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DEVELOPMENT AND CHARACTERISTICS OF TRIPLE SPECIES HYBRIDS USED TO TRANSFER RENIFORM NEMATODE RESISTANCE FROM *GOSSYPIUM LONGICALYX* TO *GOSSYPIUM HIRSUTUM*

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Abstract

Two triple-species hybrids were developed by a protocol designed to achieve a tetraploid plant (52 chromosomes) containing one complete set of 13 F genome chromosomes from *Gossypium longicalyx*, with the goal of backcrossing the hybrids with *G. hirsutum* to introgress genes from the F genome into Upland cotton. The genes of greatest interest were those conferring immunity to the reniform nematode, an important trait found in no other *Gossypium* species. The strategy was to substitute an F genome set of chromosomes for an A genome set in a triple- species hybrid of *G. hirsutum* × *G. longicalyx* × *G. amourianum* (HLA), and for a D genome set in a triple-species hybrid of *G. hirsutum* × *G. herbaceum* × *G. longicalyx* (HHL). The HLA hybrid was formed by crossing *G. armourianum* pollen onto a *G. hirustum* 'TM-1' (Deltapine 14) × *G. longicalyx* hexaploid created by Dr. Meta Brown. The HHL hybrid was formed by crossing *G. longicalyx* pollen onto a *G. hirsutum* 'Tamcot CAMD-E' × *G. herbaceum* hexaploid. Both hexaploids were obtained by colchicine treatment of vegetative buds of their triploid progenitors. The hybrids retained *R. reniformis* immunity. Nematodes infected roots but neither hybrid supported normal development or egg mass production by parasitic females on roots, and nematode populations in soil ultimately dropped to undetectable levels when hybrids were grown in pots inoculated with nematodes. The hybrids exhibit various parental traits related to growth habit and flower morphology. Both hybrids are male sterile but flowers can be pollinated by *G. hirsutum* to obtain backcross progeny, with six times as many viable embryos obtained from HLA as HHL. More than 1,100 progeny have been obtained from the hybrids, with ca. 900 characterized in relation to fertility and (or) nematode resistance. Because a small number of nematodes from the primary inoculum usually survive until the termination of nematode resistance assays, a value of 1% of the nematodes observed either in soil or on roots of the susceptible 'Deltapine 16' control was operationally defined as immunity. Among 66 BC1 progeny of HLA with various male parents, 9 were scored as immune and 8 as either immune or highly resistant. Among BC1 populations obtained by crosses of 'Acala NemX' pollen onto HLA (603 progeny) and HHL (21 progeny), 106 and 4 plants, respectively, were male-fertile with 12 and 1 plant, respectively, scored as immune to *R. reniformis*. Immunity was pollen-transferred to one-fourth (26%) of progeny within second and third *G. hirsutum* backcross generations from 'Delta and Pineland 458' bolls, suggesting inheritance by a single dominant gene.

Introduction

Since 2000, the reniform nematode (*Rotylenchulus reniformis*) has caused annual losses of 1.3 to 1.7% of the potential cotton crop in the United States and over 5% in Mississippi and Louisiana (Cotton Disease Council Loss Estimates). All of the many cultivars that have been tested support prolific reproduction by the reniform nematode and often suffer losses of 15 to 45% in heavily infested fields (Cook et al., 2002, 2003; Robinson, 1999; Robinson et al., 1999). Some tolerant breeding lines have been identified that can yield well in infested fields under optimal growing conditions in the areas to which they are adapted. Tolerant lines do not, however, reduce populations of the nematode in the soil and heavy losses can be expected if susceptible cultivars are planted after them. It is not known with certainty how much suppression of nematode reproduction is needed to prevent crop damage. If the objective is to clean up a field while growing cotton, then a very high level of resistance to this nematode will be required. Numerous studies with partially resistant rotational crops have shown that populations of *R. reniformis* rebound to damaging levels during the first half of the first season that a field is planted back to cotton. Various levels of partial resistance to *R. reniformis* occur in some but not other accessions of *G. arboreum*, *G. herbaceum* and *G. barbadense* (Yik and Birchfield, 1984) and there are protocols that have been used successfully in some cases to transfer traits from these species into Upland cotton. In pots, these sources of resistance typically reduce reniform nematode populations by 60 to 85%.

Complete resistance to *R. reniformis* occurs in the wild species *G. longicalyx* from eastern Africa (Percival et al., 1999; Yik and Birchfield, 1984). *G. longicalyx* is unusual among wild cottons in having a scandent growth habit and in being well-adapted to mesic environments and salt stress. It is genetically incompatible with *G. hirsutum* because it is a diploid with the F genome unique to *G. longicalyx*, contrasted with the amphidiploid *G. hirsutum* that contains the common A and D genomes of *Gossypium* species (Bell, 1984) We report here the development of two male-sterile triple-species hybrids that were designed and developed to contain a complete set of the F genome chromosomes in a tetraploid plant, allowing the hybrids to be used to transfer reniform nematode immunity and other unique F genome traits into *G. hirsutum*.

Methods and Materials

Approaches for developing multiple species plants and the use of these plants for transferring resistance to pests in cotton have been reviewed (Bell, 1984). The approach that worked for incorporating *G. longicalyx* chromosomes into a synthetic tetraploid was the cross between a diploid and hexaploid to yield the tetraploid. The two-species hexaploids were created by crossing an amphidiploid (tetraploid) with a diploid to first yield a sterile triploid, which then was converted to a hexaploid by colchicine treatment of axillary buds. Buds at the base of the cotyledons or first three true leaves were treated with 1% colchine in K-Y jelly or lanolin, and the plants were decapitated above the treated buds to force growth of new branches. A few hexaploid flowers occurred on some of these branches and were both self-fertile and fertile when pollinated with *G. hirsutum*. These flowers also were larger and displayed restoration of normal pollen formation and shed, compared to triploid flowers that rarely shed pollen.

The HLA triple species hybrid was formed by crossing the diploid *Gossypium armourianum* (D genome) as pollen onto a *G. hirsutum* 'TM-1' (Deltapine 14) × *G. longicalyx* hexaploid created by Dr. Meta Brown. The HHL hybrid was formed by crossing *G. longicalyx* pollen onto a *G. hirsutum* 'Tamcot CAMD-E' × *G. herbaceum* (A genome) hexaploid. Thus, the HLA hybrid is expected to have an F genome set of chromosomes substituted for an A set (i.e., AFDD genome), while the HHL hybrid has an F genome set substituted for a D set (i.e., AADF genome). Both HLA and HHL are self-sterile and failed to give seed when used as a pollen source. Over-pollination with *G. hirsutum*, however, occasionally gave bolls containing 1-4 seeds. Cuttings from both HLA and HHL root readily and were used as female parents and experimental controls in crossing studies.

In 1999, pot-bound plants of HLA, HHL, *G. longicalyx*, and 'Tamcot CAMD-E' (control) that had been grown within 500-cm^3 plastic cups were transplanted into 4-liter pots containing sand that was uniformly infested with reniform nematodes and roots that grew out into the surrounding sand from the original root ball were collected 1, 2 and 3 weeks after transplanting and fixed in FAA. The nematodes on ca. 1,250 cm of roots of each genotype (ca. 1 nematode/cm) were counted and classified developmentally. Roots were processed for serial sectioning and examined by collaborator Paula Agudelo at the University of Arkansas, to characterize histological changes induced by nematode feeding.

The first lot of 69 first-generation backcross (BC1) seed were generated in 1999-2000 by using random *G. hirsutum* pollen sources as available, including 'Paymaster H1220', 'Stoneville 373', 'Suregrow 125' 'Tamcot Sphinx', 'Auburn M-315', 'Auburn 623', 'Acala NemX', and 'Stoneville LA887' to pollinate both hybrids. Plants grown from these seed were tested for reniform and root-knot nematode resistance by a split-root technique and 20 reniform-immune or highly resistant plants were identified. Resistant BC1 plants from the first lot were pollinated with 'Acala Nem-X' and other root-knot nematode-resistant parents to obtain BC2 seed and were self-pollinated to obtain BC1S1 seed; 245 BC1 and BC2 progeny were evaluated for immunity to *R. reniformis*.

A second lot of BC1 seed with uniform parentage was generated in 2002. Forty plants of each hybrid, HLA and HHL, were pollinated daily with 'Acala Nem-X' from April 1 until July 31 to obtain 603 seed from HLA and 21 from HHL. These were planted in 500-cm^3 cups to identify male fertile and female fertile plants; male fertile plants were subsequently transplanted to 4-liter pots infested with reniform nematodes and evaluated for nematode immunity.

Immune progeny of BC1 and BC2 immune parents were used during 2003 as pollen sources to pollinate 'Delta and Pineland 458', and the BC2 and BC3 seed from these crosses were in turn planted and evaluated. Plants of 'Delta and Pineland 458' pollinated by immune BC3 plants have set bolls that are expected to produce BC4 seed by January of 2004.

Methods used to screen BC1 plants from lot 1 and appropriate controls, and results of these tests, were described previously (Robinson, 2002; Robinson et al., 2002). Progeny from those BC1 plants and plants of the second lot of BC1 were screened by starting plants in 500-cm^3 cups containing 450 ml of greenhouse mix and transplanting into sand infested with reniform nematodes in 4-liter pots. The BC2 and BC1S1 progeny and controls were transplanted as young plants (4-8 true leaves) and BC1 plants from lot 2 were transplanted as mature plants after they were first scored for self-fertility. Only self-fertile plants were screened.

Results and Discussion

The HLA and HHL hybrids both retained the scandent (climbing) growth habit of *G. longicalyx* so that branches reached several feet in length and soon become recumbent (Fig. 1). The calyxes of some hybrid flowers show accentuated division and narrow triangular elongation of lobes similar to *G. longicalyx*, but not to the same degree. Both hybrids produce flowers prolifically all year in the greenhouse and have yellow petals. The HLA hybrid has pink staminal columns and smooth stems like *G. armourianum*; the HHL hybrid has red petal spots and hairy stems similar to *G. herbaceum*. Both hybrids are extremely vigorous and readily propagated by cuttings. While the hybrids flower profusely through the year, they retain bolls only during the spring and summer months. The hybrids are female fertile, but not male fertile, and develop single seeds in bolls without hormone assistance. Seeds from HLA have green lint, while those from HHL have tan lint.

The HLA hybrid backcrossed more readily with *G. hirsutum* than did HHL. As noted, we obtained 603 seeds when 40 HLA plants were over-pollinated daily for 4 months with 'Acala NemX' and only 21 seeds (3.5% as many) from the 40 HHL plants. Our data suggest that BC1 plants from HLA also have greater self fertility than BC1 from HHL, with 104 (17%) of HLA and 4 (10%) of HHL plants being self fertile. We note however that the sample size (21) for HHL was small. BC1 from the two hybrids appear to have similar female fertility.

Data for experimental controls from BC1S1 and BC2 evaluations in 2003 illustrate the unique level of resistance consistently seen in progeny from *G. longicalyx* when contrasted with other sources of resistance to *R. reniformis* within *Gossypium* (Fig. 2). In this experiment, we included as experimental controls 121 plants of five genotypes spanning the full range of reniform nematode resistance. Among these were 27 replications of the HHL hybrid, 6 of the highly resistant *G. barbadense* 'GB-713', 32 of resistant *G. barbadense* 'TX-1348', 28 of resistant *G. arboreum* 'A2-87' and 28 of susceptible 'Deltapine 16'. The standard deviation in our assays varies directly with the mean so that the confidence interval around nematode reproduction for highly resistant genotypes is a small fraction of the range between zero and the mean level observed for susceptible genotypes. Within BC1 populations, we have observed 26-32% of the plants to show a level of resistance comparable to that in the *G. longicalyx* parent, while the remainder show a wide range of nematode reproduction levels (Fig. 3). It is important to note that BC1 plants typically are highly variable morphologically and are the survivors of a much larger population of largely aborted embryos, and prudence must be exercised in using ratios of immune and susceptible plants in BC1 populations to test hypotheses regarding resistance inheritance. Within the BC2 and BC3 populations from 'Delta and Pineland 458' bolls tested in 2003, we observed one fourth (26%) of the plants to exhibit the characteristic immunity trait. Thus, results are generally consistent with Mendelian inheritance of immunity through a single dominant gene. However, an additional weak level of resistance of unknown origin is consistently observed in some of the progeny that lack immunity.

We have initiated inheritance studies that utilize progeny testing and nematode assay improvements to resolve intermediate phenotypes. Collaborative research also has been initiated with David Stelly at Texas A&M University to examine cytogenetics in relation to immunity inheritance and develop molecular markers.

References

Bell, A.A. 1984. Morphology, chemistry and genetics of *Gossypium* adaptations to pests. Recent Advances in Phytochemistry. 18:197-230.

Cook, C.G., A.F. Robinson, A.C. Bridges, A.E. Percival, W.B. Prince, W.B., J.M. Bradford, and J.A. Bautista. 2002. 2001 Evaluation of cotton cultivars grown in reniform nematode infested soil, pp. 433-434. *In* 2002 Proceedings of the Beltwide Cotton Conferences.

Cook, C.G., A.F. Robinson, A.C. Bridges, A.E. Percival, W.B. Prince, J.M. Bradford, J.M., and J.A. Bautista. 2003. 2002 Field evaluation of cotton cultivar response to reniform nematodes. *In* 2003 Proceedings of the Beltwide Cotton Conferences.

Percival, A.E., J.F. Wendel, and J.M. Stewart. 1999. Taxonomy and germplasm resources, pp. 33-63. *In* C.W. Smith and J.T. Cothren (eds.) Cotton: Origin, History, Technology, and Production. John Wiley and Sons, Inc., New York.

Robinson, A.F. 1999. Cotton nematodes, pp. 595-615. *In* C.W. Smith and J.T. Cothren (eds.) Cotton: Origin, History, Technology, and Production. John Wiley and Sons, Inc., New York.

Robinson, A.F. 2002. Reniform nematodes: *Rotylenchulus* species, pp. 153-174. *In* J.L. Starr, J.L. Cook, and J. Bridge (eds.) Plant Resistance to Parasitic Nematodes. CAB International, London.

Robinson, A.F., A.A. Bell, and A.C. Bridges. 2002. Progress in transferring reniform nematode immunity from *Gossypium longicalyx* into upland cotton. *In* 2002 Proceedings of the Beltwide Cotton Conferences.

Robinson, A.F., C.G. Cook, and A.E. Percival. 1999. Resistance to *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3 in the major cotton cultivars planted since 1950. Crop Science 39:850-858.

Yik, C.P., and W. Birchfield. 1984. Resistant germplasm in *Gossypium* species and related plants to *Rotylenchulus reniformis*. J. Nematol. 16:146-153.

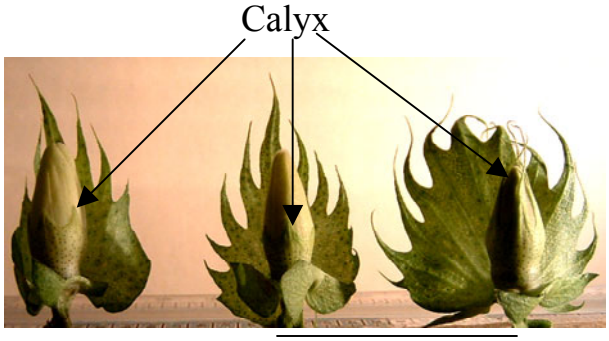
HHL hybrid – Red petal spots from *G. herbaceum*

HLA hybrid – Pink staminal filaments from *G. armourianum*

HLA

HHL

Stem Pubescence



G. hirsutum **BC1 F1**

A.A. Bell with HLA hybrids

Decumbent growth habit of HLA

Figure 1. Characteristics of triple species hybrids.

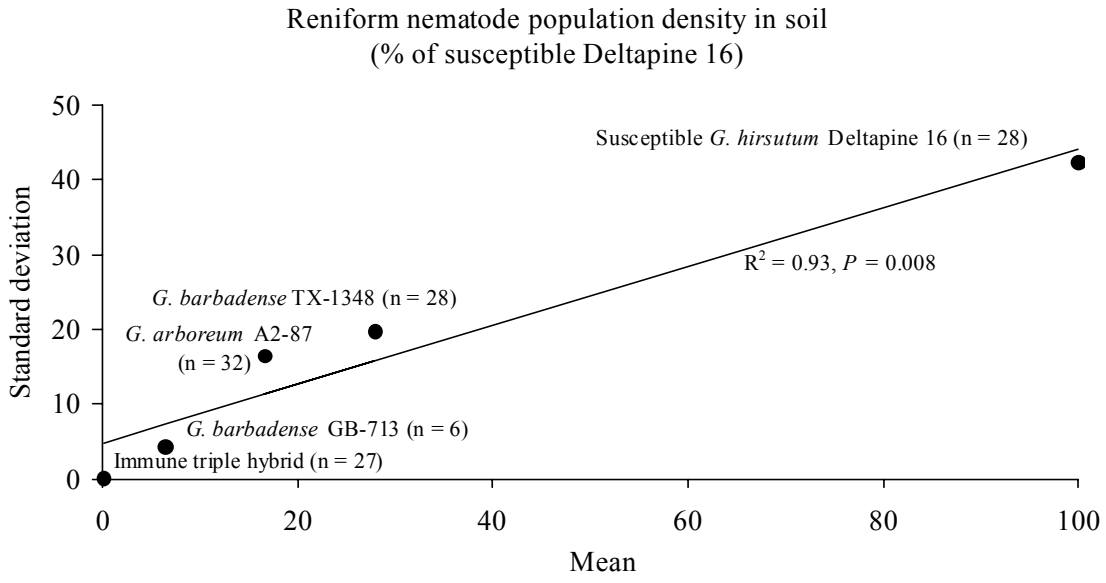


Figure 2. Relationship between standard deviation and mean of nematode reproduction for selected controls.

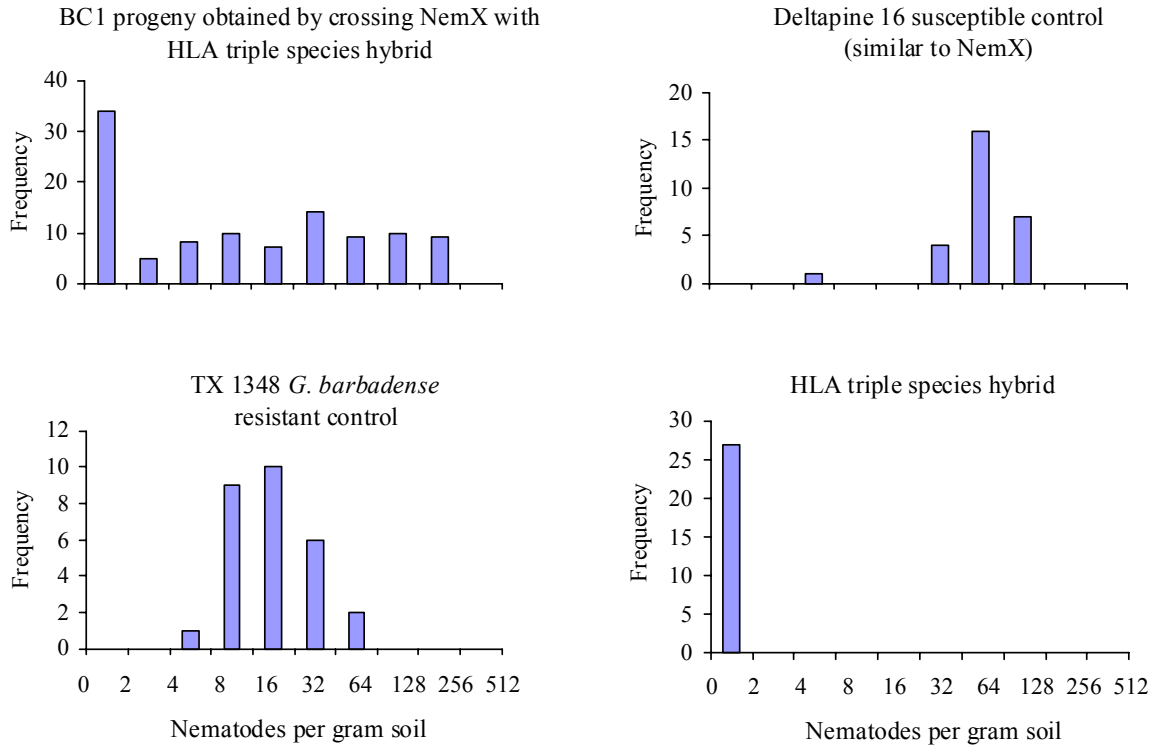


Figure 3. Inheritance of nematode resistance within BC1 progeny from HLA triple species hybrid.