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Genetics of Reniform Nematode Resistance in *Gossypium arboreum* Germplasm Line PI 529728

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Abstract Reniform nematode (*Rotylenchulus reniformis*) is a serious pathogen of cotton in the United States and management has been difficult due to the lack of resistant upland cotton (*Gossypium hirsutum*) varieties. The diploid *G. arboreum* germplasm line PI 529728 was identified as a potential new source of *R. reniformis* resistance. Line PI 529728 was crossed with the highly susceptible *G. arboreum* germplasm line PI 529729 to develop F_1, BC_1F_1, and F_2 populations that were screened for nematode resistance under controlled environmental conditions to determine the genetics of resistance. The 10 F_1, 69 BC_1F_1, and 332 F_2 plants were inoculated with 1,000 vermiform nematodes and the number of swollen females on the root systems was determined 28 days after inoculation. The F_1 plants supported more nematodes than the susceptible control genotype PI 529251 (*G. hirsutum* accession Deltapine 16) indicating resistance was a recessive trait. For the BC_1F_1 and F_2 populations, plants supporting the same or a reduced level of infection that developed on the resistant control genotype PI 163068 (*G. barbadense* accession Texas 110) were rated as resistant. Based on this classification of resistance and susceptibility, it was predicted that a single recessive gene was conferring resistance; although, the BC_1F_1 population had more susceptible plants than expected. Additionally, highly resistant plants were observed in the BC_1F_1 and F_2 populations. This information will aid in the introgression of *R. reniformis* resistance from PI 529728 into upland cotton for the development of resistant varieties.

Keywords: *cotton, germplasm, Gossypium arboreum, reniform nematode, resistance*

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1. Introduction

Reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) was first reported on cotton (*Gossypium hirsutum* L.) in 1940 [1] and has emerged as a serious pathogen of cotton in the cotton belt of the southeastern United States [2,3,4,5]. *Rotylenchulus reniformis* is an obligate plant parasite with a wide host range of more than 300 plant species [6,7,8]. Vermiform preadult females penetrate cotton roots, establishing a feeding site in the stele, where they remain as sedentary semiendoparasites [8,9,10]. Disease symptoms include plant stunting, suppressed root growth, nutritional deficiencies, fruit abortion, and delayed maturity; however, disease symptoms may not be apparent due to uniformity of symptoms across the field [11]. Reniform nematodes affect cotton production by reducing seed cotton yield, boll size, and lint percentage [12,13]. The United States produced 18.7 million bales of cotton in 2012 [14] and ranks third in the world for cotton lint production. An estimated loss of 268,698 bales of cotton to reniform nematode was reported in 2012, with an average yield loss of 2.25% for the 11 cotton producing states where this pathogen has been reported on cotton [14]. Since 2000, yield losses have ranged from 1.46 to 2.37% with an average yield loss of 1.74% over the 13 year period. The highest yield losses in 2012 occurred in Alabama, Louisiana, and Mississippi, and ranged for 4 to 5%.

Because of the lack of host-plant resistance in upland cotton varieties [15,16,17,18], disease management has relied on nematicides and crop rotation [19,20,21,22]. Nematicides are costly, have human health and environmental impacts resulting in the removal of some nematicides from the market, and some nematicides have shown inconsistent control [19,20,22,23]. Crop rotation to non-host crops has been shown to be effective in reducing nematode populations; however, the suppressed early season nematode populations will quickly rebound to economic threshold levels prior to the end of the cropping season in the subsequent cotton crop [19]. The nematode has several survival strategies to thrive in the absence of a host-plant; therefore, rotation to non-host crops for multiple years is required to substantially reduce populations in the cotton crop [11,24].

Host-plant resistance is needed to develop an effective nematode management strategy to reduce yield losses. Weak to moderate resistance has been identified in *G. hirsutum* primitive race accessions [15,25,26], but these sources of resistance have not been shown to sustainably reduce nematode populations. A few sources of resistance have been identified in tetraploid *G. barbadense* L. germplasm [18,25] and breeding lines have been released [27] for cotton improvement. The diploid cotton species

are another source of resistance genes, with *R. reniformis* resistance reported for germplasm accessions of *G. anomalum* (Wawra and Peyritsch), *G. arboreum* (L.), *G. herbaceum* (L.), *G. longicalyx* (Hutch. and Lee), *G. raimondii* (Ulbr.), *G. somalense* (Gürke), *G. stocksii* (Mast.), and *G. thurberi* (Tod.) [18]. Of 14 *G. arboreum* accessions evaluated, 46% were rated as resistant [18], suggesting that the *G. arboreum* germplasm collection would be an important source of nematode resistance. However, the cotton diploid species do not hybridize with tetraploid *G. hirsutum* and introgression of resistance from these species requires specialized breeding methods [28,29]. Additionally, the development of improved breeding lines is time-consuming due to the transfer of undesirable agronomic traits along with the nematode resistance [28,30]. Thus, information on the genetics of resistance is needed to increase the efficiency of introgression and to aid in the identification of DNA markers linked to the resistant trait for marker-assisted breeding.

Resistance has been successfully transferred from *G. longicalyx* and *G. arboreum* accessions to upland cotton using hexaploid bridging lines to obtain tetraploid trispecies hybrids [28,29]. Resistance from *G. longicalyx* has been more extensively evaluated and the four accessions representing the species in the United States Department of Agriculture (USDA), National Plant Germplasm System (NPGS) cotton collection showed immunity to reniform nematode infection. These four accessions were classified as non-hosts because reniform females penetrating the root system never matured or produced eggs [9,18]. For *G. arboreum*, the incompatible plant reaction resulted from lignification of host cells adjacent to the nematode head and cell wall lysis [10], which contributed to a significant reduction in the number of reproductive females on the root system. However, more than 1,700 accessions are maintained in the NPGS *G. arboreum* germplasm collection representing many more diverse ecogeographic regions as compared to the *G. longicalyx* collection; and thus the *G. arboreum* collection may be the best resource for identifying new sources of resistance. Screening of the *G. arboreum* collection is underway and PI 529728 was identified as a new source of reniform resistance, but to successfully use new sources of resistance for the development of resistant cotton varieties determining the genetics of resistance is essential. Therefore, the objective of this study was to determine the inheritance of *R. reniformis* resistance for the germplasm line PI 529728 by crossing the line with a highly susceptible *G. arboreum* accession, PI 529729, to develop segregating populations to determine the range of phenotypic variability that could aid genetic interpretation of resistance and introgression of resistance into upland cotton.

2. Materials and Methods

Seed samples for the *G. arboreum* germplasm lines PI 529728 (A2-100) and PI 529729 (A2-101) were supplied by the NPGS cotton collection in College Station, TX. The two lines have red colored stems and okra shaped leaves that are highly pubescent. PI 529728 flowers have a red petal spot with yellow petal color; whereas, PI 529729 flowers have white petal color with a red petal spot.

PI 529728 was rated as moderately resistant to *R. reniformis* infection in growth chamber evaluations based on the number of swollen females per gram of root; whereas, PI 529729 was rated as highly susceptible. For the genetic evaluation of resistance, the PI 529729 x PI 529728 cross was made and 10 F_1 plants were rated for *R. reniformis* resistance. Because the F_1 plants showed susceptibility (Table 1), PI 529728 was used as the female recurrent parent to develop a BC_1F_1 population to evaluate the genetics of resistance. Seeds of the F_1 generation were planted in the field and self-pollinated to develop an F_2 population for genetic evaluation. Population development and nematode screening was conducted at the United States Department of Agriculture, Agricultural Research Service, Crop Genetics Research Unit in Stoneville, MS.

Table 1. Mean Number and Range of Swollen Female Reniform Nematodes per Gram of Root and Root Weights for the *Gossypium arboreum* Parental Lines, F_1 Progeny, and Control Genotypes.

Genotype	Females per gram of root		Root fresh weight (g)	
	Mean[a]	Range	Mean[a]	Range
PI 529729 (A_2-101, susceptible parent)	175	45-446	0.45	0.28-0.66
PI 529729/PI 529728 (F_1 progeny)	66	40-108	0.51	0.23-0.70
PI 529251 (Deltapine 16, susceptible *G. hirsutum* control)	40	27-47	1.76	1.14-2.45
PI 163608 (Texas 110, resistant *G. barbadense* control)	20	19-23	1.62	1.18-2.45
PI 529728 (A_2-100, resistant parent)	19	0-42	0.76	0.10-1.10

[a]The means represent the average of five plants for the control genotypes and 10 plants for the parental genotypes and F_1 generation.

Screening of the F_1, BC_1F_1, and F_2 populations for infection by reniform nematode was conducted in a plant growth room at a constant temperature of 27°C with a 16 hour photoperiod. A single seed was planted in each container (Ray Leach SL-10 Cone-tainer, Stewe and Sons Inc., Tangent, OR) with 10 F_1, 69 BC_1F_1, and 332 F_2 seedlings screened for resistance. Each population was screened separately because of space limitations in the growth room. Two genotypes were included as controls in each screening; PI 163068 (*G. barbadense* accession Texas 110) was used as the resistant control and PI 529251 (*G. hirsutum* accession Deltapine 16) was used as the susceptible control. The controls were replicated five times in each evaluation. The two parental lines were also included in each screening and replicated 10 times in the F_1 screening and five times in the BC_1F_1 and F_2 evaluations. Containers were filled with approximately 120 cm^3 of a steam sterilized soil mix consisting of two parts sand and one part sandy loam soil. An automatic watering system was used to maintain soil moisture and adjusted as needed during the experiment to supply additional water with seedling growth. Seedlings were inoculated at 7 and 14 days after planting using 500 vermiform reniform nematodes suspended in 1 ml of tap water for each inoculation, resulting in an inoculum level of 1,000 nematodes per seedling. Mississippi isolate MSRR04 [31], maintained on tomato (*Solanum lycopersicon* cv. Rutgers), was used to evaluate all populations. Plants were removed from the containers approximately 28 days after the second inoculation. The

root system was removed from individual plants, gently agitated in tap water to remove soil, and stained with red food coloring [32]. Swollen females attached to the root system (Figure 1) were counted using a stereomicroscope [33]. The root samples were placed on paper towels to remove excess moisture and fresh weights were determined. Results were expressed as the number of females per gram of root in order to compensate for differences in root size. The disease response of the resistant control genotype was used to classify plants as resistant or susceptible. Plants that supported the same number or fewer females per gram of root compared to the mean number supported on PI 163068 were classified as resistant. Segregation ratios were tested using the Chi-square test of significance (Statistix 9, Analytical Software, Tallahassee, FL).

3. Results

The number of swollen female reniform nematodes per gram of root (FGR) showed quantitative variation for the populations. The F_1 plants supported more FGR than the resistant parent PI 529728 and the susceptible control PI 529251, but fewer FGR than the susceptible parent PI 529729 (Table 1). The F_1 generation was classified as susceptible.

For the BC_1F_1 population, the FGR ranged from 2 to 300 (Figure 2), with a mean of 74 FGR for the 69 plants. The resistant parent PI 529728 supported a mean of 46 FGR. This value is 28% lower than the mean FGR on the susceptible control PI 529251, and 61% lower than the mean FGR supported on the susceptible parent PI 529729. Thirty BC_1F_1 plants showed fewer FGR (mean 30, range 2-46) than the resistant parent PI 529728. For a single gene model, a 1:1 segregation ratio would be observed for the backcross population and classifying these 30 plants as resistant would indicate a single recessive gene is conferring resistance ($\chi^2 = 1.17$, P = 0.2786). Compared to the resistant control PI 163608, 10 BC_1F_1 plants supporting 18 or fewer FGR would be rated as resistant and these data do not suggest a single recessive gene conferring resistance ($\chi^2 = 34.8$, P = 0.0) or support a two gene model ($\chi^2 = 4.06$, P = 0.0438). For both models, more plants would be rated susceptible than expected.

For the F_2 population, disease severity was reduced based on the data for the control genotypes and parental lines with FGR ranging from 1 to 154 for the 332 F_2 plants (Figure 3), with a mean of 22 FGR. The resistant parent PI 529728 supported a mean of 27 FGR, which was 61% fewer nematodes than the mean FGR supported on the susceptible parent PI 529729 and this reduction was consistent with the data for the BC_1F_1 population. The resistant parent PI 529728 showed a slightly higher mean FGR than the susceptible control PI 529251. For a single recessive gene model, 83 F_2 plants would be predicted as resistant and 249 plants as susceptible. Based on the mean of 10 FGR supported on the resistant control PI 163608, 62 F_2 plants showed fewer FGR and 19 more had levels of infection equal to that observed on PI 163608. The classification of these 81 plants as resistant would fit a single recessive gene model ($\chi^2 = 0.08$, P = 0.7758).

Root weights were compared to determine if resistant plants had a smaller root system that would support fewer female nematodes. The tetraploid control genotypes had higher mean root weights than the *G. arboreum* genotypes (Table 1), but mean root weight of the resistant *G. arboreum* parent PI 529728 was only slightly larger than the mean for the susceptible *G. arboreum* parent PI 529729. No obvious differences in root weights were observed between BC_1F_1 progeny classified as resistant (n = 10, mean 0.45 g, range 0.25-0.76 g) or susceptible (n = 59, mean 0.44 g, range 0.15-1.04 g). In the F_2 population, root weights were similar for lines classified as resistant (n = 81, mean 1.04 g, range 0.24-2.23 g) or susceptible (n = 251, mean 0.96 g, range 0.11-2.72 g).

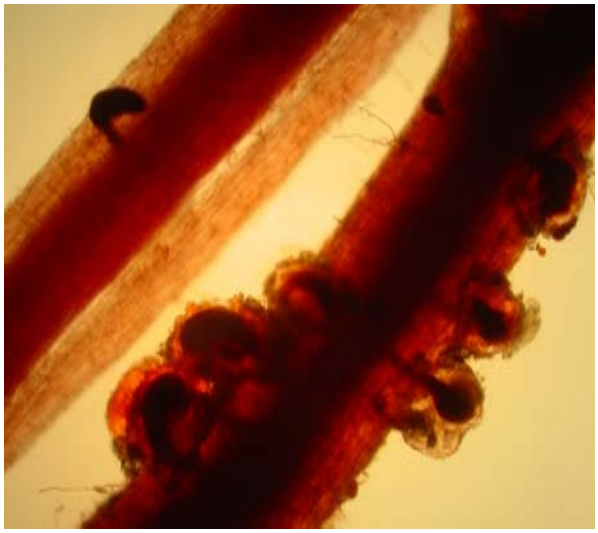

Figure 1. Relative levels of *Rotylenchulus reniformis* infection on resistant (top left) and susceptible (bottom right) F_2 *Gossypium arboreum* plants derived from the cross PI 529729/PI 529728 (50 × magnification).

4. Discussion

Understanding the genetics of *R. reniformis* resistance will provide essential information for breeding resistant cotton varieties, pyramiding multiple sources of resistance into single varieties, and assessing genetic diversity of resistance. However, determining the genetics of resistance is confounded by quantitative variation in disease response and by environmental variation in the screening procedure [17,22,29]. This variation may be more pronounced for accessions with moderate resistance as was observed for accession PI 529728 used as the resistant parent in this study.

The populations evaluated in this study showed quantitative variation in disease response and plants were classified as resistant that support the same or fewer FGR as compared to the resistant control PI 163068. Data from the F_2 population would strongly support the single recessive gene model. However, the BC_1F_1 population would have a greater frequency of susceptible genotypes and the data would suggest resistance is quantitatively inherited. The BC_1F_1 population was considerably smaller than the F_2 population and inherent variability in nematode infection could have resulted in more plants rated as susceptible. The evaluation of a large F_2 population would reduce this potential source of variation. The susceptibility of the F_1 plants confirms that a recessive gene is conferring resistance.

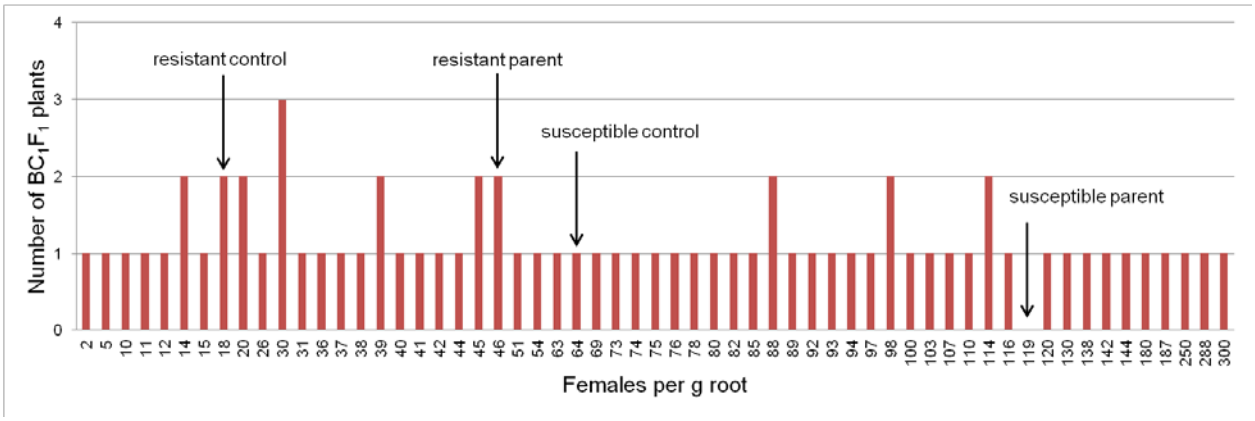

Figure 2. Distribution of 69 BC_1F_1 *Gossypium arboreum* plants derived from the cross PI 529728//PI 529729/PI 529728 based on infection by reniform nematode. Reactions for the resistant parent (PI 529728), susceptible parent (PI 529729), resistant *G. barbadense* control (PI 163608), and susceptible *G. hirsutum* control (PI 529251) are indicated by arrows and represent the mean of five plants. Plants supporting 18 or fewer females per gram of root (FGR) were classified as resistant based on mean FGR of the resistant control PI 163608.

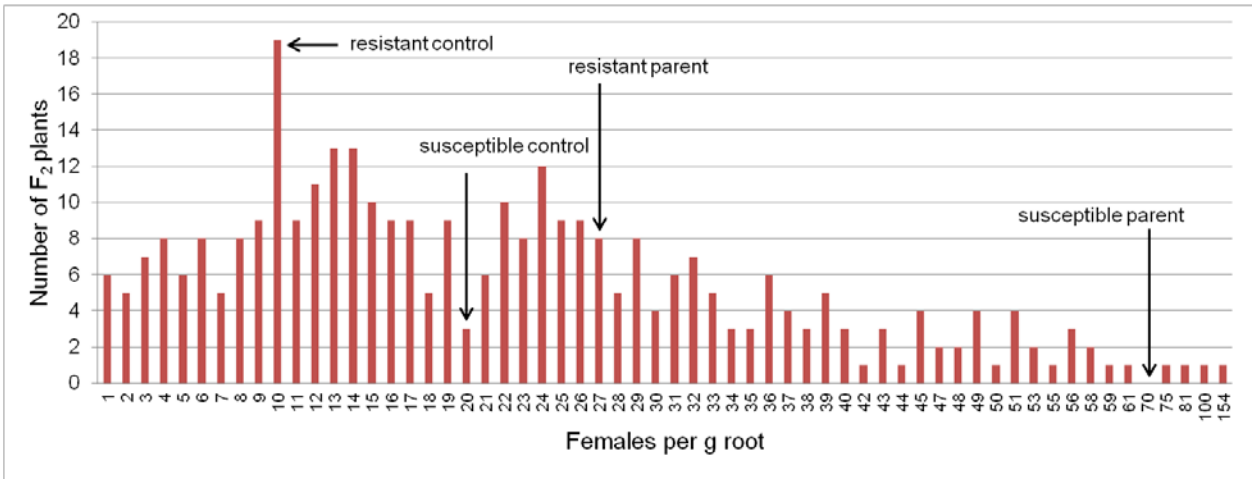

Figure 3. Distribution of 332 F_2 *Gossypium arboreum* plants derived from the cross PI 529729/PI 529728 based on infection by reniform nematode. Reactions for the resistant parent (PI 529728), susceptible parent (PI 529729), resistant *G. barbadense* control (PI 163608), and susceptible *G. hirsutum* control (PI 529251) are indicated by arrows represented by the mean of five plants. Plants supporting 10 or fewer females per gram of root (FGR) were classified as resistant based on mean FGR of the resistant control PI 163608.

The highest frequency of nematode-resistant accessions was found in the cotton diploid species germplasm collection [18]. Resistance from *G. longicalyx* has been successfully introgressed into upland cotton and Robinson et al. [28] reported nematode resistance was conferred by a single dominant gene. Agudelo et al. [9] suggested it was an incompletely dominant trait because the *G. longicalyx* resistant phenotype was not observed in hybrid combinations. Single dominant genes conferring resistance have also been reported for nematode resistance introgressed from *G. aridum* and *G. arboreum* [29,34,35]. In the present study, the BC_1F_1 and F_2 populations developed from the *G. arboreum* germplasm line PI 529728 showed no apparent bimodal clustering of genotypes into resistant and susceptible categories as was observed for populations developed from *G. longicalyx* [28] and *G. arboreum* [29]. Although, fewer lines clustered around the highly susceptible parent PI 529729 in the F_2 population. In the studies with *G. longicalyx* [28] and *G. arboreum* [29], the resistance had been introgressed into a *G. hirsutum* background prior to genetic characterization and resistance was determined based on indirect assays. Sacks and Robinson [29] reported a greater frequency of plants with the resistant phenotype in the distribution of a BC_1 population with a *G. hirsutum* line used as the recurrent parent. In comparison, the distribution for the BC_1F_1 population in the present study was more uniform. Additionally, the susceptible parent used to develop the populations in the present study was highly susceptible as compared to PI 529251, resulting in a more extensive range of quantitative variation observed for the progeny.

Screening tetraploid *G. hirsutum* and *G. barbadense* germplasm for *R. reniformis* resistance has received greater emphasis [15,16,17,25,26], as sources of resistance would be more rapidly introgressed into upland cotton varieties than resistance identified from diploid species. Despite some inconsistencies in the identification of resistant germplasm [22], only weak to moderate resistance has been identified in *G. hirsutum* germplasm with less than 0.4% of the accessions classified as resistant [17,25]. Muhammad and Jones [36] conducted a genetic evaluation of three upland cotton lines and reported that resistance was quantitatively inherited with significant epistatic gene effects and also observed transgressive segregation for susceptibility, suggesting resistance was controlled by two or more genes. Recently, McCarty et al. [37] released three germplasm lines with moderate resistance derived from the *G. hirsutum* photoperiodic

primitive race accession T2468; however, the genetics of resistance is unknown for these lines.

Compared to *G. hirsutum* germplasm, *G. barbadense* germplasm generally showed less susceptibility with 19 moderately resistant and five resistant accessions identified from the collection [25]. Starr et al. [22] reported that resistance in *G. barbadense* accession Texas 110 (PI 163068) was inherited as a dominant trait; however, the F_2 population data did not fit a one- or two-gene model, suggesting the resistance trait was polygenic. Accession GB713 (PI 608139) showed the highest level of resistance within *G. barbadense* germplasm [25]. The resistance in accession GB713 was determined to be conferred by three QTL with significant additive and dominance effects [38]. Similarly, Starr et al. [22] reported a single partially dominant gene with additive effects conferring resistance in GB713.

The PI 529728 source of resistance evaluated in this study is being introgressed into a *G. hirsutum* background to develop breeding lines for cotton improvement and for the identification of DNA markers associated with the resistant phenotype for marker-assisted selection. Multiple generations of backcrossing are typically required to introgress resistance into a *G. hirsutum* background to recover the upland cotton phenotype. Results of this study would suggest screening larger BC_xF_2 populations would be desirable to successfully recover progeny with the PI 529728 source of resistance because resistant plants would be less frequent in the population.

5. Conclusions

Accession PI 529728 was identified as a source of *R. reniformis* resistance from the germplasm collection and genetic characterization of the accession suggested resistance is conferred by a single recessive gene. This is the first report of a recessive gene conferring *R. reniformis* resistance in *G. arboreum*, suggesting genetic diversity for resistance in the germplasm collection. Additionally, highly resistant plants were frequently observed in the BC_1F_1 and F_2 populations developed from PI 529728 indicating that highly resistant breeding lines could be derived from moderately resistant sources. Thus, *G. arboreum* accessions identified with moderate resistance should not be overlooked as these accessions could be an important source of new resistance genes. For cotton diploid species, nematode resistance appears to be associated with single genes; therefore, introgression of resistance into a *G. hirsutum* background should be straightforward. More than 1,700 accessions are maintained in the NPGS *G. arboreum* germplasm collection representing many diverse ecogeographic regions and nematode-resistant accessions are frequent in the collection, thus evaluation of the collection should result in the identification of additional sources of resistance.

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Abbreviations: FGR (female reniform nematodes per gram of root).

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