes.

No records were made of the boll type number of each plant selected in either year. It was assumed, however, that plants with bolls of type 2, 3, 4, or 5 were double heterozygotes (2).

Plants in backcross progenies growing at the Cotton Breeding and Genetics Nursery in Iguala, Mexico, during the winter of 1960–61 were scored on the basis of the 5 boll types mentioned previously. Plants having bolls of type 2, 3, 4, or 5 were considered double heterozygotes. This material was in the 3rd, 4th, and 6th generations of backcrossing. Of the 664 plants identified, 361 had type 1 bolls and 303 had bolls of types 2, 3, 4, or 5. These data do not fit the expected ratio of 3 nondouble heterozygotes to 1 double heterozygote. It was thought that the plants with type 2 bolls could easily have been misclassified and were probably not double heterozygotes since the type 2 boll is difficult to distinguish from type 1. There were 475 plants with type 1 and 2 bolls and 189 with bolls of types 3, 4, and 5. This ratio approaches that expected but still does not fit it according to the chi-square goodness-of-fit test ($P=.05$ to $.02$). Self-pollinated seed from several of the plants with bolls of types 2, 3, 4, and 5 were used to determine whether or not the plants so identified were double heterozygotes. The results of these progeny tests are given in Table 1. All plants with bolls of type 3, 4, or 5 were double heterozygotes. Only 1 of the 47 plants with type 2 bolls was a double heterozygote.

In addition to boll gland number and distribution, the number and distribution of glands in the mature leaves of plants with type 2 bolls were also observed at Iguala. The number of leaf glands ranged from very few to almost normal with the exception of the single plant with type 2 bolls which proved to be a double heterozygote. No glands were observed in the mature leaves on this plant. The glands in those leaves with a reduced gland number were restricted to the areas near the leaf margins. Plants with this reduced leaf gland number and distribution were quite numerous.

It was unfortunate that the gland contents of leaves of the plants with bolls of each of the 5 types were not observed. In order to get information on the leaf gland content of plants with bolls of a type other than type 2, observations were made on gland content of mature leaves of plants growing in the breeding nursery at the U. S. Cotton Field Station, Shafter, Calif., in 1961. The leaf

Table 1—Boll type, number of plants, and number of plants producing glandless seed.

| Boll type | No. of plants | No. of plants producing glandless seed |
|---|---|---|
| 2 | 47 | 1 |
| 3 | 65 | 65 |
| 4 | 31 | 31 |
| 5 | 11 | 11 |

gland contents of plants with bolls of types 3 or 4 were compared with the leaf gland content of normally glanded cotton (boll type 1).

A very slight reduction in gland number and size in the leaf veins of the plants with bolls of type 3 or 4 was observed. There were fewer glands in the interveinal sections of the leaves of plants with bolls of type 3 or 4 than in leaves of normally glanded cotton. Little difference was seen in gland size. The few glands present in the interveinal sections of leaves of plants with bolls of type 3 or 4 were mostly located near the leaf margins.

## DISCUSSION AND SUMMARY

A system of identifying doubly heterozygous cotton plants in backcross progenies based on the gland content of the bolls has proven highly accurate. Doubly heterozygous plants have been successfully identified by this method with better than 95% accuracy in progenies with as many as 6 backcrosses. Specifically, the results indicate that only plants with bolls in the range of types 3 through 5 can be reliably considered double heterozygotes. The plants so identified may then be used as donor parents in the backcross program. This system was used to identify doubly heterozygous plants in backcross progenies grown in 1961, and 108 of the plants so identified were progeny-tested. Each of these plants was a double heterozygote.

Identification of doubly heterozygous plants by means of mature leaf gland content was also investigated and found unreliable. Numerous plants with type 2 bolls were observed at Iguala to have leaf gland numbers and distribution patterns similar to those of plants with boll types 3 and 4 observed at Shafter. Thus, if the presence of few leaf glands situated near the leaf margin was to be used as the sole criterion for identifying double heterozygotes, some plants would be incorrectly identified as double heterozygotes; namely, those plants with type 2 bolls. This system of identification would be costly in a backcross breeding program of the type described here, since too many plants which are not double heterozygotes would mistakenly be used as donor parents.

The senior author had the opportunity of classifying plants in 3 backcross progenies belonging to A. L. Smith of the Alabama Experiment Station at Auburn. The source of glandless-seed genes in each of these progenies was a genetic stock developed by McMichael at the U. S. Cotton Field Station, Shafter, Calif. The recurrent parents involved were Auburn 56, Empire, and Plains. Each progeny was in the second generation of backcrossing. Due to the system of breeding employed, several genotypes with boll gland phenotypes similar to that of the double heterozygote were considered likely to be present in each of the progenies. Therefore, the plants in each progeny were classified only as to whether or not they would produce some glandless seed. The identification of these plants was based mainly on the boll gland content criterion used in identifying doubly heterozygous plants; however, the classification of these plants was not overly restrictive in that several plants with type 2 bolls were classified as plants that would produce some glandless seed. Smith grew progenies from self-pollinated seed of each of these plants. He observed that 64 out of 80 plants, or 80%, were correctly identified. In no case did a plant which was classified as one that would produce only glanded seed produce any glandless seed.

It is the authors' opinion that the degree of accuracy would have been greatly improved had the identification been more restrictive, i.e., had plants with type 2 bolls been classified as ones that would produce only glanded seed. While these results are not conclusive, they do suggest that the method of identifying doubly heterozygous plants on the basis of their boll gland content can be applied successfully to the Upland cottons of the Southeast, Delta, and Mid-South equally as well as to the Western Acala cottons.

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