Published May, 1986

NOTES

DEVELOPMENT OF A COTTON PLANT WITH GLANDLESS SEEDS, AND GLANDED FOLIAGE AND FRUITING FORMS[1]

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Abstract

Pigment glands in cotton (*Gossypium* spp.) contain gossypol and related terpenoid aldehyde compounds and these glands are found throughout most aboveground parts, including seeds. These glands protect the plant from insect pests, but gossypol in the embryo of the seed is undesirable because it is toxic to nonruminant animals. The objectives of the present study were to identify a hexaploid cotton plant that possessed glands in most of the aboveground parts, except the seed, and initiate the transfer of this trait into a tetraploid cotton. A fertile hexaploid ($2N=78$) plant from an interspecific cross of tetraploid ($2N=52$) *Gossypium hirsutum* L. × diploid ($2N=26$) *G. sturtianum* Willis showed a phenotype having glandless seed, and glanded foliage and fruiting forms. This phenotype had flowerbud and seed gossypol percentages of 0.29 and 0.02, respectively. Fertile pentaploid F_1 plants from crossing the hexaploid and the tetraploid *G. hirsutum* Texas marker-1 (TM-1) stock appeared to produce as much seed cotton as the tetraploid Texas marker-1 at Brownsville, TX. However, the F_1 plant produced 0.31% flowerbud and only 0.02% seed gossypol. The flowerbud and seed gossypol percentages of TM-1 were 0.58 and 1.29, respectively. These results show that fertile hexaploid germplasm in cotton has been developed that possesses the unique characteristic of storing gossypol in glands in the vegetative foliar and fruiting plant parts but not in the embryo of the seed.

Additional index words: ***Gossypium hirsutum*** **L.** ***Gossypium sturtianum*** **Willis, Interspecific hybrid, Gossypol, Terpenoid aldehydes.**

DISTINCT pigment glands called black glands, oil glands, secretion cavities, internal glands, lysigenous glands, resin glands, or gossypol glands are found throughout most of the aboveground parts, including seed, of cotton (*Gossypium* spp.) and certain related genera. The glands contain a variety of terpenoid chemicals, with gossypol being the best known and most studied (1,3,13). Gossypol in the embryo of the seed is undesirable because it is toxic to nonruminant animals, including pigs, chickens, and humans. Gossypol remains in the oil when it is pressed from the seed and in the protein-rich meal that remains after oil extraction.

The glandless phenotypes developed by McMichael in the 1950s (15,16) were considered agronomically important because gossypol was not stored in the embryo. However, glandless plants are completely devoid of gossypol glands and research revealed that the gossypol present in the aboveground parts of cotton acts as a protective mechanism against certain insect pests (4,12). Research was initiated in the 1960s to increase the natural resistance of cotton to insects, specifically *Heliothis* spp., by genetically increasing the level of gossypol in the flowerbuds (11). Therefore, breeding for both increased levels of gossypol in flowerbuds and decreased levels in the seed has been practiced. The ideal phenotype appears to be a combination of low seed gossypol and high flowerbud gossypol in the same plant. This phenotype exists in several wild diploid ($2N=26$) species of *Gossypium* and in the related genus *Cienfuegosia* (9). Theoretically, a tetraploid ($2N=52$) cotton plant could be developed through ge-

[1] Cooperative investigation of Cotton Insects Res. Lab., USDA-ARS, Brownsville, TX and the Texas Agric. Exp. Stn. Received 13 Apr. 1984.

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netic manipulation, which produces glands and possibly high levels of gossypol in all aboveground parts except the embryo. The objectives of the present study were to discover, develop, and describe a nondiploid cotton plant that possessed these characteristics, and initiate the transfer of this trait into the tetraploid cotton, *G. hirsutum* L.

Materials and Methods

In 1979, I discovered a single cotton plant growing in the field (Gila Loam, which is a coarse-loamy, mixed, thermic, Typic Torrifluvents soil) at Tucson, AZ, that possessed gossypol glands in the foliage and fruiting forms but had no observable glands in embryos of its seed (8). This plant was in a population of hexaploid ($2N=78$) plants developed by H. Muramoto (14) and derived from an interspecific cross of tetraploid *G. hirsutum* ('Acala 44–10–1') and *G. sturtianum* Willis, an Australian diploid. Nine seeds from this plant were sent to me by Muramoto in the fall of 1979. Four of these seeds were scarified and placed on wet filter paper in a petri dish. Three of the four seeds germinated and the seedlings were transplanted to a greenhouse at Brownsville, TX.

Seeds from the three plants were harvested and analyzed for gossypol and related terpenoid aldehyde compounds by the method described by Pons et al. (17). Chemical analysis of the seed from the three plants revealed small amounts of gossypol in the embryo, so a few seeds from each plant were dissected and the number of functional glands were observed under magnification with a ×10 binocular dissecting microscope. A gland was considered functional if the sac contained yellow pigment when observed under the microscope. The gossypol glands that were observed under the dissecting microscope did not appear throughout the embryo of the seed as they do in seed from glanded cultivars. Conversely, most of the functional glands in the seed from the three plants appeared along the margin of the embryo in varying numbers. In 1980, seed from each of the three plants were grown at Brownsville and seeds from these plants were observed under the dissecting microscope for the presence or absence of functional glands. Seed from a single plant was found that apparently did not produce functional glands as observed under the dissecting microscope.

This plant was transplanted to a greenhouse in the fall of 1980. Flowerbud and seed gossypol percentages were determined (18) and seeds from this plant were planted in the field in 1981. Flowerbuds from derivatives of the single plant that I found in 1979 in Muramoto's hexaploid population, the glandless seeded selection from this material, and its progeny were analyzed cytologically for chromosome number at the University of Arizona, Tucson, and confirmed as hexaploids. Also, crosses were made in 1981 between the hexaploid derivative that had no seed glands and a tetraploid *G. hirsutum*, Texas marker-1, TM-1 (10). The data were analyzed statistically using paired comparisons (hexaploid vs. TM-1 and hexaploid × tetraploid hybrid vs. TM-1), on an individual plant basis.

Results and Discussion

A comparison of the mean flowerbud and seed gossypol percentages determined from the three hexaploid plants grown in the greenhouse at Brownsville in 1979, Muramoto's original material, showed the gossypol percentages to be similar (Table 1). The single hexaploid plant that did not produce functional glands in the embryo identified in the summer of 1980 at Brownsville, had significantly higher percent flowerbud gossypol than percent seed gossypol. This relationship was maintained in progeny from this plant. On the other hand, percent seed gossypol was significantly higher than percent flowerbud gossypol for the Texas marker-1, TM-1 (Table 1).

Table 1. Flowerbud and seed gossypol percentages (mean ± SD) of a *Gossypium hirsutum* × *G. sturtianum* interspecific cross and derivatives.

Ploidy	Pedigree	Gossypol	
		Flowerbud	Seed
		%	
6×† (Hexaploid)	*G. hirstum* × *G. sturtianum* Muramoto's original material	0.34 ± 0.05*	0.28 ± 0.04
6×†	*G. hirsutum* × *G. sturtianum*		
	Original individual plant selection	0.27 ± 0.05	0.03 ± 0.02
	Progeny from individual plant	0.29 ± 0.04	0.02 ± 0.01
4× (Tetraploid)	*G. hirsutum* TM-1	0.58 ± 0.11	1.29 ± 0.14
5×§ (Pentaploid)	Hexploid × *G. hirsutum*	0.31 ± 0.06	0.02 ± 0.01

* Significant at the 0.05 probability level.
† Confirmed cytologically by Endrizzi and Ramsey.
§ Have not been analyzed cytologically.

Most of the viable F_1 seed obtained from crossing the hexaploid and tetraploid TM-1 resulted from the hexaploid parent as female rather than from the reciprocal cross. However, the hybrid plants are assumed to be pentaploids because the leaf shape of the F_1 was intermediate between the okra leaf phenotype of the hexaploid and the normal leaf shape of the TM-1 tetraploid. The F_1 seed was planted in the field in 1982 and percent flowerbud and seed gossypol was similar to that of the hexaploids with gland-free seed (Table 1).

The identification of a single plant with glandless seed in a large population indicates a recessive trait or a mutant plant. The seed gossypol content of the glandless-seeded hexaploid × glanded tetraploid hybrid given in Table 1 suggests that the glandless seed trait is dominant. Beasley's (2) description of the unusual properties of a *G. sturtianum* hybrid states: That many of the *G. sturtii* [sic] characters are dominant. The flowerbud and seed gossypol percentages of the pentaploid hybrid supports Beasley's conclusion.

The wild diploid cotton species *G. sturtianum* produces gossypol glands in the aboveground plant parts, but does not store terpenoid aldehydes in the embryo. Gossypol and other terpenoid aldehydes are stored in glands in the cotyledonary leaves after germination is initiated. This same sequence occurred in the seeds of the hexaploid and the F_1 pentaploid plants. In addition, I observed that this sequence is not triggered by sunlight because seeds germinated in the absence of light continued to accumulate terpenoid aldehydes in the cotyledonary glands.

The transfer of the glandless seed–glanded plant character may require persistence, but the identification of the phenotype and the analysis for terpenoid aldehydes in flowerbuds and seeds are definitive and not as subjective as screening for such traits as disease resistance or fiber strength. These traits have required as many as 16 backcrosses and 40 yrs of crossing, in-

tercrossing, and selection for transfer from diploids into agronomically acceptable tetraploid cotton (5,6,7).

The discovery of a glandless seed-glanded plant hexaploid and the production of fertile F_1 pentaploid plants from crossing the hexaploid × tetraploid plants reported in the present paper are two important steps in the transfer of this phenotype into tetraploid cotton. The F_1 pentaploid plants appeared to be as fertile as either the hexaploid or tetraploid parents. Therefore, completing the transfer of the glandless seed-glanded plant trait into *G. hirsutum* appears to be a promising but challenging research area.

Acknowledgments

Appreciation is expressed to Dr. J.E. Endrizzi and Mr. G.M. Ramsay, University of Arizona, Tucson, for cytological analysis to determine chromosome number of the plant material.

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TECHNIQUES FOR IDENTIFYING TOLERANCE OF SOYBEAN TO PHYTOTOXIC SUBSTANCES IN WHEAT STRAW[1]

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Abstract

Experiments have shown that substances in wheat (*Triticum aestivum* L.) straw are phytotoxic to soybean [*Glycine max* (L.) Merr.]. We evaluated techniques for screening soybean germplasm to these growth-reducing substances and studied factors affecting this trait. Techniques used were: i) pots in a greenhouse, ii) large wooden flats in a greenhouse, and iii) field evaluations. The soil mixture in the greenhouse contained 20 g straw/kg soil. Results from the flat technique were highly correlated with the pot method, the technique commonly used. Field evaluations were not highly correlated with greenhouse results. Seed age and seed source affected the tolerant response, whereas seed size was not an important factor.

***Additional index words:* *Glycine max* (L.) Merr., Allelopathy, Seed source and age, Wheat residue.**

FARMERS in areas where the growing season is of sufficient length can better utilize their land and increase production by growing a small grain during the cool season and soybean, [*Glycine max* (L.) Merr.] during the summer. The major environmental factors limiting yields of double cropped soybean are length of the growing season and amount of rainfall. Another factor that has been shown to limit yield, but to a lesser extent, is the allelopathic effect of wheat (*Triticum aestivum* L.) or oat (*Avena sativa* L.) residue on soybean (4). Collins and Caviness (3) compared several soybean cultivars for tolerance and phytotoxic effects of wheat residue and found that cultivars differed significantly.

For many years it has been known that residues from one crop can give rise to phytotoxic effects on succeeding crops (1,12,13,14,15,16). A wide range of injurous effects on plants due to phytotoxins has been reported, with the most common effects being reduced growth, low seedling vigor, chlorosis, reduced germination, and suppression of root elongation.

A major question in phytotoxicity research is whether the compounds are breakdown products in plant tissue or materials synthesized by microorganisms (7,9). Some researchers have suggested that the phytotoxic effects may result from a combination of both (8,10). Guenzi and McCalla (6) reported in 1966 that phenolic com-

[1] Published with the approval of the director of Arkansas Agric. Exp. Stn. Part of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree. This study was supported in part by a grant from the Am. Soybean Assoc. Received 13 May 1985.

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