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Research Article

Inheritance of the Ovule Fuzzless Trait for *Gossypium arboreum* Germplasm Line PI 529708

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Abstract

Background: Cotton is the most important fiber crop and understanding the genetic mechanisms controlling fiber production on cotton seeds can aid in the development of improved varieties with higher lint yields and improved fiber quality. Lint and fuzz are the two types of fiber produced on the cotton seed and mutations in the genes controlling these traits can result in a lack of fiber development. The ovule fuzzless genotype PI 529708 was identified from the United States *Gossypium arboreum* germplasm collection. **Methodology:** This genotype was crossed with the fuzzy seed genotype PI 529729 to determine the genetic inheritance of the ovule fuzzless trait. **Results:** Seeds from F₁ plants showed the ovule fuzzless phenotype indicating the fuzzless trait was dominant over fuzz fiber development. The F₂ segregation data fit a three complementary gene model. For corolla color, petal spot and stem pigmentation, the F₂ segregation data for each trait fit the expected single dominant gene model indicating normal segregation for the population. **Conclusion:** Reducing the amount of fuzz fibers on the cotton seed is one strategy to improve lint yield and ginning efficiency, therefore, determining the genes controlling fuzz fiber production is required to manipulate this trait in breeding programs.

Key words: Cotton, fuzzless, germplasm, *Gossypium arboreum*, trichome

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cotton is the most important fiber crop for textile production with approximately 25.9 million Mt produced in 76 countries in 2015 (<http://www.fas.usda.gov/commodities/cotton>). China, India, United States and Pakistan are the leading producers accounting for more than 70% of global production. There are 50 species of cotton in the genus *Gossypium* with two tetraploid (*G. hirsutum* and *G. barbadense*) and two diploid species (*G. arboreum* and *G. herbaceum*) cultivated for fiber production. *Gossypium hirsutum* (upland cotton) accounts for more than 90% of global production¹. In comparison, *G. arboreum* (Asiatic cotton) accounts for less than 2% of global production due to lower fiber quality and lint yield. *Gossypium arboreum* was once widely cultivated in Asia, the Middle East and North-eastern Africa, however, the introduction of *G. hirsutum* varieties have replaced the production of *G. arboreum* in most regions². Presently, *G. arboreum* varieties are cultivated in regions not suitable for *G. hirsutum* production or under low input production systems³. The adaptability of *G. arboreum* cultivars to environmental extremes provides a source of desirable traits that could be used in *G. hirsutum* improvement programs, such as tolerance to drought and saline soils and pest and disease resistance^{2,4-6}. *Gossypium arboreum* is also a source of genetic diversity for fiber and seed quality traits⁷.

Understanding the genetic mechanisms controlling fiber production on cotton seeds can aid in the development of varieties with improved fiber yields and quality. Lint and fuzz are the two types of fiber present on cotton seeds. These fibers grow as single cell trichomes from the epidermal layer of the ovule. Growth of lint fibers is initiated at anthesis and the fibers can elongate up to 30 mm in length⁸. Lint fibers are typically removed during the ginning process and are the economically important fibers used for textile production. In comparison, fuzz fibers typically adhere to the seed coat and can be observed on the seed after ginning⁹. Fuzz fibers are shorter, elongating to approximately 0.5 mm and growth is initiated 4 days post anthesis^{8,10}. The ovule fuzzless trait is characterized by a reduction in fuzz fiber development on the seed coat. This lack of fuzz fiber development on the seed coat will result in the appearance of black seed after ginning and has been referred to as the naked-seed phenotype¹¹. Reducing the amount of fuzz fibers produced on the ovule has been suggested as a strategy to improve lint yield and reduce the amount of energy required in the ginning process¹².

Four independent genes have been reported in *G. hirsutum* with one dominant and three recessive genes controlling fuzz development on the ovule¹¹⁻¹⁵. The ovule fuzzless trait has also been reported in *G. arboreum* varieties including both dominant and recessive genes¹⁶⁻¹⁸, but less information is available on the number and diversity of genes conferring the fuzzless phenotype for this species, although, determining the genetic regulatory mechanisms for fiber production on the ovule can be simplified in diploid species as compared to tetraploid relatives^{18,19}. Recently, proteomics and transcriptomics have been employed to identify genes regulating fiber development on cotton ovules²⁰⁻²⁴. These studies have indicated a cascade of genes involved in fiber development, therefore, genetic inheritance studies are needed to identify specific genes in these pathways associated with fuzz fiber development.

More than 1,600 *G. arboreum* accessions are maintained in the United States Department of Agriculture (USDA) National Plant Germplasm System (NPGS) cotton collection (<https://npgsweb.ars-grin.gov>), however, little information is available on the genetic inheritance of the ovule fuzzless trait for accessions in the collection. Thus, the aim of this study is to determine the inheritance of the ovule fuzzless trait for the *G. arboreum* accession PI 529708. The data from this study will aid in evaluating the diversity of fiber mutants in the germplasm collection, which will contribute to a better understanding of the genetic mechanisms controlling fiber development on cotton ovules in order to improve lint yield.

MATERIALS AND METHODS

Accession PI 529708 was identified as an ovule fuzzless line during a seed increase of *G. arboreum* accessions from the NPGS cotton collection. The inheritance of the ovule fuzzless trait was determined by crossing PI 529708 as the female parent with PI 529729 as the pollen parent. Accession PI 529729 shows profuse fuzz development on the ovule, whereas, PI 529708 shows no fuzz developed on the ovule except for small tufts of fiber on the ends of the seed (Fig. 1). Additionally, accessions PI 529708 and PI 529729 differ for several other phenotypic traits including flower corolla color and size, presence of petal spots, stem pigmentation and leaf shape and size (Fig. 2). Accession PI 529708 produces flowers with a yellow colored corolla and no petal spot, stems lacking red pigmentation and super-okra shaped leaves. In contrast, accession PI 529729 produces larger flowers with a white colored corolla and a red petal spot, sun-red pigmented stems and larger laciniate shaped leaves. Accession PI 529729



Fig. 1: Ginned seeds for the *Gossypium arboreum* ovule fuzzless accession PI 529708 (left), ovule fuzzy accession PI 529729 (middle) and F_1 seeds (right) from the cross PI 529708 \times PI 529729

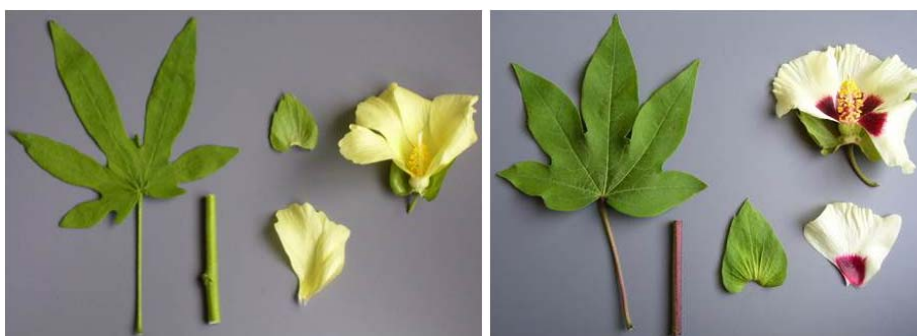


Fig. 2: Leaf, stem and flower phenotypes for the *Gossypium arboreum* accessions PI 529708 (left) and PI 529729 (right) used as parents to determine the inheritance of the ovule fuzzless trait

was also taller and later flowering than PI 529708. Phenotypic characterization of the 2 parental accessions were based on the standardized cotton descriptors (https://www.cottongen.org/data/trait/NCGC_rating_scale).

The F_1 seeds from the cross were planted in the field for F_2 seed production. The F_2 population was planted in the field at the USDA in Stoneville, Mississippi on 30 April 2015. Approximately 600 F_2 seeds were planted in a single 150 m row with approximately 0.3 m spacing between plants within the row. Individual plants were tagged at the flowering stage and stem pigmentation was recorded as reported by Hutchinson²⁵. Corolla color and the presence of a petal spot were recorded for plants with flowers as described by Hutchinson²⁶ and Harland²⁷. To evaluate the fuzzless trait, approximately 10 bolls were harvested from individual plants at maturity. The boll samples were ginned using a 10-saw laboratory gin to remove the majority of the lint fibers from the seeds. Ginned seeds were visually rated for the presence or absence of fuzz fibers on the seed coat. The fuzzy and fuzzless phenotypes were determined as described by Ware¹⁴. The fuzzless phenotype was characterized by the

appearance of black seed with a lack of fuzz development across most of the seed coat. Plants classified as fuzzy showed fuzz fiber development across most of the seed surface. The F_2 plants were classified as either fuzzy or fuzzless and were not classified for the density of fuzz fibers on the seed coat. Segregation ratios for the ovule fuzz fiber trait, corolla color, petal spot and stem pigmentation were tested using the chi-square test of significance with the software package Statistix 9 (Analytical Software, Tallahassee, FL).

RESULTS

The F_1 plants from the cross PI 529708 \times PI 529729 showed no fuzz development on the ovules indicating the fuzzless trait was dominant over the fuzzy ovule trait (Fig. 1). Seeds from the F_1 plants tended to show more pronounced tufts of fibers that adhered to the ends of the seed as compared to the fuzzless parent PI 529708. Flowers for all F_1 plants showed yellow colored corolla and red petal spots. Also, all F_1 plants showed sun-red pigmented stems. The F_2 segregation data for corolla color, petal spot and stem



Fig. 3: Ginned seeds showing the phenotypic variation in ovule fuzz fiber development for the F₂ population derived from the cross PI 529708 × PI 529729 used to determine the inheritance of the fuzzless trait for *Gossypium arboreum* accession PI 529708

Table 1: Phenotypic segregation data for corolla color, petal spot and stem pigmentation for the F₂ population derived from the *Gossypium arboreum* cross PI 529708 × PI 529729

Trait*	Phenotype	Number of plants	χ^2 (3:1 segregation ratio)	p-value
Corolla color	Yellow	207	0.400	0.5271
	White	63		
Petal spot	Present	192	2.178	0.1400
	Absent	78		
Stem pigmentation	Red	285	3.612	0.0574
	Green	117		

*Corolla color and petal spot data were recorded for 270 F₂ plants with stem pigmentation recorded for 402 plants

Table 2: Phenotypic segregation data for 425 F₂ plants derived from the *Gossypium arboreum* cross PI 529708 × PI 529729 used to determine the inheritance of the ovule fuzzless trait in PI 529708

Phenotype	Observed	Expected	χ^2	p-value
Fuzzless	190	319	208.02	0
Fuzzy	235	106	(3:1 segregation ratio)	
Fuzzless	190	239	23.01	0
Fuzzy	235	186	(9:7 segregation ratio)	
Fuzzless	190	179	1.105	0.2933
Fuzzy	235	246	(27:37 segregation ratio)	

pigmentation is presented in Table 1. The data from the F₂ population supported the single dominant gene model for corolla color, petal spot and stem pigmentation. Yellow colored corolla was dominant over white colored corolla. The presence of petal spots was dominant over the absence of petal spots and sun-red pigmented stems were dominant over green stems. Although the observed segregation ratio for stem pigmentation was not significantly different ($p = 0.05$) from the expected ratio, the frequency of plants rated as green was slightly higher than expected with 117 green stem plants observed in the population compared to an expected value of 100 plants.

For the ovule fuzz fiber trait, the majority of the F₂ plants were easily classified as fuzzless or fuzzy with some variation in the amount of fuzz development on the seed coat observed for plants rated as fuzzy (Fig. 3). Plants showing profuse fuzz fiber development similar to the fuzzy parent PI 529729 represented approximately 20% of the population. Approximately 6% of the population showed low amounts of fuzz fiber development on the seed coat and these individuals were classified as fuzzy. Several gene models were tested (Table 2), however, the F₂ segregation data did not supported

a single dominant or 2 complementary gene models for the ovule fuzzless trait as more plants were rated as fuzzy. The model that best fit the data was a three complementary gene model with the ovule fuzzless phenotype dominant over fuzzy ovules.

DISCUSSION

Three complementary genes were required for expression of the ovule fuzzless trait in accession PI 529708, however, there are no reports suggesting more than 1 gene was required for expression of the ovule fuzzless phenotype in *G. arboreum* or *G. hirsutum*. Hutchinson¹⁶ reported the ovule fuzzless phenotype in a *G. arboreum* landrace from China was conferred by a single dominant gene. This genotype was characterized as fuzzless with only the presence of small tufts of fuzz fibers on the chalazal and micropylar ends of the seed. Wang *et al.*¹⁸ also evaluated a fuzzless genotype selected from a Chinese *G. arboreum* landrace. This genotype showed the ovule fuzzless trait with some fuzz fibers on the chalazal end of the seed but the inheritance of the fuzzless trait was not reported. Rong *et al.*¹⁷

reported the ovule fuzzless phenotype for the *G. arboreum* accession PI 529740 (SMA-4) was conferred by a single recessive gene. This study showed the ovule fuzzless and fiberless phenotypes for PI 529740 where no fuzz or lint fibers were produced on the seed coat, although, tufts of fiber were observed on the seed coat. Similarly, accession PI 529708 showed the presence of small tufts of fiber on the chalazal and micropylar ends of the seed.

More genotypes have been reported to show the ovule fuzzless phenotype in *G. hirsutum*^{17,28} with at least four independent genes conferring the ovule fuzzless phenotype. Ware *et al.*¹¹ reported a recessive gene conferring the fuzzless phenotype in the cultivar 'Acadian Brown'. The F₁ seed derived from crosses with this cultivar showed less fuzz fiber development on the seed coat indicating incomplete dominance for the fuzzy ovule phenotype. A dominant gene conferring the fuzzless phenotype has also been reported by Ware¹⁴ and Ware *et al.*¹¹. Additional studies have shown the presence of the 2 genes in *G. hirsutum*^{9,15,28} and these genes have been designated N_1 and n_2 ^{29,30}. Another recessive gene conferring the fuzzless phenotype in *G. hirsutum* was reported by Turley and Kloth¹³ and designed n_3 . Bechere *et al.*¹² developed a naked-tufted genotype by chemical mutagenesis of the *G. hirsutum* cultivar 'SC 9023' and reported the ovule fuzzless phenotype was conferred by a single recessive gene.

Several studies have reported extensive variation in the amount of fuzz fibers on ovules for segregating populations^{11,12,14} suggesting quantitative inheritance, however, the genetics of this variation have not been determined. This reported range in phenotypic variation for fuzz fiber development was not observed in the PI 529708 × PI 529729 F₂ population evaluated in the present study. Most of the individuals in the population were easily classified as fuzzless or fuzzy. Some of the variation in the density of fibers observed on the seed coat could be attributed to ginning efficiency and the variation in fiber properties between the *G. arboreum* parents. Therefore, this variation was not considered a factor influencing the classification of plants in the population in the present study. Additionally, the inheritance of flower corolla color, present or absence of petal spots and stem pigmentation in the present study would indicate normal segregation for the population and these 3 traits fit a single dominant gene model as previously reported²⁵⁻²⁷. Additional studies are needed to identify the chromosomal regions associated with the ovule fuzzless trait in order to determine the function of the 3 genes and how these genes interact to inhibit fuzz fiber development.

CONCLUSION

Understanding how the 3 genes conferring the fuzzless trait interact to control fuzz fiber development will be beneficial considering the fact that fiber development and quality are regulated by the interaction of multiple genes. Reducing the amount of fuzz fiber on the seed coat is one strategy to improve lint yield and ginning efficiency, thus, determining the genetic mechanisms of fuzz development would benefit the breeding of new varieties. Additionally, the production of cotton seed lacking fuzz fiber would enhance the utilization of seed as a feedstock. Lastly, the population developed in this study can aid in the genetic mapping of the genes conferring fuzz fiber development.

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