INTROGRESSION OF RENIFORM NEMATODE RESISTANCE AND OTHER GERMPLASM FROM *Gossypium longicalyx* AND *G. armourianum* INTO *G. hirsutum*

A Dissertation

by

NILESH DEORAM DIGHE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2007

Major Subject: Plant Breeding

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ABSTRACT

Introgression of Reniform Nematode Resistance and Other Germplasm from *Gossypium longicalyx* and *G. armourianum* into *G. hirsutum*. (December 2007)

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The *Gossypium* genus includes 45 diploid and 5 tetraploid cotton species of which only 2 diploids and 2 tetraploids are cultivated in different parts of the tropics and sub-tropics, leaving the remaining diploid and tetraploid species as potential genetic sources for novel trait introgression. The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) poses significant problems to US cultivated Upland cottons (*Gossypium hirsutum* L., 2n=52), all of which lack high resistance. This work was in collaboration with the USDA-ARS team that focussed on introgressing reniform nematode-resistance from a diploid cotton species, *Gossypium longicalyx* Hutch. & Lee into *G. hirsutum* by creating a tri-species hybrid, HLA and backcross breeding (Bell et al., 2007; Robinson et al., 2007). The main objectives of this work were [1] to cytogenetically evaluate and help select superior types at each introgressed generation; [2] to identify molecular markers tightly linked to the reniform nematode-resistance gene and to map the resistance loci; [3] (A) to introgress germplasm on a genome-wide basis, (B) to evaluate introgressed germplasm for traits of economic importance other than reniform nematode resistance, and (C) to evaluate breeding methodologies in terms of this specialized breeding material.

Reniform nematode resistant plants were of diverse cytogenetic constitution but individuals that modally formed 26II chromosomal configuration were identified at BC2F1, BC3F1, BC4F1, and BC5F1 generations. Three SSR markers, BNL3279_114, BNL1066_156, and BNL836_215 and one phenotypic marker, green-colored fuzz (*Fzg*^{lon}), were identified to be tightly-linked to the resistance locus. Extension of the association analysis and linkage estimation to 16 susceptible self progeny (BC1S1, BC3S1 and BC6S1) and 374 susceptible backcross hybrids (BC2F1-BC8F1) mapped the resistance locus to chromosome 11 of cotton with BNL3279_114 on one side and *Fzg*^{lon} on the other at 0.8 cM and 2.8 cM, respectively. Besides reniform nematode-resistance introgression, genome-wide introgression efforts were also conducted. Low micronaire and high fiber strength were the two most promising traits identified in the HLA-derived introgressed generations. Most of the introgressed generations had high variability for the fiber-quality traits than the commercial checks, thus providing more opportunities for selection and improvement.

DEDICATION

To my wife, Shweta and son, Veer

ACKNOWLEDGMENTS

I would like to thank Dr. David Stelly for training and guiding me during the course of my research at the New Beasley laboratory. I am also thankful to him for helping me on my presentation skills, manuscript writing, supporting me financially, and sharing his valuable research ideas and experiences with me. I am very thankful to Dr. Forest Robinson and Dr. Alois Bell for giving me an opportunity to work with them and also for letting me use their data and research material. I take this opportunity to thank Dr. Monica Menz for training me on molecular marker technology and for her valuable guidance related to my research. I am also thankful to Dr. Wayne Smith for his support during my stay at Texas A&M University.

I take this opportunity to thank Wayne Raska for training me on how to do air-layering in cotton, taking care of my plants in the greenhouse and field, and also for helping me plant and harvest cotton. Help from my fellow graduate students, Brian Gardunia, Kelly Biddle, and Steven Todd was incredible, and I thank them for all the help. I thank Dr. Sam Yang for his help during planting and harvesting cotton. I am thankful to all the student workers from Dr. David Stelly's lab who helped me plant and harvest cotton, took care of my plants in the greenhouse and in the field, ginned fiber samples, collected flower buds, and made slides for my cytological work.

Working with Dr. Monica Menz's lab was a great experience. I am particularly thankful to Susan Hall from Dr. Menz's lab for helping me learn molecular marker techniques and her support during my stay in that lab. Dr. Wayne Smith's group was very helpful during the planting and harvesting season. I am thankful to Dawn Deno, Chris Souder, Nino Brown and several others from Dr. Smith's group. Jose Quintana and William Shattuck from the USDA-ARS were very helpful during DNA extraction, planting and harvesting cotton, and I thank them for their help. I am thankful to Cotton Incorporated for testing fiber samples related to my project, and for buying most of the primers that I used for molecular marker work. I take this opportunity to thank Dr. Roy

Cantrell, Dr. Don Jones, and Regina Horton from Cotton Incorporated for their help and guidance.

Lastly, but most importantly, I thank my wife, Shweta for her encouragement and moral support during the course of this study.

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CHAPTER I

INTRODUCTION

Cotton Cytogenetics

The cotton genus, *Gossypium* consists of 50 species, including 45 diploids (2n = 2x = 26), and 5 tetraploids (2n = 4x = 52) (Fryxell, 1992). The diploid species have been classified into 8 cytologically based genome groups as summarized by Endrizzi et al. (1985) and Stewart, (1995) and are found to exist in three primary centers of diversity including Afro-Asian species (A-, B-, F-, and E-genomes), the Australian species (C-, G-, and K-genomes), and the new world species (D-genome) (Fryxell, 1979; Small and Wendel, 2000). Only 2 diploid (*G. herbaceum* and *G. arboreum*) and 2 tetraploid species (*G. hirsutum* and *G. barbadense*) have been domesticated and are being cultivated in different parts of the world (Fryxell, 1976; Fryxell, 1992). Within these 4 cultivated species, Upland cotton, *Gossypium hirsutum* L. is produced most widely worldwide, including tropical, subtropical, and temperate latitudes (Lee, 1984). Upland cotton, *G. hirsutum* is an amphidiploid, consisting of A- and D-subgenomes and is designated as [2(AD)1] (Skovsted, 1934; Beasley, 1940, 1942).

The Asiatic A-genome and the American D-genome groups were identified as the most closely related to and are believed to be the progenitors of the A- and D-subgenomes of *G. hirsutum* (Beasley, 1942). To determine which of the two A-genome diploids and several D-genome diploids were more similar to the progenitors, several independent cytological investigations were conducted. Gerstel (1953) was the first to provide the clue on which A-genome species is more closely related to the A-subgenome of the allotetraploid cottons by studying the structural relationship between the A-subgenome of allotetraploid cotton and its diploid A-genome progenitors by analyzing the bi-specific hybrids involving *G. arboreum* and *G. herbaceum*, and *G. hirsutum* at the

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This dissertation follows the format of Crop Science.

metaphase I stage of meiosis. He observed that *G. arboreum* and *G. herbaceum* structurally differ from each other by one translocation, as indicated by consistent formation of a IV in metaphase I microsporocytes of their F1 hybrid. Furthermore, in hybrids of each species with *G. hirsutum*, he observed that *G. hirsutum* differ from *G. herbaceum* and *G. arboreum* by two and three reciprocal translocations, respectively, as indicated by the formation of two quadrivalents in the former hybrid, versus formation of a quadrivalent and a hexavalent in the latter. Gerstel (1953) concluded that *G. herbaceum* is the progenitor of the A-subgenome of the allotetraploid cotton. Molecular marker analysis has supported the cytological conclusion by Gerstel, in that *G. hirsutum* shows higher genetic similarity to *G. herbaceum* than *G. arboreum* (Kebede et al., 2007). Based on morphological characteristics and chromosomal pairing of hybrids involving the D-genome species, it is believed that *G. raimondii* is most closely related to the D-subgenome of the allotetraploid cottons (Stephens, 1944a, b; Hutchinson et al., 1945; Hutchinson et al., 1947).

Though identical in chromosome number, chromosomes of the A-genome diploids are noticeably bigger than the D-genome species and the size difference is maintained in the natural allotetraploids (Skovsted, 1934, 1937; Brown, 1951, 1954, 1958, 1961; Endrizzi and Phillips, 1960; Endrizzi, 1962). Meiotic pairing studies have revealed that the A- and D-subgenomes of the allotetraploid cotton are more fully "differentiated" from one another than are the genomes of extant species most closely related to these allotetraploid cottons (Endrizzi, 1962; Mursal and Endrizzi, 1976). This is supported by the low frequency of meiotic pairing between the A- and D-subgenomes of the haploids of allotetraploids, where an average of less than 2 bivalents per cell were observed (Beasley, 1942; Endrizzi, 1959; Sastry and Swaminathan, 1960), while the F1's of the A- and D-genome species had an average of 6-8 bivalents per cell (Endrizzi and Phillips, 1960; Mursal and Endrizzi, 1976; Skovsted, 1937).

Cotton and Impact of Reniform Nematode

In 2005-06, the total world cotton production was estimated to be 114 million bales, an increase of 16 million bales in last 4 years (Burr et al., 2006). The United States is one of the major cotton producers in the world, and was ranked only after China in cotton production by contributing 20% to the cotton production worldwide (Burr et al., 2006). Cotton is produced coast to coast in the USA primarily in 17 southern states (Smith, 1999). While, Upland cotton remains the most important fiber crop of the world, its narrow genetic base due to lack of genetic diversity in the commercially used cultivars and germplasm (Wendel et al., 1992; Brubaker and Wendel, 1994; Pillay and Myers, 1999; Khan et al., 2000; Abdalla et al., 2001; Iqbal et al., 2001; Rungis et al. 2005; Lacape et al., 2007), and use of relatively narrow genetic pool for variety development (van Esbroeck et al., 1998; Iqbal et al., 2001) has raised concerns about genetic vulnerability of cotton to biotic and abiotic stresses (Anonymous, 1972; Bowman et al., 1996), and is also believed to be the major contributor to the declined progress in developing improved cultivars for yield and fiber quality for the past 15 years (Meredith, 2000; Lewis, 2001).

It is estimated that various diseases caused 19% seedcotton yield loss in the United States in 2005 (Blasingame, 2006). In recent years, reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira), has been causing significant economic damage to cotton industry with losses exceeding $100M annually (Blasingame, 2006), which accounts for 45% of the cotton crop lost to all nematodes (Robinson, 1999). Reniform nematodes were first discovered on the roots of cowpea (*Vigna unguiculata* L. Walp. aggreg.) growing in pineapple fields in Oahu, Hawaii, USA in 1931 (Gaur and Perry, 1991) and were first reported as a pest of cotton by Smith in 1940. *Rotylenchulus reniformis* is a sedentary, semi-endoparasitic pest, feeding on the roots of wide range of food, fiber, oilseed, fruit, and plantation crops classified under herbs, shrubs and trees belonging to 30 plant families in most of the subtropical and tropical parts of the world (Varaprasad, 1986; Gaur and Perry, 1991).

In general, most of the populations are amphimictic with 40-60% males, but few populations that are parthenogenetic with very few or no males have been found (Triantaphyllou and Hirschmann, 1964; Sivakumar and Seshadri, 1971; Nakasona, 1977, 1983). The populations observed in cotton, however, are reported to be obligately amphimictic and to produce equal numbers of males and females (Robinson, 1999). Though the males and females are present in the soil, no evidence of copulation before the young females establishes a suitable feeding site and matures to a swollen kidney-shaped stage with convoluted ovaries has been observed (Gaur and Perry, 1991). Eggs are laid in a gelatinous matrix soon after its appearance, and egg-laying may continue for 1-3 weeks (Gaur and Perry, 1991). The first molt occurs within the egg (Sivakumar and Seshadri, 1971). After second-stage juveniles (J2) are well developed, the process of hatching commences, usually within 1-3 days of the formation of J2 (Gaur and Perry, 1991). The post-hatch molting from J2 to sexually differentiated adult is completed in the soil without feeding (Peacock, 1956; Nakasona, 1966, 1983; Sivakumar and Seshadri, 1971; Bird, 1984; Gaur and Perry, 1991). Bird (1983) reported reduction in the body volume with every molt. This reduction in the volume might be caused by the consumption of the glycogen and lipid reserves present in the J2 stage since the nematodes do not feed during the inter- or intra-molt periods (Gaur and Perry, 1991).

Only the adult females feed by invading the cortex of roots, mostly in the zone of elongation behind the growing tip (Birchfield, 1962; Robinson and Orr, 1980; Gaur and Perry, 1991). The vermiform females penetrate the epidermis and cortex, intracellularly and create a permanent feeding site in the endodermis and pericycle cells (Razak and Evans, 1976; Taha and Sultan, 1979; Khan and Khan, 1985). After establishing a feeding site, the vermiform female develops further and acquires the typical reniform kidney-shaped posterior that is positioned outside the root (Gaur and Perry, 1991). The life cycle of reniform nematodes varies with host suitability, temperature and other physiochemical properties of the rhizosphere (Gaur and Perry, 1991). While, Linford and Oliveira (1940) reported life cycle of 25 days, periods of 24-29 days life cycle on okra [*Abelmoschus esculentus* (L.) Moench] (Sivakumar and Seshadri, 1971) and 17-23

days on soybean [*Glycine max* (L.) Merr.] (Rebois, 1973) have also been reported. Factors contributing to emergence of this nematode as a major pathogen of cotton include its broad host range (Birchfield and Jones, 1961; Birchfield and Brister, 1962; Robinson et al., 1997), its ability to survive in a dormant state (Birchfield and Martin, 1967), to infest the soil profile to a depth of more than 1 meter (Lee et al., 2002; Newman and Stebbins, 2002) and its high population densities even in the absence of host (Lee et al., 2002).

Foliar symptoms of reniform nematode infection can be hard to detect under ideal growing conditions, but are more readily detected under stressed conditions (Robinson, 1999). The most consistent symptoms of reniform nematode infestation in cotton are uniform stunting, a one-to two-node delay in fruit set, yellowing of lower leaves, and browning of lower margins and tips as typically observed during potassium deficiency (Robinson, 1999).

Extensive screening of the *G. hirsutum* germplasm collection over the years, have confirmed that none of the Upland cotton genotypes has high resistance to reniform nematodes (Yik and Birchfield, 1984; Jones et al., 1988; Robinson and Percival, 1997; Robinson et al., 1997; Robinson et al., 1999), but moderately resistant (Robinson et al., 2004) and tolerant breeding lines (Cook and Robinson, 2005; Jones et al., 1988; Koenning et al., 2000) have been found. Currently, the control of reniform nematode in cotton has been done mainly though management options including the use of nematicides (Lawrence et al., 1990; Zimet et al., 1999; Kinloch and Rich, 2001; Overstreet and Erwin, 2003) and crop rotations (Davis et al., 2003; Gazaway et al., 1998, 2000), and by the use of moderately resistant and tolerant breeding lines (Jones et al., 1988; Koenning et al., 2000; Robinson et al., 2004). Due to increased awareness of real and perceived dangers from nematicides and legal restrictions on the nematicide use (Fassuliotis, 1979; Medina-Filho and Tanksley, 1983; Roberts et al., 1986; Cook and Evans, 1987; Cook et al., 1995; Wrather et al., 2002), low economic returns from the rotation crops as compared to cotton (Davis et al., 2003) and unreliability of the environment-dependent tolerant breeding lines, emphasis on the importance of genetic

resistance as a solution to nematode threats has greatly increased. Genetic resistance within Upland cottons might be derived by genetic engineering, inter-generic horizontal transfer of an intact system by biotechnological methods, inter-specific transfer by wide-cross introgression, induction and/or discovery intra-specifically. Naturally occurring sources of resistance would be particularly beneficial to producers as they would likely be freely deliverable to producers, without imposition of payments to commercial entities for expensive patent-protected constructs and technologies.

Though, no appreciable resistant sources have been identified in *G. hirsutum* germplasm, accessions from other *Gossypium* species, including *G. anomalum* Wawr. & Peyr., *G. arboreum* L., *G. barbadense* L., *G. herbaceum* L., *G. longicalyx* Hutch. & Lee, *G. raimondii* Ulbr., *G. somalense* (Gurke) Hutch., *G. stocksii* Mast. in Hook., and *G. thurberi* Tod. (Yik and Birchfield, 1984; Stewart and Robbins, 1995; Robinson et al., 2004) have been identified as potential resistance sources that could be used to introgress resistance into Upland cotton. Within these resistant sources, *G. longicalyx* provides the highest resistance and thus the best choice to introgress resistance into Upland cotton (Yik and Birchfield, 1984; Stewart and Robbins, 1995).

To identify resistance is itself, however, insufficient. Resistance to root-knot nematode (RKN-R) was identified in Upland cotton (Jones et al., 1988; Shepherd, 1988) but never incorporated into a highly successful cultivar. At that time, genome mapping was very primitive and markers extremely sparse, it was an immense challenge to develop a facile marker-based system for selection. However, without it, breeders were unable or unwilling to exploit RKN-R. In contrast, researchers in tomato were fortunate in detecting a close linkage between an easily scored isozyme and resistance to root-knot nematode, which enabled and led to its extensive usage in tomato cultivar-breeding programs (Rick and Forbes, 1974; Medina-Filho, 1980; Medina-Filho and Tanksley, 1983). Thus, to effectively deliver reniform resistance to the cotton producer, it is essential to provide both the genetic system in a breeder-friendly genetic background and a suitable marker-assisted breeding system that will facilitate use of the resistance gene in applied breeding programs. With a plethora of molecular marker types now

available (Reiter, 2001; Gupta and Rustgi, 2004), and a concerted effort to populate the cotton genome with portable markers (http://www.cottonssr.org), the development of a marker-assisted selection system is quite feasible.

Early, rapid degeneration of the syncytia (Rebois et al., 1970, 1975; Carter, 1981; Agudelo et al., 2005), formation of wall deposits (Rebois et al., 1970; Carter 1981) and lack of hypertrophy of pericycle cells (Carter, 1981; Agudelo et al., 2005) have been proposed as mechanisms for plant resistance to reniform nematodes in the plant species studied. To better understand the plant-nematode interaction, the reniform nematode resistance mechanisms and to more efficiently manipulate the resistance gene and other plant disease resistance genes, it is important to clone the reniform nematode resistance genes (R-genes). Such cloning may also eventually lead to transformation of crops for which a resistance source is not available. Map-based cloning has been used to clone the resistance genes in the past (Rommens et al., 1989; Arondel et al., 1992; Giraudat et al., 1992; Chang et al., 1993; Martin et al., 1993; Zhang et al., 1994), and usually entails (1) precise linkage mapping of the trait of interest, (2) establishing the relationship between genetic and physical distance, (3) "chromosome walking" and finally (4) gene identification. In pursuit of cloning the *G. longicalyx* R-gene, it will be important to determine the number of alien segments carrying the R-genes and to delimit their positions as accurately as possible. Several approaches are possible and may include linkage mapping and molecular cytogenetics. Large insert bacterial artificial chromosome (BAC) libraries in conjunction with fluorescent i*n situ* hybridization (FISH) has been used to physically map molecular markers to sorghum chromosomes (Islam-Faridi et al., 2002; Kim et al., 2002; Kim et al., 2005). Besides physically mapping molecular markers to pachytene chromosomes, BAC-FISH has been successfully used in physically localizing genes to chromosomes (Zwick et al., 1998). For example, in tomato, root-knot nematode resistance gene (*Mi-1*) has been mapped to the pericentromeric heterochromatin region of the short arm of chromosome 6 (Zhong et al., 1999). This technique commonly referred to as BAC-FISH could be used to physically map the R-gene(s) to chromosome, thus giving an estimate of the number and

the size of the alien segment(s) carrying the R-gene(s). Because genes located in euchromatic regions are typically subjected to high rates of recombination, they are much more amenable to map-based cloning than genes in heterochromatic and other regions with low recombination rates. Without high rates of recombination, it is typically much more difficult to attain fine delimitation of a target gene's location, which is essential for map-based cloning.

Introgression of valuable traits from diploid cottons into tetraploid cotton

Germplasm of diploid cotton species remains under-utilized mainly due to their poor agronomic performances, ploidy barriers in making hybrids, differences in meiotic affinities, lower rates of recombination between the genomes if a hybrid is created, undesirable linkages, fertility issues due to unbalanced gametes, and time constraints in developing a breeding line or germplasm (Fryxell, 1976; Stewart, 1995; Miller and Rawlings, 1967; Meredith and Bridge, 1971; Ndungo et al., 1988). Despite the above reasons that dissuade most breeders from utilizing the potential traits of the diploid species, a few workers across the world have been working with these diploids. Several potential traits including resistances to diseases, pest, and nematodes, as well as fiber quality and yield-related traits have been identified in the exotic diploid cotton species and a few of them have been introgressed into Upland cotton by the use of different breeding methods that involve chromosome doubling and the use of bridging species to overcome ploidy barriers (Fryxell, 1976; Stewart, 1995; Mergeai, 2004). While summarizing 40 years of work in the inter-specific hybridization in the genus *Gossypium* at the Gembloux Agricultural University in Belgium, Mergeai (2004) reported that hybridization barriers in cotton are less important than in other genera and that viable seeds can be obtained from almost all possible crosses by carrying out large numbers of pollinations and applying adequate hormone mixture at pollination, the only exceptions being *G gossypioides*, *G. davidsonii*, *G. klotzschianum*, and the "sanguineum" race of *G. arboreum* because of the presence of complementary lethal genes that condition embryo

or seedling death in hybrids with *G. hirsutum* (Grestel, 1954; Phillips, 1962; Phillips, 1963).

Some of the traits that have been introgressed from exotic, diploid species into tetraploid cotton include, bacterial blight resistance genes from *G. arboreum*, *G. herbaceum*, and *G. anomalum* (Endrizzi et al., 1985); cytoplasms and restorer factors of *G. harknesii* (Meyer, 1975) and *G. trilobum* (Stewart, 1992) conditioning cytoplasmic male sterility; resistance to jassid due to hairiness transferred from *G. raimondii* (Stewart, 1995); insect resistance attributed to D2 smoothness from *G. armourianum* (Meyer, 1957); a gene controlling terpenoid aldehyde methylation from *G. sturtianum* (Bell, 1984; Bell et al., 1987; Bell et al., 1994); higher fiber strength from *G. thurberi* (Miller and Rawlings, 1967; Meredith and Bridge, 1971; Harrell and Culp, 1979); fiber quality parameters (Ndungo et al., 1988).

Cotton breeders are fortunate enough to have diverse germplasm resources from which new genes can be introgressed into commercial cultivars (Stewart, 1995). Though these valuable resources are underutilized currently, they shall certainly be valuable in the future breeding efforts by creating genetic variation from which selections to develop improved cotton cultivars can be done efficiently. Studies of interspecific crosses have demonstrated that it is possible to identify and introgress novel alleles or genes from wild species that enhance quantitatively inherited traits even if the alien source is grossly inferior overall for that trait (Eshed and Zamir, 1994; Tanksley and Nelson, 1996).

Escalating pressure of reniform nematodes on US cotton growing regions and unavailability of the appreciable resistance in the *G. hirsutum* germplasm led to the initiation of the "reniform nematode-resistant introgression" project by the USDA scientists in College Station (Bell and Robinson, 2004; Robinson et al., 2007) that involved two wild diploids, *G. longicalyx* (reniform nematode-resistance source) and *G. armourianum* and Upland cotton, *G. hirsutum*. The project was significantly expanded at the early generations of various lineages (F1, BC1 and BC2), at which time the introgression efforts became a collaborative effort by the USDA-ARS, Texas Agricultural Experiment Station and Cotton Incorporated. The plant material used in

this study was derived from this reniform resistance-introgression project. The main objectives of my work have been [1] to cytogenetically evaluate and help select superior types at each introgressed generation; [2] to identify molecular markers tightly linked to the reniform nematode-resistance gene and to map the resistance loci; [3] (A) to introgress germplasm on a genome-wide basis, (B) to evaluate introgressed germplasm for traits of economic importance other than reniform nematode resistance, and (C) to evaluate breeding methodologies in terms of this specialized breeding material.

CHAPTER II

CYTOGENETICS OF THE SYNTHETIC TRI-SPECIES TETRAPLOID, [(*Gossypium hirsutum* x *G. longicalyx*)^2 x *G. armourianum*] (HLA) AND ITS BACKCROSSED DERIVATIVES

Introduction

The cotton genus, *Gossypium*, consists of 50 species, including 45 diploids (2n = 2x = 26), and 5 tetraploids (2n = 4x = 52) (Fryxell, 1992). The diploid species have been classified into eight cytologically based genome groups as summarized by Endrizzi et al. (1985) and Stewart (1995) and are found to exist in three primary centers of diversity including Afro-Asian species (A-, B-, F-, and E-genomes), the Australian species (C-, G-, and K-genomes), and the new world species (D-genome) (Fryxell, 1979; Small and Wendell, 2000). Two diploid (*G. herbaceum* and *G. arboreum*) and two tetraploid species (*G. hirsutum* and *G. barbadense*) have been domesticated and are being cultivated in different parts of the world (Fryxell, 1976; Fryxell, 1992), leaving the remaining species as potential sources of novel genetic traits for cultivar development (Stewart, 1995). Limited genetic diversity (Wendel et al., 1992; Pillay and Myers, 1999; Khan et al., 2000; Abdalla et al., 2001; Iqbal et al., 2001; Rungis et al. 2005) and predominant use of relatively narrow genetic pool for cultivar development (van Esbroeck et al., 1998; Iqbal et al., 2001) has raised concerns about genetic vulnerability of cotton to biotic and abiotic stresses (Anonymous, 1972; Bowman et al., 1996). Thus the need to increase the genetic base of cotton by utilization of wild, unused germplasm from both diploid and tetraploid cotton species has been recently been emphasized (Stewart, 1985).

Stewart (1995), while describing the potential for crop improvement using exotic germplasm points out that the successful utilization of the potential genetic resources in commercial breeding programs depends on two events. Firstly, a fertile hybrid between the diploid donor and the tetraploid recipient genotypes must be created and secondly,

genetic recombination between the donor and the recipient chromosomes in a hybrid must occur. Stewart (1995) also pointed out that the extent to which these two events occur depend on the genomic "relatedness" between the donor and recipient genotypes and further delineated cotton germplasm into primary, secondary and tertiary germplasm pools, based on the ease with which the genes could be transferred from the donor source to the crop species (cultivated tetraploid cottons), a concept first described by Harlan and deWet (1971). The primary germplasm pool contains genetic resources that can be easily crossed with the breeding lines and will result in direct genetic recombination between the parental genomes in a hybrid. All natural allotetraploids, including breeding stocks, obsolete cultivars, landraces, and the wild tetraploid species fall into this pool (Stewart, 1995). The secondary pool consists of the A, D, E, and F genomes, in which A and D are the progenitor sub-genomes of the tetraploid cotton, while B and F genomes have chromosomes that are structurally similar to those of the A-genome and, thus have close homeology to the A-genome (Phillips, 1966; Phillips and Strickland, 1966; Stewart, 1995). While ploidy barriers prevent direct hybridization with the breeding lines (cultivated tetraploid cotton), these barriers can be overcome with techniques like chromosome doubling and use of bridge species (Stewart, 1995). Once a hybrid is created, the frequency of genetic recombination is high (Stewart, 1995; Brubaker et al., 1999). The tertiary germplasm pool, which consists of Australian genomes (C, G, and K) and E-genome from Africa, is the most difficult to create hybrids with the cultivated tetraploid cottons. Moreover, it has a very low rate of homeologous recombination, thus making it the most difficult group from which genes can be introgressed into cultivated cottons (Stewart, 1995).

Several potentially useful traits possessed by these underutilized, primary, secondary, and tertiary germplasm pools including resistance to diseases and pests, have been identified and a few of them have been reported to be introgressed into *G. hirsutum* breeding lines (Fryxell, 1976; Stewart, 1995). In recent years, reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) has been causing significant economic damage to the cotton industry with losses exceeding $100M annually (Blasingame,

2006), which accounts for 45% of the cotton crop lost to all nematodes (Robinson, 1999). The growing problem of the reniform nematode in cotton production has led to the desire to incorporate genetic resistance to the reniform nematode in Upland cotton genotypes. Two major extensive screenings of the *G. hirsutum* germplasm confirmed that none of the Upland cotton genotypes has high resistance to reniform nematodes (Yik and Birchfield, 1984; Robinson et al., 2004). However, one or more accessions of *G. longicalyx* Hutch. & Lee, *G. somalense* Hutch., and *G. stocksii* Mast., *G. arboreum* L., and *G. barbadense* L. have been identified to be resistant to reniform nematodes with *G. longicalyx* showing the highest resistance among all *Gossypium* germplasm (Yik and Birchfield, 1984; Stewart and Robbins, 1996).

Gossypium longicalyx, which was first described as a new species of cotton by Hutchinson and Lee in 1958 was first found in the central Tanganyika (United Republic of Tanzania). It was later classified into the F-genome of cotton by Phillips and Strickland (1966) based on meiotic metaphase I chromosome pairing. A long calyx, scandent growth habit, and lack of petal spot are some of the distinctive morphological features of *Gossypium longicalyx*, which is the only diploid cotton species that occurs in mesophytic areas (Hutchinson, 1959; Fryxell, 1984, p. 189-197). While the extreme resistance of *G. longicalyx* to reniform nematodes makes it the most attractive genetic source of resistance, barriers to introgress into *G. hirsutum* include ploidy differences, reduced chromosome homology and thus meiotic recombination, and the possible influence of structural differences, e.g., inversions. Use of a pseudophyletic introgression method (Mergeai, 2004) that involves crossing of *G. hirsutum* with one of the diploids, chromosome doubling of the triploid and then crossing of the obtained hexaploid to another diploid to create a tetraploid was used. This led to the creation of two tri-species hybrids, designated as HLA (|*G. hirsutum*|*G. longicalyx*||*G. armourianum*|) and HHL (|*G. hirsutum*|*G. herbaceum*||*G. longicalyx*|), where *G. armourianum* and *G. herbaceum* were used as bridge species in the respective hybrids (Bell and Robinson, 2004). Introgression of reniform nematode resistance from *G. longicalyx* into Upland cotton was accomplished by recurrent backcrossing of the HLA

and HHL tri-species hybrids to *G. hirsutum* (Bell et al., 2007; Robinson et al., 2007). To facilitate transfer of this reniform nematode-resistant germplasm to different *G. hirsutum* cultivars and breeding lines without having to screen for reniform nematodes, tightly-linked markers flanking the resistant locus have been identified (Chapter III). This chapter provides cytogenetic evaluation of the tri-species hybrid, HLA and its successive generations created by backcrossing reniform nematode-resistant plants to *G. hirsutum*.

Materials and Methods

Reniform Nematode Screening

Nematode resistance was defined as a low rate of nematode reproduction on healthy roots. Reproduction was evaluated directly by counting mature females and eggs on roots, or indirectly by counting vermiform nematodes within soil (Robinson et al., 2006). In the direct assay, nematode-free plants were grown 8 to 12 weeks until pot-bound within 500-ml cups. Then the root "ball" of each cup was removed, slipped into a close-fitting cup-shaped sleeve fashioned from fiberglass window screen fabric, and transplanted into a 3-liter pot containing soil infested with *R. reniformis*. The pot was then placed in a controlled environment chamber for 3 weeks, when the root balls were gently lifted from pots. New roots that had grown out through the screen from the root ball into the infested soil were cut off with scissors, collected, placed in fixative and examined microscopically to evaluate female nematode development and presence or absence of associated eggs. In the indirect and most frequently used assay, seeds were scarified by nicking the seed coat, germinated in moistened, rolled blotter paper, transplanted individually into 500-ml cups, which were held in a greenhouse for 10 to 14 days, and then inoculated with 4,000 nematodes per plant. After inoculation, plants were held in a controlled environment chamber for 7 weeks, at which time three soil cores weighing 40 g total were removed from each cup, and analyzed to measure the concentration of active, vermiform *R. reniformis* in the soil. In both methods, selected plants were retained for breeding and/or leaf tissue generation for DNA studies by transplanting to larger pots containing nematode-free potting medium. The results were

compared with *G. hirsutum* cv. Deltapine 16 as a susceptible control and *G. barbadense* GB-713 as a resistant control.

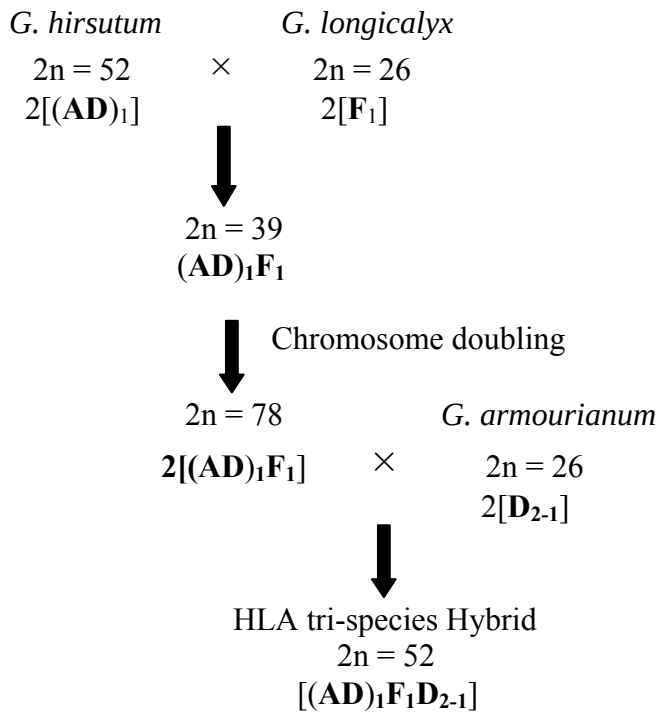

Figure 1. Inter-specific breeding scheme used to create HLA tri-species hybrid.

Plant Material

The breeding scheme used in developing the HLA tri-species hybrid is explained in Fig. 1. *G. hirsutum* inbred 'TM-1' was used as the female parent in crosses with *G. longicalyx*. The product was a sterile triploid plant, which upon chromosome-doubling by colchicine treatment yielded a fertile hexaploid (6X) of the genomic composition $2[(AD)_1F_1]$. The hexaploid was crossed with *G. armourianum* (D_{2-1}) to obtain a tetraploid (4X) of the genomic composition $[(AD)_1F_1D_{2-1}]$. The balance between genomic groups in this hybrid, specifically AFDD, was expected to allow for high meiotic homology (A-F and D-D), recombination and fertility.

Tri-species hybrid, HLA was pollinated with various *G. hirsutum* genotypes to create the BC1F1 generation. The HLA-derived BC1F1 plants were screened for reniform nematodes to identify resistant plants, which were repeatedly backcrossed, screened and selected for reniform nematode resistance at each generation to create BC2F1, BC3F1, BC4F1, and BC5F1 generations and then self pollinated to create BC1S1 generation (Robinson et al., 2007). Approximately, 500 HLA-derived BC1F1 plants that were identified to be self sterile in greenhouse conditions and not screened for reniform nematode resistance were hand-transplanted to the F&B field in College Station in June of 2003. Twenty of these self-sterile BC1F1 plants were also included in the cytological analysis (A-1). A total of 200 plants, including the HLA tri-species hybrid, 45 BC1F1, 26 BC1S1, 89 BC2F1, 22 BC3F1, 15 BC4F1, and 2 BC5F1 were analyzed cytologically for chromosome number and configurations (Table 1). Generations, BC1F1, BC2F1, BC4F1, and BC5F1 were created by using the resistant plants from the preceding generation as female parents and *G. hirsutum as* male parents, while BC3F1 was created by pollinating *G. hirsutum* cultivars with pollen from the resistant plants of the preceding generation. Tri-species hybrid, HLA and all backcrossed plants, with the exception of the 20 self sterile BC1F1 plants were maintained in the greenhouse during the time of bud collection for cytological analysis.

Meiotic Chromosome Preparation

Floral buds were collected between 9 am and 12 pm, slit open using a razor blade and fixed in Carnoy's fluid-I fixative (3 parts of 95% ethanol and 1 part of glacial acetic acid). The fixative was changed after 3 days. For slide preparations, the fixed buds were rinsed well with water, soaked in running water for 1 hour, and then several anthers were dissected from a given size-selected bud and transferred onto a glass slide. After applying a drop of 45% acetocarmine solution, the anthers were gently "popped", and preliminarily screened under the dissecting and/or compound microscope to check the developmental stage. If at metaphase I, the somatic anther tissue was removed and a clean cover slip was applied and heated mildly either on a hot plate or on a flame to

differentiate chromosome stain and free the protoplasts. Once free of walls and stain-differentiated, the protoplasts were squashed and then screened for chromosome number and configuration, usually with just brightfield illumination. On an average, eight microsporocytes (PMCs) were analyzed per plant, but ranging from 2-37 in individual plants (A-1). PMCs with chromosome numbers different from the modal types for individual plants were considered as observation errors and were excluded from the analysis.

Table 1. Chromosomal counts and percentages of successive generations produced by repeated backcrossing of reniform nematode-resistant plants with susceptible agronomic *Gossypium hirsutum.*

Generation	Reniform Resistance status	Number of chromosomes						
		≤ 51		52		≥ 53		Total
BC1F1	Res & Sus[†]	1	2%	33	73%	11	25%	45
BC1S1	Res & Sus	0	0%	18	69%	8	31%	26
BC2F1	Res & Sus	17	19%	62	70%	10	11%	89
BC3F1	Res	0	0%	22	100%	0	0%	22
BC4F1	Res	0	0%	15	100%	0	0%	15
BC5F1	Res	0	0%	2	100%	0	0%	2
Total		18		152		29		199

[†]Res indicate resistant plants; Sus indicate susceptible plants.

Statistical Analysis

Generation least square means (LSMEANS) were calculated for each chromosomal configuration using PROC GLM of SAS v9.1.3 (SAS Institute, Cary, NC). The multiple comparisons were tested for significance at $P \leq 0.05$ using Tukey-Kramer adjusted least significant difference (LSD) and the output was condensed into letter grouping using SAS macro, PDGLM800 (Saxton, 1998).

Table 2. Meiotic metaphase I chromosomal configurations in microsporocytes of the HLA tri-species hybrid.

Cell No.	I[†]	II	III	IV	V	VI	Chrom.[‡]
1	26	11	0	1	0	0	52
2	12	20	0	0	0	0	52
3	4	22	0	1	0	0	52
4	16	16	0	1	0	0	52
5	16	16	0	1	0	0	52
6	11	14	3	1	0	0	52
7	9	10	2	3	1	0	52
8	8	20	0	1	0	0	52
9	7	21	1	0	0	0	52
10	9	17	3	0	0	0	52
11	6	15	4	1	0	0	52
12	7	21	1	0	0	0	52
13	6	17	2	0	0	1	52
14	7	21	1	0	0	0	52
15	5	20	1	1	0	0	52
16	11	17	1	1	0	0	52
17	8	20	0	1	0	0	52
18	14	16	2	0	0	0	52
19	5	18	1	2	0	0	52
20	10	18	2	0	0	0	52
21	7	21	1	0	0	0	52
22	5	22	1	0	0	0	52
23	11	16	0	1	1	0	52
24	8	22	0	0	0	0	52
25	3	18	3	1	0	0	52
26	4	21	2	0	0	0	52
27	6	21	0	1	0	0	52
28	12	18	0	1	0	0	52
29	14	16	2	0	0	0	52
30	3	21	1	1	0	0	52
31	7	21	1	0	0	0	52
32	6	18	2	1	0	0	52
33	10	21	0	0	0	0	52
34	5	22	1	0	0	0	52
35	8	19	2	0	0	0	52
36	6	15	0	4	0	0	52
37	4	24	0	0	0	0	52
38	8	20	0	1	0	0	52
39	6	21	0	1	0	0	52
Mean	8.46	18.64	1.03	0.69	0.05	0.03	52
Min.-Max.	3-26	10-24	0-4	0-4	0-1	0-1	

Average number of chromosomes paired: 43.56

[†]I, II, III, IV, V, VI represents univalent, bivalent, trivalent, quadrivalent, pentavalent, and hexavalent chromosomal configurations respectively.

[‡]Chromosome number.

Results

Analysis of 39 PMCs of the HLA tri-species hybrid indicated that it had 52 chromosomes with mean metaphase I chromosomal configurations of 8.46I + 18.64II + 1.03III + 0.69IV + 0.05V + 0.03VI, where I, II, III, IV, V, and VI denote univalent, bivalent, trivalent, quadrivalent, pentavalent, and hexavalent configurations, respectively (Table 2). In the tri-species hybrid, an average of 43.56 chromosomes paired at metaphase I, but the chromosomal configurations ranged from 3-26 univalents, 10-24 bivalents, 0-4 trivalents, 0-4 quadrivalents, 0-1 pentavalents, and 0-1 hexavalents (Fig. 2; Table 2).

In generations BC1F1 and BC1S1, 73% and 69% of the plants had 52-chromosome complements, and the remaining 27% and 31% plants were aneuploids, respectively, the majority of the generations being hyper-aneuploids (Table 1). Similar results were observed in the BC2F1 generation, in which 70% of the plants had 52 chromosomes and 30% were aneuploids. The only differences between these generations were in the aneuploid distribution, where BC1F1, and BC1S1 generations had higher percentages of hyper-aneuploids than hypo-aneuploids, while BC2F1 had higher percentages of hypo-aneuploids than hyper-aneuploids (Table 1). All the plants in BC3F1, BC4F1, and BC5F1 generations had the 52-chromosome complement (Table 1).

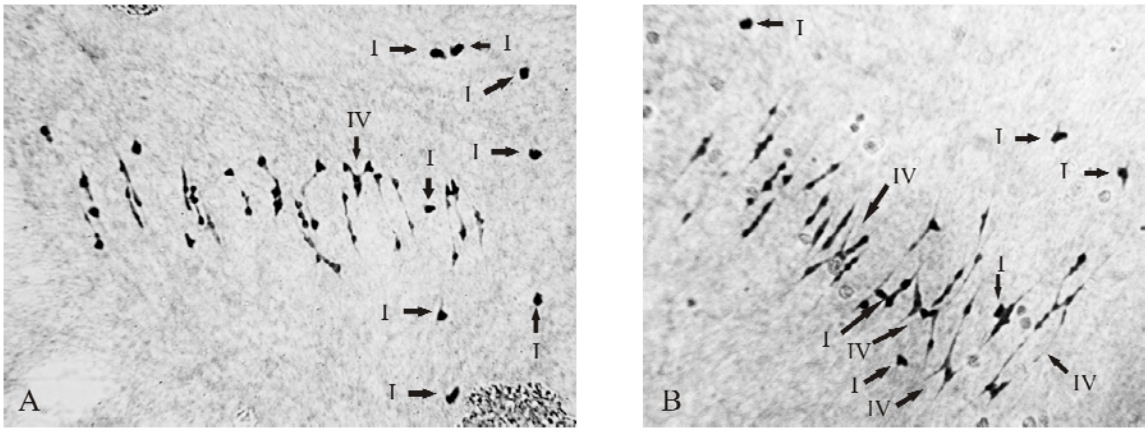

Figure 2. Meiotic metaphase I cells of the HLA tri-species hybrid (A) showing 8I + 20II + IV chromosomal configuration and (B) showing 6I + 15II + 4IV chromosomal configuration.

Table 3. Least square means for the meiotic chromosomal configurations in the HLA and its successive backcrossed generations using cells from all the plants in the respective generation.

Gen.	I[†]		II		III		IV		V		VI		Chrom.[‡]		Cells	Plants
HLA	8.46	A[§]	18.64	E	1.03	A	0.69	A	0.05	A	0.03	A	52.00	C	39	1
BC1F1	4.49	B	23.57	D	0.06	B	0.14	B	0.00	B	0.00	B	52.35	A	301	45
BC1S1	1.66	C	25.16	B	0.03	B	0.06	BC	0.00	B	0.00	B	52.28	AB	229	26
BC2F1	1.91	C	24.80	C	0.04	B	0.07	C	0.00	B	0.00	B	51.91	C	773	89
BC3F1	0.30	D	25.65	A	0.00	B	0.10	BC	0.00	B	0.00	B	52.00	C	119	22
BC4F1	0.57	D	25.55	AB	0.00	B	0.08	BC	0.00	B	0.00	B	52.00	C	87	15
BC5F1	0.00	D	25.70	AB	0.00	B	0.15	BC	0.00	B	0.00	AB	52.00	BC	20	2

[†]I, II, III, IV, V, VI represents univalent, bivalent trivalent, quadrivalent, pentavalent, and hexavalent chromosomal configurations, respectively.

[‡]Chromosome number.

Significant differences between the least square means tested with Tukey-Kramer adjusted LSD.

[§]Means within columns followed by the same letter are not different at $p \leq 0.05$

Statistical comparisons of chromosomal configuration frequencies revealed that all backcrossed generations (BC1F1, BC1S1, BC2F1, BC3F1, BC4F1, and BC5F1) had lower univalents, and higher bivalents, trivalents, quadrivalents, pentavalents, and hexavalents than HLA, with an exception of hexavalent frequencies in HLA and BC5F1, which were not different ($P \leq 0.05$; Table 3). Frequencies of univalents decreased and bivalents increased each generation until the BC3F1 generation was reached. No differences in the univalent and bivalent frequencies were observed among BC3F1, BC4F1, and BC5F1 generations. There were no differences observed between the HLA-derived backcross generations for the mean trivalent, quadrivalent, pentavalent, and hexavalent configurations, with one exception, where higher quadrivalents were observed in BC1F1 than BC2F1.

The modal types observed in the backcrossed generations are described in Table 4. The two modal types observed in BC1F1 were 25II + 2I (52 chromosomes) and 24II + 4I (52 chromosomes), each type having 9 plants out of total 45 analyzed, which is 20% each or a total of 40% of the plants analyzed, while in the BC1S1, the modal type was 25II + 2I (46%). The modal type observed in BC2F1, BC3F1, BC4F1, and BC5F1 generations was 26II, where 28%, 68%, 73%, 100% of the plants had the modal configuration, respectively. Though the modal type in BC2F1 generation was 26II, the percentage was comparatively low, i.e., 28%. The second most common chromosomal type observed in BC2F1 was 25II + 2I, which was observed in 16% of the plants. In addition, there were 6% of the plants that had 26II and 25II + 2I as their modal types. Thus, in all, 50% of the BC2F1 plants had 26II or 25II + 2I or both as their modal chromosomal configurations. The percentage of plants that had a modal configuration of 26II increased each backcross generation.

Table 4. Modal meiotic chromosomal configurations in the successive generations produced by repeated backcrossing of the reniform nematode-resistant plants to *G. hirsutum*.

Gen	I[†]	II	III	IV	V	VI	Chrom.[‡]	Mode[§]	Cells	% Cells[¶]	Plants	% Plants[††]
BC1F1	2	25	0	0	0	0	52	36	78	46	9	20
	4	24	0	0	0	0	52	33	67	49	9	20
	6	23	0	0	0	0	52	14	23	61	5	11
	4	25	0	0	0	0	54	16	28	57	5	11
	8	22	0	0	0	0	52	6	10	60	3	7
	10	21	0	0	0	0	52	12	20	60	3	7
	3	24	0	0	0	0	51	8	8	100	1	2
	3	23	1	0	0	0	52	2	3	67	1	2
	14	19	0	0	0	0	52	2	5	40	1	2
	2/8/10	25/22/21	0/0/0	0/0/0	0/0/0	0/0/0	52	3	3	100	1	2
	6/8/12/14	23/22/20/19	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	52	4	4	100	1	2
	3	23	0	1	0	0	53	2	3	67	1	2
	3	25	0	0	0	0	53	10	20	50	1	2
	7	23	0	0	0	0	53	5	11	45	1	2
	1/1	24/26	0/0	1/0	0/0	0/0	53	8	10	80	1	2
	1/5[‡‡]	26/24	0/0	0/0	0/0	0/0	53	2	2	100	1	2
	2	26	0	0	0	0	54	3	6	50	1	2
Total								166	301	55	45	100
BC1S1	2	25	0	0	0	0	52	84	114	74	12	46
	1	26	0	0	0	0	53	28	34	82	5	19
	0	26	0	0	0	0	52	22	30	73	3	12
	1	24	1	0	0	0	52	3	8	38	1	4
	0/2	24/23	0/0	1/1	0/0	0/0	52	2	2	100	1	4
	0/2	26/25	0/0	0/0	0/0	0/0	52	17	18	94	1	4
	3	25	0	0	0	0	53	2	4	50	1	4
	1/1	24/26	0/0	1/0	0/0	0/0	53	9	11	82	1	4
	2	26	0	0	0	0	54	5	8	63	1	4
Total								172	229	75	26	100
BC2F1	0	26	0	0	0	0	52	161	226	71	25	28
	2	25	0	0	0	0	52	94	145	65	14	16
	3	24	0	0	0	0	51	46	65	71	8	9
	4	24	0	0	0	0	52	37	77	48	7	8
	1	25	0	0	0	0	51	45	52	87	6	7
	0/2	26/25	0/0	0/0	0/0	0/0	52	54	56	96	5	6
	1	26	0	0	0	0	53	16	19	84	3	3
	1/3	25/24	0/0	0/0	0/0	0/0	51	8	10	80	2	2
	6	23	0	0	0	0	52	11	23	48	2	2

Table 4. Continued.

Gen	I[†]	II	III	IV	V	VI	Chrom.[‡]	Mode[§]	Cells	% Cells[¶]	Plants	% Plants[††]
	0/4	26/24	0/0	0/0	0/0	0/0	52	4	4	100	2	2
	3	25	0	0	0	0	53	11	18	61	2	2
	1/3	24/25	0/0	1/0	0/0	0/0	53	8	11	73	2	2
	0	25	0	0	0	0	50	2	2	100	1	1
	0	24	0	1	0	0	52	9	13	69	1	1
	8	22	0	0	0	0	52	3	5	60	1	1
	2/4	25/24	0/0	0/0	0/0	0/0	52	12	13	92	1	1
	2/6	25/23	0/0	0/0	0/0	0/0	52	6	8	75	1	1
	2/8	25/22	0/0	0/0	0/0	0/0	52	2	2	100	1	1
	8/10	22/21	0/0	0/0	0/0	0/0	52	2	2	100	1	1
	0/6	24/23	0/1	1/0	0/0	0/0	52	4	6	67	1	1
	5	24	0	0	0	0	53	5	10	50	1	1
	3/4	25/23	0/1	0/0	0/0	0/0	53	4	4	100	1	1
	7/9	23/22	0/0	0/0	0/0	0/0	53	2	2	100	1	1
Total								546	773	71	89	100
BC3F1	0	26	0	0	0	0	52	85	93	91	15	68
	2	25	0	0	0	0	52	10	10	100	3	14
	0	24	0	1	0	0	52	7	8	88	2	9
	2/0	25/26	0/0	0/0	0/0	0/0	52	2	2	100	1	5
	0/0	24/26	0/0	1/0	0/0	0/0	52	6	6	100	1	5
Total								110	119	92	22	100
BC4F1	0	26	0	0	0	0	52	53	63	84	11	73
	2	25	0	0	0	0	52	13	15	87	3	20
	0	24	0	1	0	0	52	4	9	44	1	7
Total								70	87	80	15	100
BC5F1	0	26	0	0	0	0	52	17	20	85	2	100
Total								17	20	85	2	100

[†]I, II, III, IV, V, VI represents univalent, bivalent trivalent, quadrivalent, pentavalent, and hexavalent chromosomal configurations respectively.

[‡]Chromosome number.

[§]Modal value for the respective chromosomal configuration.

[¶]% Cells with modal chromosomal configuration.

[††]Percentage plants with modal chromosomal configuration.

[‡‡]Represents two or more types of modal chromosomal configurations. For example, 1/5I, 26/24II, 0/0III, 0/0IV, 0/0V, and 0/0VI represent 1I + 26II + 0III + 0IV + 0V + 0VI and 5I + 24II + 0III + 0IV + 0V + 0VI as the modal types.

Although BC1F1, BC1S1, and BC2F1 generations had about the same percentage of plants with 52 chromosomes (Table 1), they differed in their modal configurations (Table 4), where the most advanced backcross generation had more plants with normal 26II pairing than the previous generation. Examination of individual plant data revealed that there was not a single plant of the BC1F1 generation exhibiting normal 26II pairing, while BC1S1 and BC2F1 had 12% and 28% of the plants with 26II pairing, respectively. The configuration data also distinguished among the BC3F1, BC4F1, and BC5F1 generations, in which all of the plants had 52 chromosomes. Nevertheless, they differed markedly in chromosome pairing, where 68%, 73%, and 100% of the plants had a modal 26II chromosomal configuration, respectively (Tables 2, 4).

Higher univalent and lower bivalent frequencies were observed in the self-sterile BC1F1 population than the self-fertile BC1F1 population, but no differences were observed in the multivalent frequencies ($P \leq 0.05$; Table 5). Presence of comparatively higher univalents in the sterile BC1F1 plants than the fertile plants could lead to higher number of unbalanced gametes in the sterile plants, thus explaining part of the sterility in some of these plants. Observations at anaphase I in the PMCs of the tri-species hybrid have shown that these lagging univalents tend to form micronuclei during the tetrad stage, leading to unbalanced gametes, but no data on the occurrence of micronuclei in the introgressed-backcross generations were collected.

Table 5. Least square means for the meiotic chromosomal configurations in the self-sterile and self-fertile plants of the BC1F1 population.

Gen.	I[†]		II		III		IV		V		VI		Chrom[‡]		Cells	Plants
Sterile BC1F1	5.08	A	23.21	A	0.04	A	0.12	A	0.00	A	0.00	A	52.08	A	121	20
Fertile BC1F1	4.09	B	23.81	B	0.07	A	0.15	A	0.00	A	0.00	A	52.53	B	180	25

[†]I, II, III, IV, V, VI represents univalent, bivalent trivalent, quadrivalent, pentavalent, and hexavalent chromosomal configurations respectively.

[‡]Chromosome number.

Significant differences between the least square means tested with Tukey-Kramer adjusted LSD.

[§]Means within columns followed by the same letter are not different at $p \leq 0.05$

Table 6. Average and modal meiotic chromosomal configurations in the resistant BC2F1 plants with resistance scores ≤ 5% of the susceptible control, DP-16.

Family	Plant ID	%DP-16[††]	Cells	AVERAGE							MODE								
				I[†]	II	III	IV	V	VI	Chrom.[‡]	I	II	III	IV	V	VI	Chrom.	Mode[§]	% Cells[¶]
HLA-A85	70	0	6	3.17	23.50	0.17	0.33	0.00	0.00	52	0/6[‡‡]	24/23	0/0	1/0	0/0	0/0	52	4	67
HLA-A85	129	0	37	1.84	24.89	0.05	0.05	0.00	0.00	52	2	25	0	0	0	0	52	25	68
HLA-A122	22	1	4	3.50	24.00	0.50	0.00	0.00	0.00	53	3/4	25/23	0/1	0/0	0/0	0/0	53	4	100
HLA-A85	101	1	3	3.00	24.00	0.00	0.00	0.00	0.00	51	3	24	0	0	0	0	51	3	100
HLA-A85	176	1	21	0.57	25.05	0.19	0.19	0.00	0.00	52	0	26	0	0	0	0	52	9	43
HLA-A122	43	2	2	8.00	22.50	0.00	0.00	0.00	0.00	53	7/9	23/22	0/0	0/0	0/0	0/0	53	2	100
HLA-A99	140	2	15	4.00	23.33	0.13	0.13	0.00	0.07	52	4	24	0	0	0	0	52	6	40
HLA-A123	146	2	8	1.50	24.75	0.00	0.00	0.00	0.00	51	1	25	0	0	0	0	51	6	75
HLA-A132	111	3	10	5.40	23.60	0.00	0.10	0.00	0.00	53	5	24	0	0	0	0	53	5	50
HLA-A132	178	3	3	0.00	26.00	0.00	0.00	0.00	0.00	52	0	26	0	0	0	0	52	3	100
HLA-A103	38	4	14	1.43	25.79	0.00	0.00	0.00	0.00	53	1	26	0	0	0	0	53	11	79
HLA-A122	18	5	13	4.31	23.85	0.00	0.00	0.00	0.00	52	6	23	0	0	0	0	52	6	46
HLA-A103	49	5	13	0.31	24.31	0.00	0.77	0.00	0.00	52	0	24	0	1	0	0	52	9	69
HLA-A77	112	5	13	1.92	24.92	0.08	0.00	0.00	0.00	52	2	25	0	0	0	0	52	8	62
HLA-A4	139	5	8	4.00	24.00	0.00	0.00	0.00	0.00	52	2/6	25/23	0/0	0/0	0/0	0/0	52	6	75
HLA-A77	167	5	10	0.00	26.00	0.00	0.00	0.00	0.00	52	0	26	0	0	0	0	52	10	100
HLA-A85	181	5	6	2.00	25.00	0.00	0.00	0.00	0.00	52	2	25	0	0	0	0	52	6	100

[†]I, II, III, IV, V, VI represents univalent, bivalent trivalent, quadrivalent, pentavalent, and hexavalent chromosomal configurations respectively.

[††] %Deltapine-16.

[‡]Chromosome number.

[§]Modal value for the respective chromosomal configuration.

[¶]% Cells with modal chromosomal configuration.

[‡‡]Represents two or more types of modal chromosomal configurations. For example, 0/6I, 24/23II, 0/0III, 0/0IV, 0/0V, and 0/0VI represent 0I + 24II + 0III + 0IV + 0V + 0VI and 6I + 23II + 0III + 0IV + 0V + 0VI as the modal types.

Since the cytological data on the BC2F1 generations were collected on a large number of plants that were also screened for reniform nematode-resistance, they provided an opportunity to estimate crude rate of recombination between the *G. longicalyx* and *G. hirsutum* chromosomes in the resistant plants, based on bivalent and multivalent formations in metaphase I. Evaluation of the reniform nematode-resistant plants (reniform nematodes less than 5% of the susceptible check, DP-16) showed that two plants out of 17 had 26II chromosomal configuration in 100% of their PMCs, thus indicating a proximal recombination event in *ca*. 12% of megasporocytes of BC1F1 resistant plants (Table 6). Cytological data were collected from only the reniform nematode-resistant plants in the advanced generations, BC3F1, BC4F1, and BC5F1 to help select resistant plants with normal pairing for creating further backcrosses in an effort to introgress reniform nematode-resistance into *G. hirsutum*. Fifteen out of 22 (68%) plants in BC3F1, 11 out of 15 (73%) plants in BC4F1 and 2 out of 2 (100%) plants in BC5F1 had a modal chromosomal configuration of 26II, which further indicated the feasibility of recovering 52-chromosome reniform nematode-resistant plants with normal pairing (Table 4).

Chromosome numbers and modal configurations of some of the reniform nematode-resistant parents and their resistant backcross progeny for some of the HLA-derived BC1 families are described in Table 7. It was observed that for most of the lineages, there was a tendency towards an increased chromosome pairing in the reniform-resistant backcross progeny as compared to their resistant parents, either in the form of bivalents or multivalents. Images of Fig. 3 demonstrate recombination between chromosomes in the reniform nematode-resistant plants. Chromosomal configurations in the PMCs of one of the highly resistant BC2F1 plants (Plant ID-176) were 26II (43%), 24II + IV (19%), 23II + III + I (19%), and 25II + 2I (19%). Thus, no recombination occurred between two chromosomes in 19% of the cells, yet recombination was manifested either in the form of multivalent formation or complete 26II pairing in the remaining 81% of cells (Fig. 3A-D). Another Fig. 3 example that demonstrates the evidence of recombination between chromosomes is BC1S1-84-1-11, which had 46% of

the cells with normal 26II and an additional unpaired univalent (26II + I), while that univalent had undergone a recombination event with a bivalent to form a trivalent, thus leading to a 25II + III chromosomal configuration in 36% of the cells (Fig. 3E-F). In BC2-2-3, 46% of the PMCs had two univalents that failed to pair (25II + 2I), while those two univalents paired through recombination to form a bivalent in 50% of the PMCs leading to normal 26II pairing (Fig. 3G-H).

Discussion

In developing the HLA-based approach to introgression of *G. longicalyx*-based reniform nematode resistance, the D-genome species, *G. armourianum* ($D_{2\text{-}1}$) served as a "bridge" that made possible the synthesis of an FADD hybrid (HLA) with workable levels of female fertility. In HLA, meiotic pairing occurs between the D-subgenome of *G. hirsutum* (D_h) and the $D_{2\text{-}1}$ genome (*G. armourianum*), and between the A-subgenome of *G. hirsutum* (A_h) and F_1 genome (*G. longicalyx*). This interpretation of meiotic observations reported here is based on observed similarities of bivalent components and previously established homeology between the D-D (Brown and Menzel, 1952; Endrizzi, 1957; Phillips and Strickland, 1966) and A-F genomes (Phillips, 1966; Phillips and Strickland, 1966; Stewart, 1995). On an average, 8.46I, 18.64II, 1.03III, 0.69IV, 0.05V, and 0.03VI were observed per cell in the tri-species hybrid, HLA. While use of genomic *in situ* hybridization (GISH) would expectedly improve precision of the data by revealing the genomic affiliation of the univalents, it is possible to differentiate D-genome chromosomes from A- and F-genome chromosomes at the metaphase I stage due to the noticeable size differences between them where the D-genome chromosomes are relatively smaller than the A- and F-genome chromosomes (Kimber, 1961; Phillips and Strickland, 1966). Univalents at metaphase-I were relatively large and indicated that they belong to A and F genomes (Fig. 2).

Table 7. Pedigree sheet describing chromosome number and modal configuration of the reniform-resistant parents and their resistant progeny.

BC1F1				BC1S1/BC2F1[†]				BC3F1				BC4F1			
Plant ID	Conf[§]	Chr[‡]	% Cells	Plant ID	Conf	Chr	% Cells	Plant ID	Conf	Chr	% Cells	Plant ID	Conf	Chr	% Cells
HLA-A84				84-3-4 (03)	26II	52	35	3-25 (03)	24II + IV	52	67	81-4 (04)	26II	52	67
												81-6 (04)	26II	52	70
								3-27 (03)	26II	52	100				
								3-5 (03)	26II	52	86				
								3-22 (03)	26II	52	100				
HLA-A83	24II + 3I	51	100	7-4 (03)	24/25II + 1/0IV + 1/3I	53	57								
				5-4 (04)	24II + 3I	51	56								
HLA-A85				BC1S1-30 (03)	24II + III + I	52	38	14-4 (04)	24II + IV	52	100				
				70 (03)	24/23II + 0/1III + 1/0IV + 0/6I	52	67	13-9 (04)	26II	52	80				
				129 (03)	25II + 2I	52	68	11-10 (04)	26II	52	100				
				101 (03)	24II + 3I	51	100	10-9 (04)	26/25II + 0/2I	52	100				
				176 (03)	26II	52	43	12-5 (04)	26II	52	100				
HLA-A77								1-14 (03)	26II	52	100	51-7 (04)	26II	52	78
HLA-A103	25II + 4I	54	67	BC1S1-44 (03)	25II + 3I	53	50								
				BC1S1-87 (03)	26II + I	53	67								
				BC1S1-102 (03)	26II	52	58								
				BC1S1-110 (03)	24/23II + 1/1 IV + 0/2I	52	100								
				BC1S1-115 (03)	26II + I	53	86								
				BC1S1-122 (03)	26II + 2I	54	63								
				2-3 (03)	26II/25 + 0/2I	52	95	33-2 (04)	26II	52	100				
				38 (03)	26II + I	53	79	1-9 (04)	25II + 2I	52	100				
				49 (03)	24II + IV	52	69								
				60 (03)	25II + 2I	52	57								
				15-11 (04)	25II	50	100								
				15-8 (04)	26II	52	100								
HLA-B91	25II + 2I	52	31	43-5 (04)	26II	52	100								

[†]Selfed plants are prefixed with BC1S1, while plant IDs with no prefix indicates BC2F1.
[‡]Chromosome number.
[§]Modal metaphase I configuration.

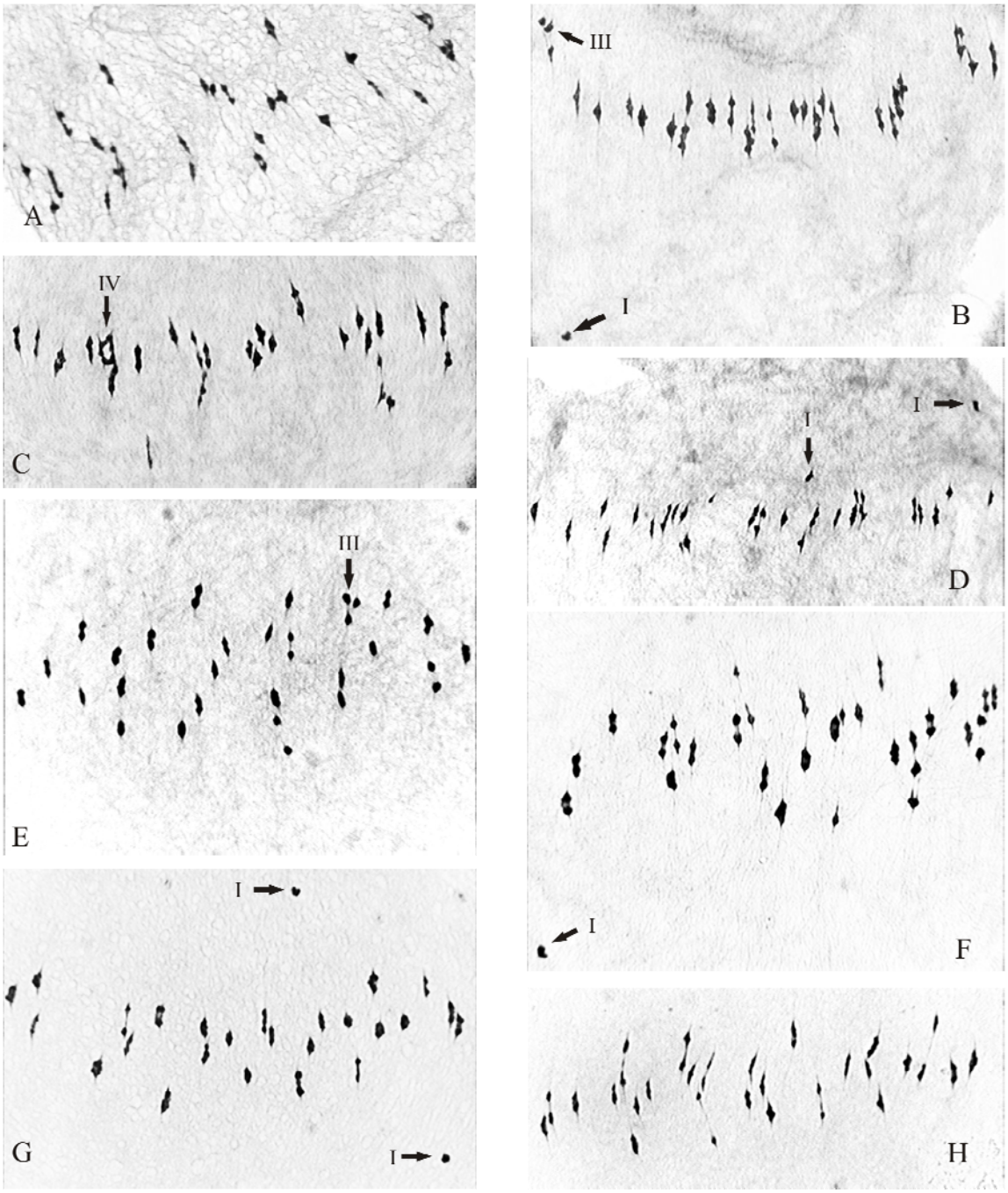

Figure 3. Examples demonstrating recombination between chromosomes in the reniform nematode-resistant plants. (A-D) Four types of metaphase I configurations in a highly resistant BC2F1 plant (BC2-176) where (A) shows complete normal 26II configuration (modal type), while (B), (C) and (D) shows cells with I + 24II + III, 24II + IV, and 2I + 25II chromosomal configurations, respectively. (E-F) Two common cell types in a highly resistant BC1S1 plant (BC1S1-1-11), where (E) has 25II + III, while (F) has I + 26II. (G-H) Two cells of a highly resistant BC2F1 plant (BC2-2-3) showing differences in recombination, one (G) with 2I + 25II versus (H) with 26II.

Moreover, D_h-subgenome of *G. hirsutum* retains sufficient homology with the D-genome chromosomes to form bivalents (Brown and Menzel, 1952; Endrizzi, 1957; Phillips and Strickland, 1966), supporting the view that the unpaired chromosomes probably belong to the A and F genomes. Multivalents in the HLA tri-species hybrid can probably be attributed to the residual homology existing between the A_h-, F_1-, $D_{2\text{-}1}$, and D_h-genomes present in the HLA hybrid due to segmental interchanges during the *Gossypium* genome evolution (Kammacher, 1956; Marechal, 1974). However, since none of the D-genome species have been found to be structurally different from the D-subgenome of *G. hirsutum* (Menzel and Brown, 1954), grossly no multivalents would be expected involving $D_{2\text{-}1}$, and D_h-genome chromosomes.

Cytological evaluation of another tri-species hybrid, HTL (|*G. hirsutum*|*G. thurberi*||*G. longicalyx*|) showed an average of 14.13I + 15.10II + 1.03III + 0.9IV + 0.03V + 0.13VI (Konan et al., 2007). The only difference between HLA and HTL tri-species hybrids lies in the selection of bridge species where *G. armourianum* ($D_{2\text{-}1}$) was used in HLA, while *G. thurberi* (D_1) was used in HTL. The two studies mainly differ in the mean number of univalents and bivalents, where HLA had fewer univalent and more bivalent frequencies than HTL. Average multivalents in the HTL and HLA were 2.0 and 1.8 respectively. The HLA tri-species hybrid was male sterile but had some female fertility (Bell and Robinson, 2004). Major causes of sterility in HLA and HTL presumably include aneuploids, unbalanced gametes due to the duplications and deficiencies caused by multivalent formations and also due to lagging univalents. The lagging univalents were observed to form micronuclei at the sporad stage in HLA (data unavailable), HHL (*G. hirsutum* + *G. herbaceum* + *G. longicalyx*) (unpublished data), as well as in HRS (*G. hirsutum* + *G. raimondii* + *G. sturtianum*) and TSH (*G. thurberi* + *G. sturtianum* + *G. hirsutum*) tri-species hybrids (Vroh et al., 1998). Based upon the above explanation, one would expect incomplete chromosomal complements in both male and female gametes formed by HLA. Difference in female (high) and pollen (low) fertility probably reflect differing levels of reliance on gametophytic gene transcription. Pollen grains with any such incomplete chromosomal complement would most likely lack some

vital genes governing nutrient uptake or other important functions essential for pollen development or tube growth, causing male sterility, while the chances of such unbalanced egg cell to be functional are relatively high due to the fact that female gametophytes are relatively protected and nonautonomous from surrounding maternal cells.

Hyper-aneuploids and hypo-aneuploids among HLA-derived BC1F1 and BC2F1 plants can be respectively attributed to the aneuploidy of functional egg cells of HLA, because in both cases the female parents were pollinated with normal *G. hirsutum* pollen. Generations, BC1F1 and BC1S1 had higher percentages of plants that were hyper-aneuploids than hypo-aneuploids. This can be explained by the death of hypo-aneuploid progeny in the zygotic or early embryonic stage due to severe reduction in the gene dosage, while survival of the hyper-aneuploids can be attributed to the fact that plants can bear additional gene dosage contributed by extra chromosomes. Higher percentages of hypo-aneuploids observed in the BC2F1 could be due to an increase in the percentage of the *G. hirsutum* genome, which in turn compensates for the deficient genes on the missing chromosomes.

Based on the overall genetic map size of *G. hirsutum*, which is estimated to be *ca*. 5,500 cM (Nguyen et al., 2004), each chromosome size can be estimated to be *ca*. 200 cM, which involves a minimal average of 4 crossovers (XO) (1XO = 50 cM). Phillips and Strickland (1966) observed 6.78 bivalents in a *G. hirsutum* x *G. longicalyx* triploid which is close to the 5.64 bivalents formed between A_h-subgenome chromosome of *G. hirsutum* and F genome-chromosome (*G. longicalyx*), included in the HLA tri-species hybrid, presuming that the additional 13 bivalents are formed due to pairing between D_h-subgenome of *G. hirsutum* and $D_{2\text{-}1}$ (*G. armourianum*) (Table 2). Thus, 5.64 or *ca*. 6 out of 13 chromosomes from *G. longicalyx* would be expected to be involved in forming bivalents with the A_h-subgenome of *G. hirsutum*. In other words, one out of two *G. longicalyx* chromosomes were involved in pairing with the A_h-genome, which is 1/2 XO per chromosome, assuming single crossover per bivalent as evident in the metaphase I configuration of HLA where rod bivalents were prevalent (Fig. 2). These

data suggest that F-A homeologous pairs average an 8X or more reduction in pairing relative to A-A pairs.

This report provides the cytological evidence of recombination between the A_h subgenome of *G. hirsutum* and F genome, which is the immune source to reniform nematodes (Yik and Birchfield, 1984). It also demonstrates increase in percentage of plants with normal 26II pairing with successive backcrossing of the reniform nematode-resistant plants to *G. hirsutum*. This work has facilitated in identification of highly resistant plants with 26II chromosomal complement, which were used to derive advanced backcross plants that were used in releasing the reniform nematode-resistant germplasm (Bell et al., 2007).

In future work, it would be highly durable to clearly define and minimize the alien R-gene segment that confers resistance to reniform nematodes. Linkage data can be used to minimize but not to determine the physical size. The most direct means of doing so will likely involve molecular cytogenetic analysis. Molecular markers tightly linked to the reniform resistance gene from *G. longicalyx* have been identified (Chapter III). These markers can be used to screen F-genome BAC library to pull BAC clones carrying the resistance gene. Potential BAC clones carrying the target gene sequence can be used as a probe to determine the size of the alien segment carrying the resistance gene using a technique such as BAC-FISH. Information on the physical size of the alien segment would help in deciding the most efficient approach to clone the reniform nematode-resistance gene in cotton.

CHAPTER III

TAGGING AND GENETIC MAPPING OF UPLAND COTTON RESISTANCE TO RENIFORM NEMATODE INTROGRESSED FROM *Gossypium longicalyx*

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important textile fiber crop of the world, and the current U.S. lint production accounts for nearly 20% of the world supply (Burr et al., 2006). High-yielding Upland cotton cultivars have been considered to have low genetic variation relative to other plant taxa (Brubaker and Wendel, 1994) and thus are vulnerable to potential pathogen or insect epidemics (Anonymous, 1972; Bowman et al., 1996). A distribution survey led Lawrence and McLean (1995) to conclude that plant-parasitic nematodes present the most difficult pest problem encountered in cotton production due to ineffective management practices caused by underestimation of nematode population densities and/or misidentification of the nematode species.

The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira), which was first reported as a pest of cotton by Smith in 1940, is now a major pest of cotton in the southeastern and central United States (Overstreet and McGawley, 1997; Robinson et al., 1997; Lawrence, 1999; Overstreet, 1999; McLean and Lawrence, 2000; Lawrence and McLean, 2001; Koenning et al., 2004). Factors contributing to emergence of this nematode as a major pathogen of cotton include its broad host range (Birchfield and Jones, 1961; Birchfield and Brister, 1962; Robinson et al., 1997), its ability to survive in dormant state (Birchfield and Martin, 1967), to infest the soil profile to a depth of more than 1 meter (Lee et al., 2002; Newman and Stebbins, 2002) and its high population densities even in the absence of hosts (Lee et al., 2002). According to Blasingame (2006), the estimated cotton production loss in the United States due to reniform nematode in the year 2005 was 115 x 10^6 kilograms (526 thousand bales), conservatively worth one dollar kg^{-1} (http://www.cotton.org/econ/prices/monthly.cfm).

The growing problem of reniform nematode in cotton production has led to the desire to incorporate genetic resistance to the reniform nematode in Upland cotton genotypes. Extensive screenings of the *G. hirsutum* germplasm collection over the years have confirmed that none of the Upland cotton genotypes has adequate resistance to reniform nematodes (Yik and Birchfield, 1984; Jones et al., 1988; Robinson and Percival, 1997; Robinson et al., 1997; Robinson et al., 1999). Some of the wild diploid *Gossypium* species are highly resistant to the reniform nematode, but introgression of the trait into Upland cotton is extremely difficult due to the difference in their ploidy levels (Koenning et al., 2004). Upland cotton (2n-52) is a tetraploid, and has the genomic constitution $2[AD]_1$. Several related species share a similar AD-genomic architecture, whereas there are about 45 2n=26 species that contain genomes of diverse size and meiotic affinity. The species most resistant to reniform nematode is *Gossypium longicalyx*, which has near immunity to reniform nematode (Yik and Birchfield, 1984), but is diploid (2n=26) and contains an F-genome. An effort to extract the resistance trait from *G. longicalyx* was undertaken in the early 1990s, using monosomic addition lines developed by Ehou (1983). Screening of the 12 available segregating addition lines failed to reveal discernibly high levels of resistance among them, although the failure may have been procedural rather than genetic in nature (Frerich 1995).

Successful introgression of the reniform nematode resistance trait of *G. longicalyx* into Upland cotton commenced with development of two tri-species hybrids [*G. hirsutum*|*G. longicalyx*||*G. armourianum* (designated HLA) and *G. hirsutum*|*G. herbaceum*||*G. longicalyx* (designated HHL)]. While *G. longicalyx* (F_1) served as donor of the resistance trait, *G. armourianum* Kearney ($D_{2\text{-}1}$) or *G. herbaceum* L. (A_1) essentially served as bridge species to overcome ploidy differences relative to *G. hirsutum*. By recurrent backcrossing these tri-species hybrids with *G. hirsutum*, backcross populations were created and screened for reniform nematode resistance. In all advanced generations derived from the 28 resistant families, backcross progeny segregated 1:1 for resistant:susceptible and, where studied, self progeny segregated 3:1 (Robinson et al., 2007). These results indicated that the reniform nematode resistance

trait is inherited as a monogenic trait, suggesting that it is conferred by a single dominant gene or, if not, by very closely linked genes.

Classification of reniform nematode resistance by phenotypic screening is time-consuming, expensive and sensitive to screening conditions, any of which can dissuade breeders from incorporating and utilizing such a trait in their breeding programs. Conversely, usage of genetic resistance by breeders could be fostered by the availability of means for indirect selection that are accurate, inexpensive, rapid and facile, e.g., marker-assisted selection (MAS). In tomato (*Lycopersicon esculentum* L.), for example, close linkage (*ca*. 1 cM) between the *Aps-1* locus governing an acid phosphatase isozyme and an introgressed alien locus for resistance to root-knot nematodes by Rick and Fobes (1974) enabled extensive use of the resistance in breeding programs. Today, the mapping of a resistance gene relative to marker loci can also lead to high-resolution mapping, cloning, sequence identification, molecular analysis, expression analysis, molecular manipulation, and further definition of plant self-defense systems.

Such efforts are facilitated by availability of several genetic maps of cotton that have been constructed using different molecular markers like amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and restriction fragment length polymorphism (RFLP) (Reinisch et al., 1994; Shappley et al., 1998; Ulloa and Meredith, 2000;Abdalla et al., 2001; Zhang et al., 2002; Ulloa et al., 2002; Lacape et al., 2003; Rong et al., 2004; Mei et al., 2004; Nguyen et al., 2004; Lacape et al., 2005; Ulloa et al., 2005). These maps collectively include large numbers of mapped public SSR markers that have been assembled into the Cotton Microsatellite Database (Blenda et al. 2006), which renders them all more suitable for development of marker-assisted selection (MAS) in cotton improvement. The public SSRs provide fairly comprehensive genomic coverage that affords efficient genome scanning and progressively finer mapping. Moreover, the mapped SSRs have locus identity that affords portability, their rates of polymorphism and multi-allelism afford relatively good rates of discrimination, and they are amenable to simple or high-throughput PCR procedures.

Several lines of evidence indicate that *G. longicalyx* is more closely related to the A-subgenome of *G. hirsutum* than it is to the D-subgenome of *G. hirsutum*. In addition to geographic proximity of wild species bearing the A and F genomes, the A-subgenome chromosomes of *G. hirsutum* are more similar in size and exhibit much greater meiotic affinity to the F genome in *G. hirsutum*×*G. longicalyx* triploid and hexaploid plants (Phillips and Strickland, 1966). Similar meiotic preference was noted (Ehou, 1983; Stelly, unpublished; Dighe, unpublished) for the alien *G. longicalyx* chromosomes in *G. hirsutum* monosomic alien addition plants developed independently by Ehou (1983) and in the course of this introgression effort (Robinson et al. 2007). The observations indicate that chromosome pairing and subsequent crossovers involving the relevant chromosome regions of *G. longicalyx* resistance would likely cause incorporation of the resistance gene(s) into an A- rather than a D- subgenome chromosome of *G. hirsutum*. Thus, the search for markers linked to the introgressed trait would be most wisely directed to marker loci in A-subgenome linkage groups.

Here, we report results from a set of experiments aimed at rendering the resistance gene introgressed from *G. longicalyx* more amenable to breeding manipulations, and more approachable for fine-mapping, cloning and molecular analysis. In these experiments, we used publicly available SSR markers and linkage maps, especially those of Lacape et al. (2003), Nguyen et al. (2004), and Lacape et al. (2005), to localize and enumerate the gene conferring the resistance trait, to determine linkage, to select heterozygotes after backcrossing and to select homozygotes after self-pollination. The results support a single-locus or single-haplotype model for inheritance in *G. hirsutum* of the resistance trait introgressed from *G. longicalyx*, identify closely linked flanking markers that could be used to screen for the resistance gene and employ MAS and potential fine-mapping, identify chromosomal location of the reniform nematode resistance gene, and in concert with a companion paper (Robinson et al., 2007), demonstrate the utility of the most closely linked loci for MAS.

Materials and Methods

Reniform Nematode Screening

Nematode resistance was defined as a low rate of nematode reproduction on healthy roots. Reproduction was evaluated directly by counting mature females and eggs on roots, or indirectly by counting vermiform nematodes within soil (Robinson et al., 2006). In the direct assay, nematode-free plants were grown 8 to 12 weeks until pot-bound within 500-ml cups. Then the root "ball" of each cup was removed, slipped into a close-fitting cup-shaped sleeve fashioned from fiberglass window screen fabric, and transplanted into a 3-liter pot containing soil infested with *R. reniformis*. The pot was then placed in a controlled environment chamber for three weeks, when the root balls were gently lifted from pots. New roots that had grown out through the screen from the root ball into the infested soil were cut off with scissors, collected, placed in fixative and examined microscopically to evaluate female nematode development and presence or absence of associated eggs. In the indirect and most frequently used assay, seeds were scarified by nicking the seed coat, germinated in moistened, rolled blotter paper, and transplanted individually into 500-ml cups, which were held in a greenhouse for 10 to 14 days, then inoculated with 4,000 nematodes per plant. After inoculation, plants were held in a controlled environment chamber for seven weeks, at which time three soil cores weighing 40 g total were removed from each cup, and analyzed to measure the concentration of active, vermiform *R. reniformis* in the soil. In both methods, selected plants were retained for breeding or leaf tissue generation for DNA studies by transplanting to larger pots containing nematode-free potting medium.

Plant Materials

Plant materials included two tri-species hybrids consisting of *G. hirsutum* (AD_1) as the recipient species, *G. longicalyx* (F_1) as the donor parent, and two wild diploids, *G. armourianum* ($D_{2\text{-}1}$), and *G. herbaceum* (A_1) as respective bridge species. These hybrids were designated by the initials HLA for [(*G. hirsutum*×*G. longicalyx*) doubled×*G. armourianum*] and HHL for [(*G. hirsutum*×*G. herbaceum*) doubled×*G.*

longicalyx] (Fig. 1). The chromosome complements of the intermediate triploid bi-species hybrids were doubled by treatment with colchicine. The hexaploid used to create HLA tri-species hybrid was developed by Dr. Meta Brown of the Texas Agricultural Experiment Station (TAES), while the hexaploid used to create HHL tri-species hybrid was made at the USDA-ARS, College Station. The developmental details of the two tri-species hybrids are summarized by Bell and Robinson (2004). Both HLA and HHL tri-species hybrids were involved in the reniform nematode resistance introgression efforts via backcross breeding.

Twenty-eight highly resistant BC1F1 plants were used to advance the reniform nematode resistance germplasm by backcrossing, which included 22 plants from the HLA and 6 from the HHL tri-species hybrids and are referred to as families (Table 8; Robinson et al., 2007). To develop these 28 families, two breeding sets were created. In the first set, the tri-species hybrid HLA was randomly cross pollinated with single flowers from a pool of *G. hirsutum* cultivars [Paymaster (PM)-1220 RR, Tamcot Sphinx, SureGrow (SG)-125 and Stoneville (STV)-373] to create 8 BC1F1 families (HLA-A-2, 77, 83, 84, 85, 103, 122, and 132) which were carried to advanced backcross generations (Table 8; Robinson et al., 2007). In the second set, HLA and HHL tri-species hybrids were cross pollinated with *G. hirsutum* cv. Acala NemX to create 14 and 6 BC1F1 families respectively (Table 8), which also were carried to the advanced backcross generations as described by Robinson et al. (2007). Besides these 28 families, another 6 families (HLA-A4, 99, 110, 117, 119, and 123) were also created from HLA, but were never advanced beyond BC2F1 generation (Table 8).

Four of these families were also self-pollinated to create a population of 88 plants that were phenotyped for the reniform nematode resistance; their detailed pedigree is described in Fig. 4 and by Robinson et al. (2007). In developing these self populations, resistant plants, BC1F1-103 and BC1F1-85 were self pollinated to create a total of 12 BC1S1 plants that were analyzed for the reniform nematode resistance. In another set, a highly resistant BC3F1 plant from family HLA-A77 was self pollinated, and 14 of its progeny were classified for resistance and their resistance status was confirmed by

testcross analysis (TC). In addition, 28 BC3S1 and 34 BC6S1 plants from family, HLA-A84 were also classified for the reniform nematode resistance, and the resistance status of 28 BC6S1 plants was confirmed by testcross (TC) analysis. All the plants were maintained in the greenhouse where they were hand-emasculated a day prior to pollination and hand-pollinated early on the day of pollination in cases of backcrosses and self pollinated by hand in cases of self populations.

DNA Panels

We created three types of DNA panels for progressive screening and evaluation of markers, i.e., first to assay for parental polymorphism, then to scan for association with resistance and lastly to confirm the assocation and estimate the linkage intensity with the gene(s) conferring resistance. The polymorphism-detection panel consisted of the parental species, i.e., *G. hirsutum, G. longicalyx*, *G. armourianum*, and HLA tri-species hybrid. The trait-association panel consisted of the parents (*G. longicalyx*, *G. armourianum*, HLA tri-species hybrid, a three-cultivar pooled DNA samples of *G. hirsutum*), plus individual samples from the 12 most resistant and the 12 most susceptible plants from a HLA-derived BC2F1 population of 125 plants that had been screened for reniform nematodes. DNAs from three *G. hirsutum* cultivars [Auburn (Aub)-623, Acala NemX, and Delta Pine (DP)-458], were bulked to represent the collective SSR diversity of susceptible *G. hirsutum* parent. The linkage detection panel included individual DNA samples from 896 backcrossed (BC2F1, BC3F1, BC4F1, BC5F1, BC6F1, BC7F1, and BC8F1) and 88 self pollinated plants (BC1S1, BC3S1, and BC6S1) derived from the HLA and HHL tri-species hybrid, as summarized in Table 9. The lineages and structures of these populations are detailed in Robinson et al., 2007.

Table 8. Parental details of the designated families and their most advanced backcross and self generations that were selected for the reniform nematode resistance and used in mapping the resistance gene.

Tri-species	Parents	BC1F1 (Family)	Advanced Generation
HHL	HHL x *G. hirsutum* cv. Acala NemX	HHL-3	BC5F1
	HHL x *G. hirsutum* cv. Acala NemX	HHL-5	BC5F1
	HHL x *G. hirsutum* cv. Acala NemX	HHL-7	BC5F1
	HHL x *G. hirsutum* cv. Acala NemX	HHL-11	BC5F1
	HHL x *G. hirsutum* cv. Acala NemX	HHL-14	BC5F1
	HHL x *G. hirsutum* cv. Acala NemX	HHL-17	BC5F1
HLA	HLA x *G. hirsutum*†	HLA-A2	$BC6F_1$
	HLA x *G. hirsutum*†	HLA-A77	BC7F1, BC3S1
	HLA x *G. hirsutum*†	HLA-A83	$BC6F_1$
	HLA x *G. hirsutum*†	HLA-A84	BC8F1, $BC6F_1$
	HLA x *G. hirsutum*†	HLA-A85	BC6F1, BC1S1
	HLA x *G. hirsutum*†	HLA-A103	$BC6F_1$, BC1S1
	HLA x *G. hirsutum*†	HLA-A122	$BC6F_1$
	HLA x *G. hirsutum*†	HLA-A132	$BC6F_1$
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B4	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B18	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B21	BC4F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B26	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B34	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B35	BC4F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B45	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B61	BC4F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B75	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B77	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B81	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B91	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B99	BC5F1
	HLA x *G. hirsutum* cv. Acala NemX	HLA-B103	BC4F1
	HLA x *G. hirsutum*†	HLA-A110	BC2F1
	HLA x *G. hirsutum*†	HLA-A119	BC2F1
	HLA x *G. hirsutum*†	HLA-A123	BC2F1
	HLA x *G. hirsutum*†	HLA-A117	BC2F1
	HLA x G. hirsutum cv. Acala NemX	HLA-A4	BC2F1
	HLA x *G. hirsutum*†	HLA-A99	BC2F1

†Single flowers from a pool of *G. hirsutum* cultivars [Paymaster (PM)-1220 RR, Tamcot Sphinx, SureGrow (SG)-125 and Stoneville (STV)-373] were used as male parents.

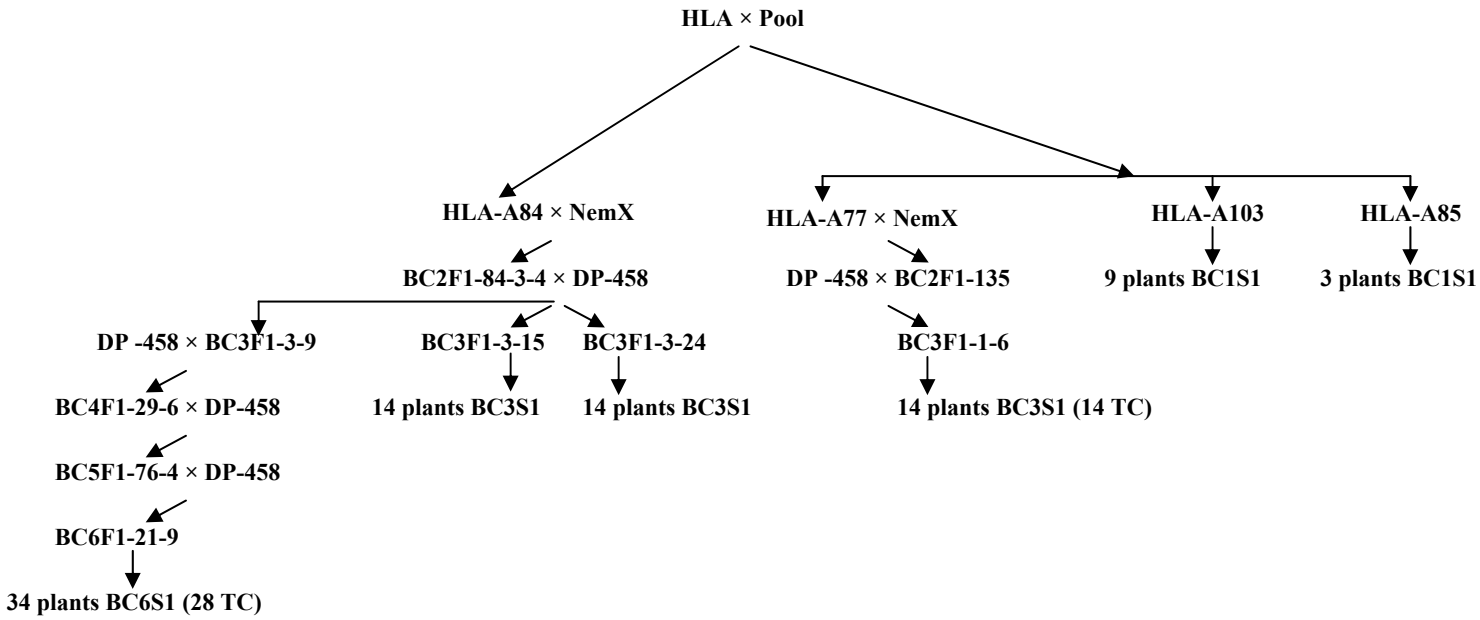

Figure 4. Flow diagram describing the development of BC1S1, BC3S1, and BC6S1 populations that were phenotyped for the reniform nematode resistance and genotyped for flanking markers. "Pool" includes four *G. hirsutum* cultivars: PM-1220RR, Tamcot Sphinx, SG-125, and STV-473. TC indicates that the resistant status of the plants was confirmed by phenotypic analysis of their testcross progenies. NemX, Acala NemX; DP, Delta and Pine Land; SG, SureGrow; PM, PayMaster; STV, Stoneville; RR, Roundup Ready.

DNA Extraction

DNA was extracted from the fresh, folded or newly unfolded leaves by a mini-prep method (Zhang and Stewart, 2000). The DNA yield was estimated using TD-360 Fluorometer (Turner Designs, Sunnyvale, CA, USA).

Table 9. Summary of the backcrossed and self-pollinated populations derived from HLA and HHL tri-species hybrids that were screened with SSR markers linked to the reniform nematode-resistance gene.

Generation	Plants Analyzed		
	HLA	HHL	Total
BC2F1	125	0	125
BC3F1	48	6	54
BC4F1	123	23	146
BC5F1	177	60	237
BC6F1	148	0	148
BC7F1	166	0	166
BC8F1	20	0	20
BC1S1	12	0	12
BC3S1	42	0	42
BC6S1	34	0	34
Total	895	89	984

Markers, Trait Association and Mapping

SSR Markers

Sampling of SSRs to detect association with the resistance trait was based on subgenome targeting and progressively deeper sampling of SSRs in A-subgenome linkage groups on a per-need basis. We focused on A-subgenome linkage groups, reflecting propensity of the A subgenome to pair with the alien F genome. We initially selected 48 SSR primer

pairs from the thirteen A-genome linkage groups reported by Lacape et al. (2003) and Nguyen et al. (2004), averaging three primers per linkage group. After two primers from the A03 linkage group (LG) indicated putative linkage to the reniform nematode resistance gene, another 10 SSR primers from LG A03, and four SSR primers from its homeologous LG D02, were selected from the published maps (Lacape et al., 2003; Nguyen et al., 2004; Frelichowski et al., 2006) making a total of 62 SSR primers that included 52 BNL (Brookhaven National Laboratory), four CIR (CIRAD), and six MUSB primer pairs. LG A03 and D02 are now identified as chromosomes 11 and 21, respectively (Wang et al. 2006b).

All 62 SSR primers used in this study were initially screened against a parental polymorphism screening panel that consisted of *G. longicalyx* (F_1), *G. hirsutum* (AD_1) (bulked), *G. armourianum* ($D_{2\text{-}1}$) and HLA-tri-species hybrid. For a given primer pair, a specific polymorphism was denoted herein by appending the bp length to the primer designation, e.g., BNL1066_156 for the 156-bp amplicon using primer pair BNL1066. Primer pairs that revealed polymorphism between the resistant group (F_1 and HLA) and susceptible group (AD_1 and $D_{2\text{-}1}$) were selected and screened on the trait-association panel consisting of the 12 most resistant and the 12 most susceptible plants in a BC2F1 population to identify markers linked to the reniform resistance gene. These putatively linked SSR primers were then screened on linkage detection panel consisting of phenotypically classified plants from the backcrossed and self generations described in Table 9 to identify the markers most tightly linked to the resistance gene, and to map the resistance gene.

All 62 primer pairs used in this study were synthesized for the polyacrylamide gels run on dual-dye LI-COR 4200 IR^2 gel detection system (LI-COR Inc., Lincoln, NE). The SSR primer sequences used in this study are described in Cotton Microsatellite Database (http://www.cottonssr.org). Each forward primer was labeled with infrared fluorescent dye (IRD 700 or IRD 800, MWG Biotech AG). PCR amplifications were performed in 8 µl volume containing 10 ng genomic DNA, 10× PCR Buffer, 3 mM $MgCl_2$, 0.3 mM dNTPs, 0.04 µM IRD-labeled forward primer, 0.04

μM reverse primer, 0.4 U *Taq* polymerase (Promega, Madison, WI, USA). All PCR amplifications were performed using a PCR system 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: an initial denaturation step at 94 ºC for 5 min, followed by 25 cycles of denaturation at 94 ºC for 1 min, annealing at 55 ºC for 1 min, and extension at 72 ºC for 1 min 30 sec. A final extension step of 7 min at 72 ºC was included. After denaturing at 94 ºC for 4 min, the PCR products were loaded onto 25 cm gels (0.25mm spacer thickness) containing 6% Gene-PAGE PLUS ™ (6% Modified Acrylamides, 7M Urea, 0.089M Tris, 0.089M Boric Acid, 0.002M EDTA) (Amresco Inc., Solon, Ohio, USA). Electrophoresis was performed on a dual-dye LI-COR 4200 IR^2 gel detection system (LI-COR Inc., Lincoln, NE). Fragment sizes were determined relative to 50-700 bp IRD labeled (IRD 700 and IRD 800) molecular standard (LI-COR Inc., Lincoln, NE).

Phenotypic Marker

In the early backcross generations, we observed that a seed trait, grey-green seed fuzz, from *G. longicalyx* (Saunders, 1961), referred here to as Fzg^{lon} (Fzg^{lon} = fuzz green from *Gossypium longicalyx*), co-segregated with the reniform nematode resistance locus. This result was confirmed after scoring 34, reniform-classified BC6S1 plants for seed fuzz color (Fig. 4). To map Fzg^{lon} relative to the resistance locus, phenotypes were scored on the linkage detection panel consisting of 708 backcrossed and self plants across generations (Table on page 55).

Green Fuzz Ratings

The green fuzz character was rated on a scale of 0-3, where 0 = no green fuzz, 1 = faint green fuzz, 2 = intermediate level of green fuzz, and 3 = most intense green fuzz. Comparisons were made among siblings of backcrosses or selfs, the last backcross cultivar grown under the same conditions, and a comparable progeny from a HLA-A85 family line which consistently showed distinct segregation of the green fuzz character.

Usually, 20-30 g of seed from the plant being rated were placed in clear, 10-cm wide Petri dishes and placed alternatively on black and white backgrounds. Ratings of 0-1 were considered negative, while 2-3 ratings were considered positive for seed fuzz classification.

Linkage Estimation

Linkage estimates were based on segregation data from a number of multi-generation lineages, each including multiple opportunities for recombination (Table 9; Figure 4). Plants were classified as resistant if the %DP-16 (%susceptible control) value was 12 or less than 12, while a plant with more than 12% was classified as susceptible. Although 984 resistance-classified plants were available for linkage estimation, the linkage estimation for each marker was based only on progeny from parents that were heterozygous for the resistance and that marker. In effect, this precluded the statistical bias of linkage estimates that would have arisen if analyzed data were to have included progeny that inherited the crossover product of a *pre-parental* recombination event rather than a parental event. Thus, slightly different subpopulations of the overall population of 984 plants were used to calculate linkage between the individual markers and the resistance gene. Given their pedigrees, the alien markers were assumed to be in coupling with each other and the resistance gene (Table 8; Figure 4).

Molecular marker data and reniform resistance classifications of individual plants in various backcross and self generations (Table on page 55) were analyzed collectively for linkage and marker order by using Carthagene software (Givry et al., 2005). Data from the backcross and self populations were merged using the "dsmergen" command. Markers were assigned to a linkage group by analysis of the merged population using the 2-point LOD with a LOD threshold of 3.0 and distance threshold of 30 cM. Recombination fractions were converted into centiMorgans (cM) based on Haldane's mapping function (Haldane, 1919). The linkage map was constructed by executing the "build" function, which uses multipoint maximum likelihood estimates (MLE) to determine locus order and estimate distances among loci.

Results

Marker Analysis and Chromosomal Localization of the Reniform Nematode Resistance Gene

Initially 48 SSR primers (BNL) drawn from thirteen A-subgenome linkage groups were scanned for polymorphism between the resistant (*G. longicalyx* and HLA) and susceptible (*G. hirsutum* and *G. armourianum*) parents used in developing the HLA tri-species hybrid. Polymorphisms were observed for 17 (35%) of the primers. When run against the trait-association panel to scan for linkage to the resistance gene(s), highly coincident distributions were noted for amplicons BNL1066_156 bp and BNL836_215 bp (Fig. 5), which indicated their linkage to the reniform nematode resistance gene(s). This inference was strongly reinforced by linkage of these marker loci (Lacape et al., 2003; Nguyen et al., 2004; Lacape et al., 2005), as well as their synteny and physical proximity (Gao et al. 2004, 2006). The presence and absence of marker BNL1066_156 coincided exactly with resistant and susceptible BC2F1 plants, whereas that of BNL836_215 was present for 10 out of 12 resistant plants and absent in all 12 susceptible plants.

Guided by association of the SSR loci BNL1066 and BNL836, we used the linkage maps of LG A03 and its homeologous linkage group, D02, to identify SSR markers that might be tightly linked to the resistance gene introgressed from *G. longicalyx* into *G. hirsutum*. Fourteen additional SSR primer pairs (4 BNL, 4 CIR, and 6 MUSB) from linkage groups A03 and D02 defined by Lacape et al. (2003), Nguyen et al. (2004), and Frelichowski et al. (2006) were selected and screened for parent polymorphism. Of these 14 primer pairs, only BNL3279, from the D02 group (Lacape et al., 2003; Nguyen et al., 2004), amplified co-dominant markers, 112 bp and 114 bp, that were polymorphic between the resistant parents (*G. longicalyx* and HLA) and the susceptible parents (*G. hirsutum* and *G. armourianum*).

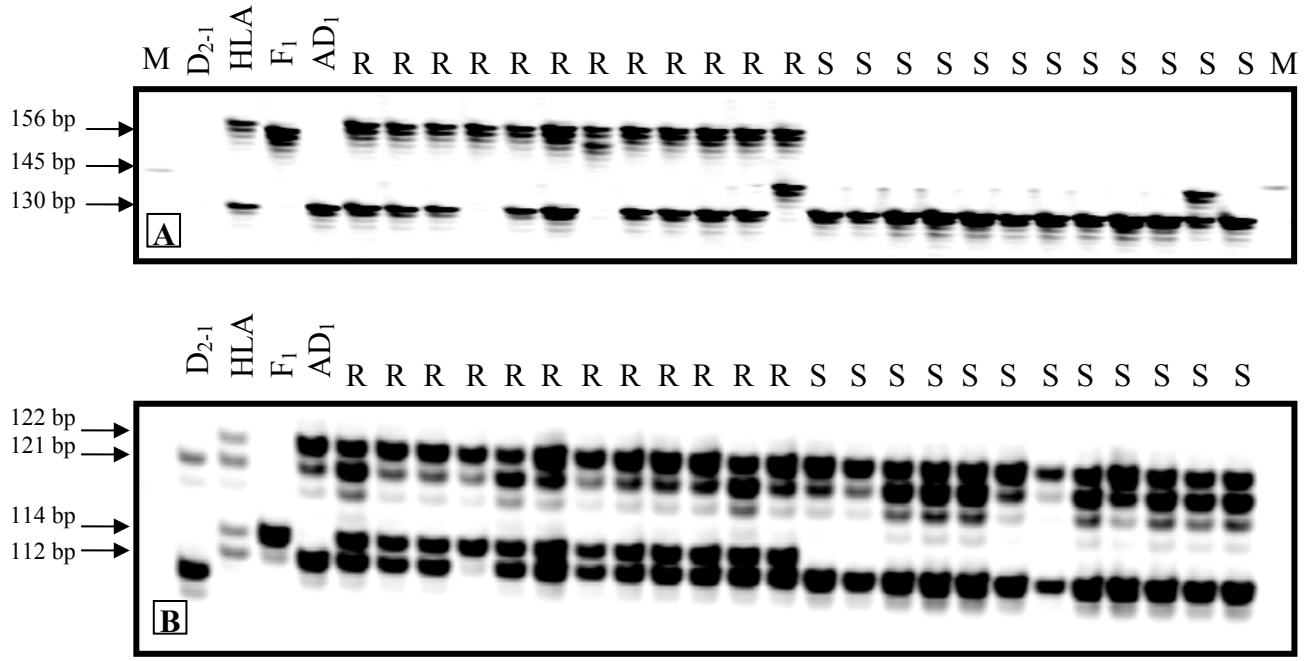

Figure 5. The PCR products amplified by the SSR primers BNL1066 (A) and BNL3279 (B) on a trait-association panel consisting of 4 parents, and 12 resistant and 12 susceptible BC2F1 plants from a reniform nematode resistance classified BC2F1 population. $D_{2\text{-}1}$, *G. armourianum*, a bridging species; HLA, resistant tri-species hybrid; F_1, *G. longicalyx*, the resistance donor species; AD_1, *G. hirsutum*, susceptible recurrent parent; R, resistant; S, susceptible; M, molecular weight standard.

Table 10A. Segregation pattern of the BNL3279 locus in the backcrossed generations across the reniform nematode-resistant families.

Family	Generations	Marker present (O)	Marker absent (O)	Expected (E)	O-E‡	$\chi^{2\dagger}$ 1:1	P value
HLA-A2	BC6F1	4.00	5.00	4.50	0.50	0.11	0.74
HLA-A77	BC6F1	3.00	7.00	5.00	2.00	1.60	0.21
HLA-A83	BC6F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-A84	BC6F1,BC7F1,BC8F1	39.00	36.00	37.50	1.50	0.12	0.73
HLA-A103	BC6F1	5.00	5.00	5.00	0.00	0.00	1.00
HLA-A122	BC6F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-A132	BC6F1	5.00	5.00	5.00	0.00	0.00	1.00
HLA-B4	BC5F1	8.00	2.00	5.00	3.00	3.60	0.06
HLA-B18	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-B21	BC4F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-B26	BC5F1	5.00	3.00	4.00	1.00	0.50	0.48
HLA-B34	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-B35	BC4F1	3.00	7.00	5.00	2.00	1.60	0.21
HLA-B45	BC5F1	6.00	4.00	5.00	1.00	0.40	0.53
HLA-B61	BC4F1	3.00	7.00	5.00	2.00	1.60	0.21
HLA-B75	BC5F1	3.00	6.00	4.50	1.50	1.00	0.32
HLA-B77	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-B81	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-B99	BC5F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-B103	BC4F1	3.00	7.00	5.00	2.00	1.60	0.21
HHL-3	BC5F1	5.00	5.00	5.00	0.00	0.00	1.00
HHL-5	BC5F1	5.00	5.00	5.00	0.00	0.00	1.00
HHL-7	BC5F1	5.00	5.00	5.00	0.00	0.00	1.00
HHL-11	BC5F1	6.00	4.00	5.00	1.00	0.40	0.53
HHL-14	BC5F1	7.00	3.00	5.00	2.00	1.60	0.21
HHL-17	BC5F1	3.00	7.00	5.00	2.00	1.60	0.21
χ^2 for overall popln (1df)		159.00	162.00	160.50	1.50	0.03	0.87
Heterogeneity (25 df)						22.50	0.60
Sum of χ^2 (26 df)						22.53	0.66

† χ^2, chi square statistics

‡ O indicates observed, E indicates expected

Table 10B. Segregation pattern of the green fuzz locus, *Fzg*^{lon} in the backcrossed generations across the reniform nematode-resistant families.

Family	Generation	Green (O)	White (O)	Expected (E)	O-E‡	$\chi^{2\dagger}$ 1:1	P value
HLA-A2	BC6F1	5.00	4.00	4.50	0.50	0.11	0.74
HLA-A83	BC6F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-A84	BC6F1, BC7F1, BC8F1	40.00	35.00	37.50	2.50	0.33	0.57
HLA-A85	BC6F1	4.00	5.00	4.50	0.50	0.11	0.74
HLA-A103	BC6F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-A122	BC6F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-A132	BC6F1	5.00	5.00	5.00	0.00	0.00	1.00
HLA-B4	BC5F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-B18	BC5F1	3.00	6.00	4.50	1.50	1.00	0.32
HLA-B21	BC4F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-B34	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-B45	BC5F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-B61	BC4F1	3.00	6.00	4.50	1.50	1.00	0.32
HLA-B75	BC5F1	2.00	8.00	5.00	3.00	3.60	0.06
HLA-B77	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-B81	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HLA-B91	BC5F1	5.00	4.00	4.50	0.50	0.11	0.74
HLA-B99	BC5F1	7.00	3.00	5.00	2.00	1.60	0.21
HLA-B103	BC4F1	3.00	7.00	5.00	2.00	1.60	0.21
HHL-3	BC5F1	4.00	6.00	5.00	1.00	0.40	0.53
HHL-5	BC5F1	5.00	5.00	5.00	0.00	0.00	1.00
HHL-11	BC5F1	6.00	3.00	4.50	1.50	1.00	0.32
HHL-14	BC5F1	6.00	4.00	5.00	1.00	0.40	0.53
HHL-17	BC5F1	3.00	7.00	5.00	2.00	1.60	0.21
χ^2 for overall popln (1df)		149.00	150.00	149.50	0.50	0.003	1.00
Heterogeneity (23 df)						21.26	0.57
Sum of χ^2 (24 df)						21.27	0.62

† χ^2, chi square statistics

‡ O indicates observed, E indicates expected

Upon screening the remaining 13 primers across a BC6S1 population of 34 plants, however, we found that three of the primers, BNL1231, CIR003, and CIR196 yielded dominant markers linked in repulsion with the resistance locus. When screened against the trait-association panel, BNL3279 differentiated all 12 resistant from all 12 susceptible BC2F1 plants, with a 114-bp band being present only in the resistant plants.

This marker is designated herein as BNL3279_114 (Fig. 5). The segregation pattern of the BNL3279 in the backcrossed generations across the resistant families supported mendelian inheritance ($\chi^2 = 0.03$; $P = 0.87$) with homogeneity among the families ($\chi^2 = 22.5$; $P = 0.60$ (Table 10A).

The reniform-nematode resistant plants were noted to form green seed, due to the presence of green-colored seed fuzz, i.e., short integument-derived fibers, irrespective of their lint color. This trait has been reported previously for *G. longicalyx* (Saunders, 1961). The segregation pattern of the green fuzz in the backcrossed generations across the resistant families supported mendelian inheritance ($\chi^2 = 0.003$; $P = 1$) with homogeneity among the families ($\chi^2 = 21.26$; $P = 0.57$) (Table 10B). The strong modality of phenotypic segregation for green versus white fuzz indicated qualitative inheritance, and the presence of green-fuzzed backcross hybrids indicated that expression of the segregating green fuzz allele(s) was dominant or codominant. The association between *Fzg*^{lon} and the reniform nematode resistance gene was confirmed after scoring 34 fully classified BC6S1 plants for green fuzz (Fig. 4). Moreover, the green fuzz character was usually more intense in homozygous than heterozygous resistant plants but apparent in both (figure on page 59). It distinguished 12 homozygous, 14 heterozygous and 8 susceptible plants as classified by nematode bio-assays and marker BNL3279_114 interpretations (figure on page 59), thus, making it a co-dominant marker. This led to the desire to determine the linkage estimate between *Fzg*^{lon} and the resistance locus.

To determine the linkage distance between the putatively linked SSR markers, BNL3279_114, BNL1066_156, and BNL836_215 and the reniform nematode resistance gene using both resistance and susceptible classes, primers of the linked markers were screened on secondary screen panels consisting of 896 backcross progeny (BC2F1, BC3F1, BC4F1, BC5F1, BC6F$_1$, BC7F1, BC8F1) and 88 self-progeny (BC1S1, BC3S1, BC6S1) plants (Table 9) that were phenotypically classified for the reniform nematode resistance. A total of 984 plants (backcrosses and self) from 34 families were screened with BNL3279_114, BNL1066_156, and BNL836_215 (Tables 9; 11A and B; 12A and

B). Because most of the susceptible segregates in the early generations were discarded from the breeding population before harvest, i.e., before fuzz color was determined, or the resistant plants with *Fzg*^{lon} in the early backcross generation were used as male parents due to which no segregation for green fuzz was observed since it is a maternal trait expressed in the seed coat, green fuzz classification data were collected only on 642 backcrossed and 66 selfed plants (Table 11B), leaving other 276 plants unclassified.

Linkages for each marker with the resistance gene were estimated after first differentiating between the products from *de novo* recombination events, i.e., where the parent contained both the resistance gene and the marker of interest (Table 11A and B), from *pre-parental* recombination events, i.e., from resistant parents that lacked the marker of interest (Table 12A and B). Progeny falling in the latter class were dubbed "post-initial recombination" products. Accordingly, the total number of backcrossed and selfed plants screened with all three SSR markers was 984, and linkage estimate calculations for BNL3279_114, BNL1066_156, and BNL836_215 were based on 930, 909, and 770 plants, respectively; and the linkage estimate for *Fzg*^{lon} was based on 668 of the 708 plants for which seed were available (Table 11A and B).

During the early backcrossed generations, inheritance of the resistance and linkage relationships were not known, so plants were selected for resistance to reniform nematodes based solely on nematode bio-assays, usually 1-3% of the level in the susceptible control DP-16 (Robinson et al., 2007). Retrospective analysis, however, revealed that homologous recombination led to cryptic loss of one or more markers in 13 lineages and sub-lineages where resistant progenies were carried to advanced backcross generations (Table 12A and B). Upon loss of a marker, further detection of recombination between the marker and resistance gene was precluded by lack of discernible polymorphism, so subsequently formed progeny were cast into the post-initial recombinant category for that marker, i.e., excluded from the respective column of Table 11A and B and instead placed in Table 12A and B. For example, only one plant from the HLA-B91 lineage was included for linkage estimates for all three markers, because the entire lineage descended from a single BC2F1 plant that contained an initial

recombination event between the resistance locus and the three linked SSR markers, BNL3279_114, BNL1066_156, and BNL836_215. Resistant progeny in subsequent generations thus lacked these three flanking markers and were correspondingly excluded from linkage calculations for the respective markers (Table 11A and B). In contrast, the same BC2F1 plant was non-recombinant between the resistance locus and *Fzg*^{lon} and thus the progenies derived from that plant were included in calculating linkage estimates for *Fzg*^{lon} and thus in the respective column of Table 11B.

Linkage analysis based on the resistant and susceptible plants indicated that the reniform nematode resistance gene is flanked bilaterally by BNL3279_114 and *Fzg*^{lon}, 1.4 cM and 4.5 cM from the reniform nematode resistance gene, respectively; while BNL1066_156 and BNL836_215 are 2.0 and 4.4 cM from the resistance gene, respectively (figure part B on page 61). In 27 of the 28 family-derived reniform nematode resistant lineages taken to advanced backcross generations, the co-dominant marker BNL3279_114 remained present and was sufficiently closely linked to the R-gene for marker-based selection for reniform nematode resistance. It distinctly classified resistant plants from susceptible plants in backcrossed populations and separates homozygous resistant, heterozygous resistant and susceptible plants in self-pollinated populations. The only family in which progenies could not be screened with BNL3279_114 was HLA-B91, because it included only one lineage, and that one lost BNL3279_114 by way of recombination in an early generation. In the HLA-B91 lineage however, *Fzg*^{lon} was able to distinguish resistant and susceptible plants.

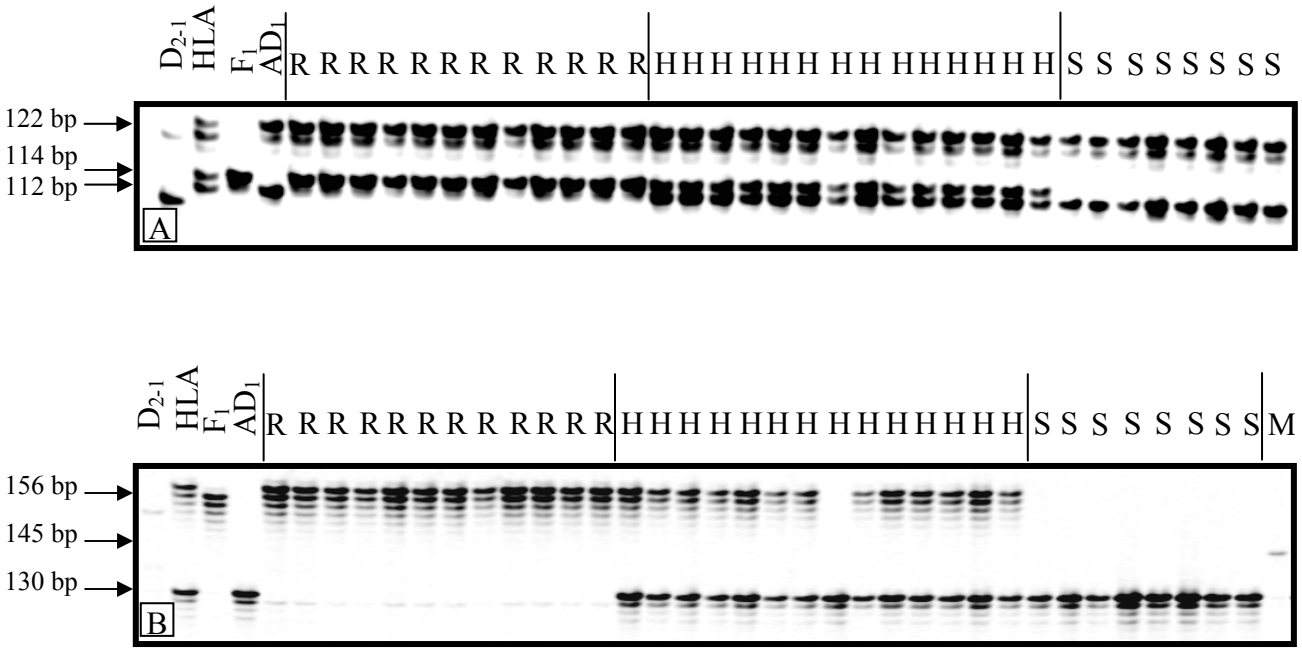

Figure 6. The PCR products amplified by the SSR primers BNL3279 (A), and BNL1066 (B) on a DNA panel consisting of 4 parents, 12 homozygous resistant, 14 heterozygous resistant and 8 susceptible BC6S1 plants from a BC6S1 population that was classified for reniform for nematode resistance. $D_{2\text{-}1}$, *G. armourianum*, a bridging species; HLA, resistant tri-species hybrid; F_1, *G. longicalyx*, the resistance donor species; $(AD)_1$, *G. hirsutum*, susceptible recurrent parent; R, resistant; S, susceptible; M, molecular weight standard.

Table 11A. Phenotypic classification data from backcrossed and self-pollinated plants in pedigreed lineages that were used to estimate linkage of the resistance gene to reniform nematodes with BNL3279_114 and BNL1066_156.

Family	BNL3279_114								BNL1066_215							
		NCO	XO	NCO	XO					NCO	XO	NCO	XO			
	Total	R:M[¶]	R:NM	S:NM	S:M	Total XO	%Recom	Notes[†]	Total	R:M	R:NM	S:NM	S:M	Total XO	%Recom	Notes
HLA-A2	25	15	1	9	0	1	4.0	ab	26	16	1	9	0	1	3.8	ab
HLA-A77	80 (14)[§]	45 (12)	1	32 (2)	2	3	3.8	ac	61 (14)	32 (12)	3	24 (2)	2	5	8.2	a[‡]
HLA-A83	41	29	0	12	0	0	0.0	a	41	29	0	12	0	0	0.0	a
HLA-A84	273 (63)	168 (50)	4	101 (13)	0	4	1.5	abc	267 (63)	164 (49)	6 (1)	97 (13)	0	6 (1)	2.2	ab[‡]
HLA-A85	57 (2)	29 (1)	1	27 (1)	0	1	1.8	ae[‡]	56 (2)	26 (1)	2	28 (1)	0	2	3.6	ae[‡]
HLA-A103	37 (9)	26 (8)	0	11 (1)	0	0	0.0	a	39 (9)	28 (8)	0	11 (1)	0	0	0.0	a
HLA-A122	36	25	0	11	0	0	0.0	ab	36	25	0	11	0	0	0.0	ab
HLA-A132	29	19	0	10	0	0	0.0	ab	29	19	0	10	0	0	0.0	ab
HLA-B4	17	14	0	3	0	0	0.0	a	17	14	0	3	0	0	0.0	a
HLA-B18	13	6	0	7	0	0	0.0	a	13	6	0	7	0	0	0.0	a
HLA-B21	15	10	0	5	0	0	0.0	ab	15	10	0	5	0	0	0.0	ab
HLA-B26	10	6	0	4	0	0	0.0	ae	10	6	0	4	0	0	0.0	ae
HLA-B34	19	11	0	8	0	0	0.0	ab	19	11	0	8	0	0	0.0	ab
HLA-B35	18	9	0	9	0	0	0.0	ae	18	9	0	9	0	0	0.0	ae
HLA-B45	15	9	1	5	0	1	6.7	a	15	9	1	5	0	1	6.7	a
HLA-B61	13	4	0	9	0	0	0.0	a	13	4	0	9	0	0	0.0	a
HLA-B75	13	5	0	8	0	0	0.0	a	15	6	0	9	0	0	0.0	a
HLA-B77	16	7	1	8	0	1	6.3	a	16	7	1	8	0	1	6.3	a
HLA-B81	16	8	0	8	0	0	0.0	ab	16	8	0	8	0	0	0.0	ab
HLA-B91	1	0	1	0	0	1	100.0	a[‡]	1	0	1	0	0	1	100.0	a[‡]
HLA-B99	14	10	0	4	0	0	0.0	a	13	9	0	4	0	0	0.0	a
HLA-B103	15	6	0	9	0	0	0.0	a	13	4	0	9	0	0	0.0	a
HHL-3	14	7	1	6	0	1	7.1	a	14	7	1	6	0	1	7.1	a
HHL-5	15	9	0	6	0	0	0.0	a	15	9	0	6	0	0	0.0	a
HHL-7	15	8	0	7	0	0	0.0	ae	15	8	0	7	0	0	0.0	ae
HHL-11	14	9	0	5	0	0	0.0	a	14	9	0	5	0	0	0.0	a
HHL-14	15	9	0	5	1	1	6.7	a	16	10	0	5	1	1	6.3	a
HHL-17	15	6	0	9	0	0	0.0	a	15	6	0	9	0	0	0.0	a
HLA-A110	4	0	0	4	0	0	0.0	a	4	0	0	4	0	0	0.0	a
HLA-A119	1	0	0	1	0	0	0.0	a	1	0	0	1	0	0	0.0	a
HLA-A123	42	4	0	38	0	0	0.0	a	41	4	0	37	0	0	0.0	a
HLA-A117	1	1	0	0	0	0	0.0	a	1	1	0	0	0	0	0.0	a
HLA-A4	16	1	0	15	0	0	0.0	a	18	1	0	17	0	0	0.0	a
HLA-A99	5	0	0	5	0	0	0.0	a	6	0	0	6	0	0	0.0	a
Plants analyzed	930 (88)	515 (71)	11	401 (17)	3	14	1.5		909 (88)	497 (70)	16 (1)	393 (17)	3	19 (1)	2.1	
No data	18	---- 11 ----		---- 7 ----		na	na		15	---- 11 ----		---- 4 ----		na	na	
Post-recom[‡]	36	0	23	13	0	23	63.9		60	0	36	24	0	36	60.0	
Total	984 (88)								984 (88)							

[†]Under each marker heading, the right-most column (Notes) indicates which markers were segregating, and if post-recombination data (see Table 12A and B) were excluded from the respective calculations: a, plants that arose from parents having the markers, *Fzg*^{lon} , BNL3279_114, BNL1066_156, and BNL836_215; b, plants that arose from parents having the markers, *Fzg*^{lon} , BNL3279_114, and BNL1066_156 only in coupling with R-gene; c, plants that arose from parents having the markers, *Fzg*^{lon} , and BNL3279_114 only in coupling with R-gene; d, plants that arose from parents having *Fzg*^{lon} only in coupling with R-gene; e, plants that arose from parents having the markers, BNL3279_114, BNL1066_156, and BNL836_215 only in coupling with R-gene.

[¶]%Recom, % recombination; R:M, resistant and marker present; R:NM, resistant and marker absent; S:NM, susceptible and marker absent; S:M, susceptible and marker present; NCO, no crossover; XO, crossover; na, not applicable.

[‡]Post-initial recombination data are not shown here and not used in the calculations of linkage, but are described in Table 12A and B.

[§]Number in the bracket indicates selfed plants.

Table 11B. Phenotypic classification data from backcrossed and self-pollinated plants in pedigreed lineages that were used to estimate linkage of the resistance gene to reniform nematodes with BNL836_215, and *Fzg*^{lon}.

	BNL836_215								*Fzg*^{lon}							
		NCO	XO	NCO	XO	Total				NCO	XO	NCO	XO	Total		
Family	Total	R:M[¶]	R:NM	S:NM	S:M	XO	%Recom	Notes	Total	R:M	R:NM	S:NM	S:M	XO	%Recom	Notes
HLA-A2	3	2	1	0	0	1	33.3	a[‡]	18	10	2	5	1	3	16.7	ab
HLA-A77	58 (14)[§]	26 (10)	6 (2)	24 (2)	2	8 (2)	13.8	a[‡]	40 (14)	26 (11)	4 (1)	9 (1)	1 (1)	5 (2)	12.5	ac
HLA-A83	41	29	0	12	0	0	0.0	a	25	21	0	4	0	0	0.0	a
HLA-A84	255 (63)	156 (49)	8 (1)	90 (12)	1 (1)	9 (2)	3.5	a[‡]	232 (45)	144 (33)	5 (1)	82 (11)	1	6 (1)	2.6	abc
HLA-A85	55 (1)	25	3 (1)	27	0	3 (1)	5.5	ae[‡]	33	24	2	6	1	3	9.1	ad[‡]
HLA-A103	38 (8)	27 (8)	1	9	1	2	5.3	a	29 (6)	22 (6)	2	5	0	2	6.9	a
HLA-A122	5	0	3	2	0	3	60.0	a[‡]	27	20	0	7	0	0	0.0	ab
HLA-A132	9	5	2	2	0	2	22.2	a[‡]	23	15	2	5	1	3	13.0	ab
HLA-B4	17	14	0	3	0	0	0.0	a	16	13	1	2	0	1	6.3	a
HLA-B18	13	6	0	7	0	0	0.0	a	13	7	0	6	0	0	0.0	a
HLA-B21	1	0	1	0	0	1	100.0	a[‡]	12	9	0	3	0	0	0.0	ab
HLA-B26	11	7	0	4	0	0	0.0	ae	1	0	1	0	0	1	100.0	a[‡]
HLA-B34	2	1	1	0	0	1	50.0	a[‡]	17	11	0	6	0	0	0.0	ab
HLA-B35	18	9	0	9	0	0	0.0	ae	0	0	0	0	0	0	0.0	[‡]
HLA-B45	16	10	1	5	0	1	6.3	a	13	10	0	3	0	0	0.0	a
HLA-B61	13	4	0	9	0	0	0.0	a	10	4	0	6	0	0	0.0	a
HLA-B75	14	5	0	9	0	0	0.0	a	12	4	1	7	0	1	8.3	a
HLA-B77	13	6	1	6	0	1	7.7	a	14	7	1	6	0	1	7.1	a
HLA-B81	1	0	1	0	0	1	100.0	a[‡]	13	7	0	6	0	0	0.0	ab
HLA-B91	1	0	1	0	0	1	100.0	a[‡]	15	10	0	4	1	1	6.7	ad
HLA-B99	14	9	1	4	0	1	7.1	a	13	9	1	3	0	1	7.7	a
HLA-B103	13	4	0	9	0	0	0.0	a	11	4	0	7	0	0	0.0	a
HHL-3	11	5	1	5	0	1	9.1	a	13	7	1	5	0	1	7.7	a
HHL-5	15	9	0	6	0	0	0.0	a	14	9	0	5	0	0	0.0	a
HHL-7	15	8	0	7	0	0	0.0	ae	3	1	2	0	0	2	66.7	a[‡]
HHL-11	14	9	0	5	0	0	0.0	a	12	9	0	3	0	0	0.0	a
HHL-14	16	10	0	5	1	1	6.3	a	14	10	0	4	0	0	0.0	a
HHL-17	15	6	0	9	0	0	0.0	a	13	6	0	7	0	0	0.0	a
HLA-A110	4	0	0	4	0	0	0.0	a	1	0	0	1	0	0	0.0	a
HLA-A119	1	0	0	1	0	0	0.0	a	0	0	0	0	0	0	0.0	
HLA-A123	43	4	0	39	0	0	0.0	a	7	0	0	7	0	0	0.0	a
HLA-A117	1	0	1	0	0	1	100.0	a	0	0	0	0	0	0	0.0	
HLA-A4	18	1	0	17	0	0	0.0	a	3	0	0	3	0	0	0.0	a
HLA-A99	6	0	0	6	0	0	0.0	a	1	0	0	1	0	0	0.0	a
Plants analyzed	770 (86)	397 (67)	33 (4)	335 (14)	5 (1)	38 (5)	4.9		668 (65)	419 (50)	25 (2)	218 (12)	6 (1)	31 (3)	4.6	
No data	23 (2)	----	17	----	----	6 (2)	----	na	na	276 (23)	----	78 (19)	----	----	198 (4)	----	na	na
Post-recom[‡]	191	0	115	76		0	115	60.2		40	0	23	17	0	23	57.5		
Total	984 (88)								984 (88)							

[†]Under each marker heading, the right-most column (Notes) indicates which markers were segregating, and if post-recombination data (see Table 12A and B) were excluded from the respective calculations: a, plants that arose from parents having the markers, *Fzg*^{lon} , BNL3279_114, BNL1066_156, and BNL836_215; b, plants that arose from parents having the markers, *Fzg*^{lon} , BNL3279_114, and BNL1066_156 only in coupling with R-gene; c, plants that arose from parents having the markers, *Fzg*^{lon} , and BNL3279_114 only in coupling with R-gene; d, plants that arose from parents having *Fzg*^{lon} only in coupling with R-gene; e, plants that arose from parents having the markers, BNL3279_114, BNL1066_156, and BNL836_215 only in coupling with R-gene.

[¶]%Recom, % recombination; R:M, resistant and marker present; R:NM, resistant and marker absent; S:NM, susceptible and marker absent; S:M, susceptible and marker present; NCO, no crossover; XO, crossover; na, not applicable.

[‡]Post-initial recombination data are not shown here and not used in the calculations of linkage, but are described in Table 12A and B.

[§]Numbers in the bracket indicates selfed plants.

Table 12A. Numerical distributions of resistance and marker(s) genotypes among backcross-derived plants that were classified as post-initial recombination events, i.e., among the progeny of parents heterozygous for a prior recombination product between the resistance gene and markers, BNL3279_114 and BNL1066_156.

Family	BNL3279_114							BNL1066_156						
	NCO[†]	XO	NCO	XO	Total	Total XO	%Recom[§]	NCO	XO	NCO	XO	Total	Total XO	%Recom
	R:M[‡]	R:NM	S:NM	S:M				R:M	R:NM	S:NM	S:M			
HLA-A85	0	14	7	0	21	14	66.67	0	14	7	0	21	14	66.67
HLA-B91	0	9	6	0	15	9	60.00	0	9	6	0	15	9	60.00
HLA-A77								0	11	8	0	19	11	57.89
HLA-A84								0	2	3	0	5	2	40.00
HLA-A2														
HLA-A122														
HLA-A132														
HLA-B21														
HLA-B34														
HLA-B81														
HLA-B26														
HLA-B35														
HHL-7														
Total	0	23	13	0	36	23	63.89	0	36	24	0	60	36	60.00

[†]Classification codes for recombination product classes: NCO, no crossover; XO, crossover.

[‡]Classification codes for phenotypic (genotypic) classes: R:M, resistant and marker present; R:NM, resistant and no marker (absent); S:NM, susceptible and no marker (absent); S:M, susceptible and marker present.

[§]%Recom, percent recombination.

Table 12B. Numerical distributions of resistance and marker(s) genotypes among backcross-derived plants that were classified as post-initial recombination events, i.e., among the progeny of parents heterozygous for a prior recombination product between the resistance gene and markers, BNL836_215 and *Fzg*^{lon}.

Family	BNL836_215							*Fzg*^{lon}						
	NCO[†]	XO	NCO	XO	Total	Total XO	%Recom[§]	NCO	XO	NCO	XO	Total	Total XO	%Recom
	R:M[‡]	R:NM	S:NM	S:M				R:M	R:NM	S:NM	S:M			
HLA-A85	0	14	7	0	21	14	66.67	0	4	1	0	5	4	80.00
HLA-B91	0	9	6	0	15	9	60.00							
HLA-A77	0	11	8	0	19	11	57.89							
HLA-A84	0	7	8	0	15	7	46.67							
HLA-A2	0	14	9	0	23	14	60.87							
HLA-A122	0	22	9	0	31	22	70.97							
HLA-A132	0	12	8	0	20	12	60.00							
HLA-B21	0	9	5	0	14	9	64.29							
HLA-B34	0	10	8	0	18	10	55.56							
HLA-B81	0	7	8	0	15	7	46.67							
HLA-B26								0	5	3	0	8	5	62.50
HLA-B35								0	9	8	0	17	9	52.94
HHL-7								0	5	5	0	10	5	50.00
Total	0	115	76	0	191	115	60.21	0	23	17	0	40	23	57.50

[†]Classification codes for recombination product classes: NCO, no crossover; XO, crossover.

[‡]Classification codes for phenotypic (genotypic) classes: R:M, resistant and marker present; R:NM, resistant and no marker (absent); S:NM, susceptible and no marker (absent); S:M, susceptible and marker present.

[§]%Recom, percent recombination.

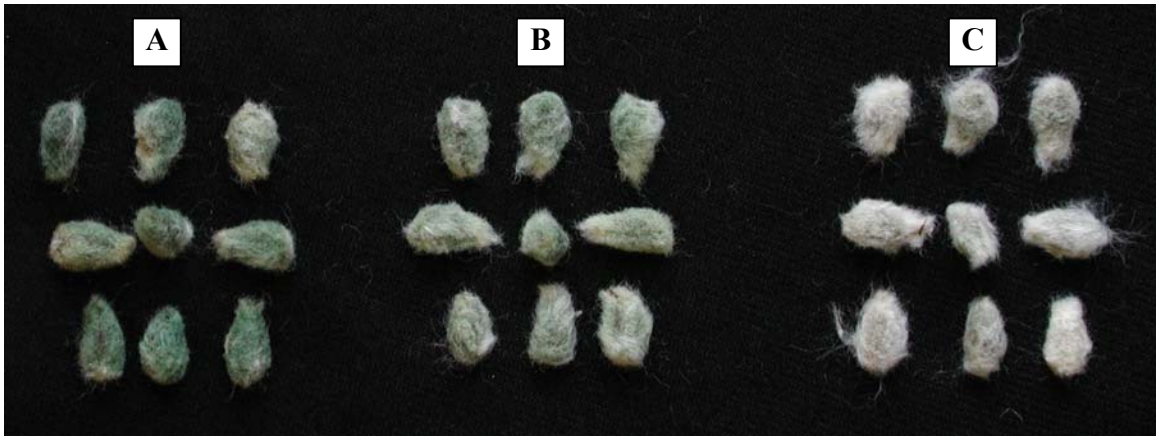

Figure 7. Ginned seed from (A) homozygous reniform nematode-resistant plant, exhibiting dark green fuzz (*Fzg*^{lon}*Fzg*^{lon}), (B) heterozygous resistant plant, exhibiting light green fuzz (*Fzg*^{lon}*fzg*) and (C) reniform nematode-susceptible plant, exhibiting white fuzz (*fzgfzg*), obtained after selfing reniform nematode-resistant BC6F1 plant.

In many situations, the reliability of linkage estimation is determined by the accuracy of phenotypic classification. To determine this reliability, phenotypic classifications of some plants among self-pollinated progeny families were confirmed by testcrossing the plants and then conducting nematode bio-assays on test-cross progeny (Robinson et al., 2007). One such set that was included consisted of 34 BC6S1 plants derived from a highly resistant BC6F1 plant from family HLA-A84, where the reniform nematode resistance status of 28 plants was confirmed by quantifying testcrosses (Fig. 4). In this set, BNL3279_114 and *Fzg*^{lon} markers differentiated homozygous resistant, heterozygous resistant and susceptible plants in 1:2:1 ratio respectively ($\chi^2 = 2.0$, $P = 0.37$) as predicted by the reniform nematode bio-assays (Fig. 6; 7), thus supporting the conclusion that the resistance gene is controlled by a single dominant gene or haplotype (Robinson et al., 2007). On the same set, BNL1066_156 (Fig. 6) and BNL836_215 had one and three recombinants, respectively. Besides these 34 BC6S1 plants, all three linked SSR markers were also screened on 12 BC1S1 and 42 BC3S1 resistance-classified plants. Seed fuzz color (*Fzg*^{lon}) was scored on 6 BC1S1 and 25 BC3S1 plants, where the resistance status of 14 BC3S1 plants was confirmed by analyzing testcrosses via nematode bio-assays (Fig. 4). BNL3279_114 and BNL1066_156 clearly identified homozygous resistant, heterozygous resistant and susceptible plants in agreement with

the nematode bio-assays in all 14 of the 42 BC3S1 plants, while BNL836_215 and *Fzg*^{lon} had two recombinants each.

Table 13. Susceptible plants used in calculating linkage between the linked markers and the reniform nematode-resistance locus.

	BNL3279_114	BNL1066_156	BNL836_215	*Fzg*^{lon}
Mapping population size	373	364	307	212
Pre-parental recombinants	13	22	75	16
Plants with no data	4	4	8	162
Total plants	390	390	390	390
Recombinants	3	3	5	6
% Recombination	0.8	0.82	1.63	2.83

While phenotyping the testcrosses to confirm the status of a plant remains an ideal solution to prevent any misclassification, it often is delimited by the number of testcrosses that can be performed, especially when the size of the population is relatively large as is seen in this project which has 984 classified plants. To overcome this limitation, a map based only on the susceptible plants ("susceptible based-map") was constructed to get more accurate linkage estimates since the susceptible data would be more reliable than the resistance data as described in the discussion. Combined analysis of 2,373 plants that were phenotyped for reniform nematode led to a bimodal distribution involving resistant and susceptible classes, with a median plant value of 33% (Robinson et al., 2007). The median plant value of 33% obtained in this companion study was used as a criterion in selecting susceptible plants. Accordingly, 374 susceptible backcrossed and 16 susceptible selfed plants with %DP-16 values more than 33% were selected from the complete data set described in Table 9 for determining linkage. Due to either *pre-parental* recombination events between the resistance loci and the 3 linked molecular-markers or failure to amplify DNA, the actual mapping population used for creating susceptible based-map was 373, 364, and 307 for 3279_114, 1066_156, and 836_215, respectively (Table 13). Because either some of the susceptible segregates were discarded before the fuzz data were collected or green fuzz, which is a

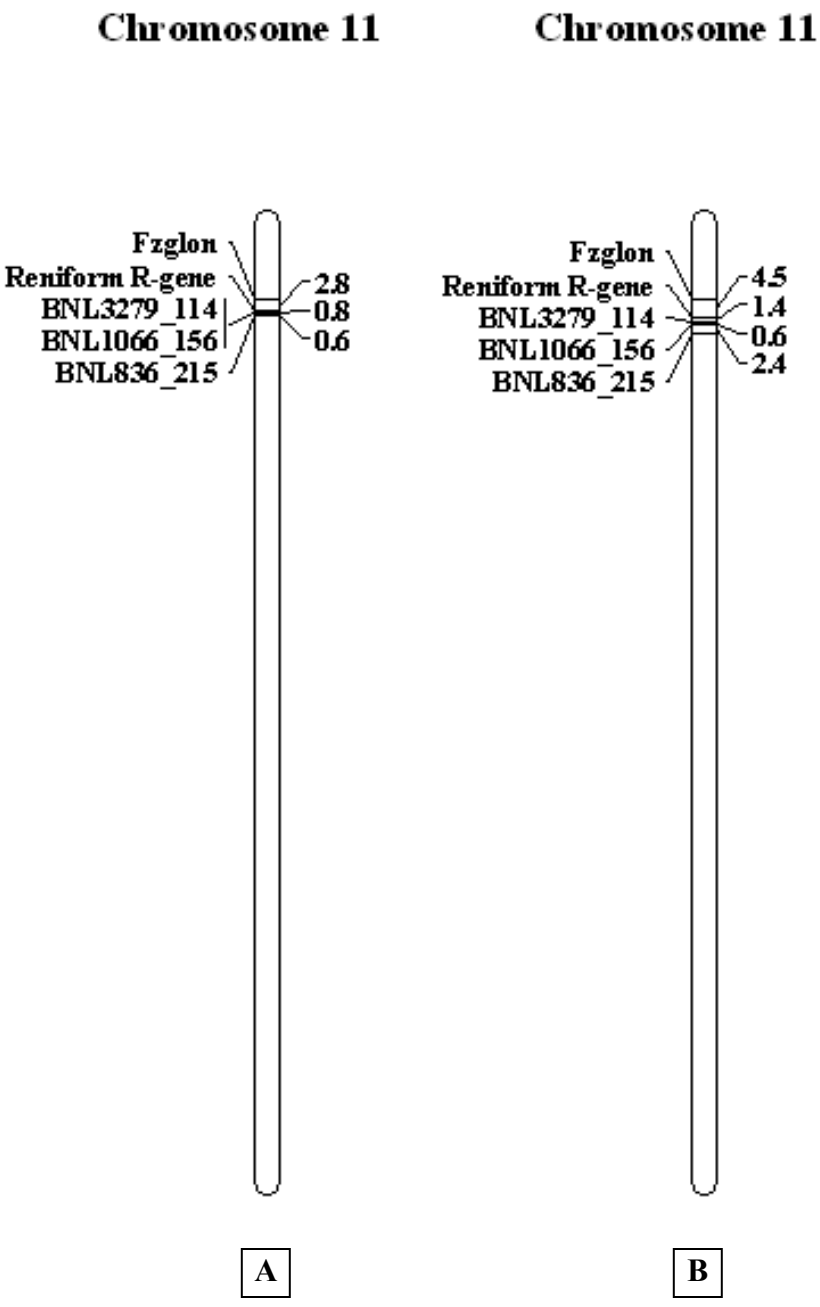

Figure 8. Map position of the gene conferring resistance to reniform nematode on chromosome 11 (A) based only on the susceptible plants that have %DP-16 values more than 33% and (B) based on complete set, including resistant and susceptible plants.

seed trait was not expressed because the resistant plant were not used as a female parent, the fuzz data were collected only on 216 backcross and 12 selfed plants, leaving other 162 plants unclassified in the susceptible-based mapping population. *Pre-parental* recombination in 16 of the backcrossed plants led to 200 backcrossed and 12 selfed plants that were used in calculating linkage of *Fzg*^{lon} to the resistance locus (Table 13). Linkage estimates based on the susceptible based-map did not differentiate markers, BNL3279_114 and BNL1066_156 from each other, mapping them 0.8 cM away from the resistant locus, while, BNL836_215 and *Fzg*^{lon} were 1.4 and 2.8 cM distance from the resistant locus, respectively, where *Fzg*^{lon} was on the other side of the resistant locus relative to the 3 molecular markers (Fig. 8A).

Discussion

Although the germplasm breeding materials here were developed for breeding purposes, they were highly amenable to the three-panel strategy employed here, which entailed stepwise application of progressively larger panels for screening progressively fewer loci. The efforts culminated in association of molecular markers with the responsible resistance gene or haplotype, mapping them to a specific chromosome, and rendering the trait amenable to marker-assisted selection, as well as further mapping.

Classification data from backcross and self progenies indicated that 12 of the 34 families (HLA-A83, HLA-B18, HLA-B61, HLA-B103, HHL-5, HHL-11, HHL-17, HLA-A110, HLA-A119, HLA-A123, HLA-A4, and HLA-A99) included no products from recombination between the alien segment carrying the resistance locus and the 4 linked markers (Table 11). Among the other families, there were 14, 19, 38 and 31 recombinants between the resistance gene and BNL markers 3279_114, 1066_156, 836_215, and *Fzg*^{lon}, respectively. Interestingly, the population of recombinants was skewed heavily toward the resistant class, specifically 11:3, 16:3, 33:5 and 25:5, respectively. However, among the overall population of nonrecombinants in these same families, the ratio of resistant:susceptible plants was also skewed (Table 11), confounding the interpretation that more recombinants were observed in the resistant

class. Nevertheless, we do suspect that some of the recombinants from resistant group, especially the ones that had lost all 4 markers, are in fact susceptible and were misclassified due to environmental errors. This is supported by performing testcross analysis on five putatively recombinant plants that lack all four markers but were classified as highly resistant based on nematode assays, which yielded resistance scores ranging from 1-4% of the susceptible control. Progeny from the testcross were susceptible, indicating the parents had indeed been misclassified, as suggested by the marker genotypes. This interpretation was also supported by correct assignment of 3 out of 34 BC6S1 plants that were initially misclassified as resistant via bio-assays but were later confirmed via test cross evaluation as susceptible as predicted by BNL3279_114 (Robinson et al., 2007; Fig. 6). This deduction is concordant with the finding that misclassification of resistance phenotypes is higher among susceptible types and decreased to zero as resistance increased because observed incidence of misassignment for Deltapine 16 (susceptible control), GB-713 (resistant control), and *G. longicalyx* (immune) was 5.4, 3.6, and 0.0%, respectively (Robinson et al., 2007). The examples given above indicate that the possibility of misclassifying a susceptible plant as resistant is higher than misclassifying a resistant plant as susceptible, thus we emphasize the linkage analysis be based only on the susceptible plants and the map is referred to as "susceptible based-map". Linkage analysis based on the susceptible plants indicate that BNL3279_114 and BNL1066_156 which are mapped together to be the most tightly linked (0.8 cM) to the gene conferring resistance to reniform nematode. Though the susceptible based-map did not differentiate BNL3279_114 and BNL1066_156, confirmed resistant BC6S1 plants based on the testcross evaluation have differentiated these two markers, making BNL3279_114 the most tightly linked-marker (Fig. 6). Being codominant relative to most if not all other Upland germplasm, BNL3279_114 has the capability to distinguish resistant plants from susceptible plants in backcross generations and to identify homozygous resistant, heterozygous resistant and susceptible plants in self generations. Association of Fzg^{lon} and BNL3279_114 enabled the resistance locus to be flanked by tightly-linked co-dominant markers, thus making the

screening process more efficient and reliable. The markers BNL3279_112 and BNL3279_122 that are amplified by primer set BNL3279, as well as green fuzz from *G. barbadense* have been mapped on the D02 linkage group by Lacape et al. (2003) and Nguyen et al. (2004). Although a genomic sequence amplifiable with primer set BNL3279 was mapped to the D02 group, the association of BNL3279_114 with markers BNL1066_156, and BNL836_215 from A03 linkage group indicates that the gene for reniform nematode resistance is located on the A03 linkage group (Chromosome 11) and that primer BNL3279 enables amplification of a homeologous gene in the A03 group.

A gene for root-knot nematode resistance (*rkn1*) from Acala NemX was recently mapped to the A03 linkage group of cotton (Wang et al., 2006a). Tight linkage was discovered between SSR marker CIR316_221 and *rkn1*, about 2.1-3.3 cM. Screening of primer set CIR316 on four parents (*G. longicalyx*, HLA tri-species hybrid, *G. armourianum*, and *G. hirsutum*) used in this study showed polymorphism between the resistant group and susceptible group with a band present in susceptible group and absent in resistant group (repulsion phase). Screening of primer set CIR316 on 88 self-progeny (12 BC1S1, 42, BC3S1, and 34 BC6S1) (Table 9; Fig. 4) derived from the reniform nematode resistant families HLA-A77, HLA-A84, HLA-A85, and HLA-A103 showed no linkage to the reniform nematode resistance gene. Because Acala NemX was used as one of the parents in early and late generations in some of these families, it will likely be possible to combine NemX-derived root-knot nematode resistance and *G. longicalyx*-derived reniform nematode resistance into one genotype using MAS for both traits, i.e., selection for BNL316_221 and BNL3279_114, or similar sorts of markers.

The availability of flanking, MAS-suitable markers that are tightly linked to the reniform nematode resistance gene will encourage plant breeders to transfer the reniform nematode resistance gene into different cultivars without having to go through cumbersome phenotypic screening at each segregating generation, saving time and resources. The map position data can be used to identify markers that are linked even more tightly to the resistance gene and/or more amenable to high-throughput technologies. Growers will benefit from the improvements in their economic returns,

and society from reduced nematicide usage. Map-based cloning and identification of this resistance gene seems desirable for basic research and potential commercial applications.

CHAPTER IV

INTROGRESSION OF EXOTIC GERMPLASM FROM *Gossypium longicalyx* AND *G. armourianum* INTO *G. hirsutum*

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the major agricultural commodities produced worldwide in tropical, subtropical, and temperate latitudes (Lee, 1984) with an estimated total world production of 114 million bales in 2005-06, an increase of 16 million bales in last 4 years (Burr et al., 2006). The United States followed only after China in lint production for the year 2005, and contributed 20% of the world's total production (Burr et al., 2006). However, there has been a declined progress in developing improved cultivars for yield and fiber quality for the past 15 years due in part to lack of genetic diversity in the commercially used cotton cultivars (Meredith, 2000; Lewis, 2001). Genetic diversity studies based on isozyme and molecular markers suggests comparatively low levels of genetic diversity in cotton cultivars (Wendel et al., 1992; Pillay and Myers, 1999; Khan et al., 2000; Abdalla et al., 2001; Iqbal et al., 2001; Rungis et al. 2005). This lack of genetic diversity along with the use of relatively narrow genetic pool for variety development (van Esbroeck et al., 1998; Iqbal et al., 2001) has also raised concerns about genetic vulnerability of cotton to biotic and abiotic stresses (Bowman et al., 1996). Efforts were thus mounted to increase in the genetic base of cotton by utilization of wild, unused germplasm from both diploid and tetraploid cotton species.

The cotton genus, *Gossypium*, consists of 50 species (Fryxell, 1992), including 45 diploid species, which are classified into 8 groups, designated as A-G, and K and 5 tetraploid species, which are designated as AD (Endrizzi et al., 1985; Stewart, 1995). However, only 2 of the diploid (A-genome species, *G. herbaceum* and *G. arboreum*) and 2 of the tetraploid species (AD genome, *G. hirsutum* and *G. barbadense*) have been domesticated (Fryxell, 1992), leaving the remaining species as potential sources of novel

genetic traits for cultivar development (Stewart, 1995). While there remains tremendous potential for introgressing valuable traits from the unused tetraploid germplasm, including breeding stocks, obsolete cultivars, land races and feral accessions, and wild tetraploid species (Stewart, 1995), this work focuses on the introgression potential of the wild diploid species.

Ploidy differences make it difficult to hybridize diploid and tetraploid species, and low rates of recombination between alien and commercial tetraploid chromosomes in a hybrid are two common obstacles that make the utilization of alien species very challenging for cotton improvement. While ploidy remains a big obstacle in utilization of the diploid germplasm, there have been successful cases of novel trait introgression from exotic, diploid species into tetraploid cotton using techniques that involve chromosome doubling using colchicine or other chemicals (Fryxell, 1976; Stewart, 1995; Brubaker et al., 1999; Bell and Robinson, 2004). Some of the traits that have been introgressed from exotic, diploid species into tetraploid cotton include reniform nematode resistance gene from *G. longicalyx* (Robinson et al., 2007); bacterial blight resistance genes from *G. arboreum*, *G. herbaceum*, and *G. anomalum* (Endrizzi et al., 1985); cytoplasms and restorer factors of *G. harknesii* (Meyer, 1975) and *G. trilobum* (Stewart, 1992) conditioning cytoplasmic male sterility; resistance to jassid due to hairiness transferred from *G. raimondii* (Stewart, 1995); insect resistance attributed to D2 smoothness from *G. armourianum* (Meyer, 1957); a gene controlling terpenoid aldehyde methylation from *G. sturtianum* (Bell, 1984; Bell et al., 1987; Bell et al., 1994); higher fiber strength from *G. thurberi* (Harrell and Culp, 1979); fiber quality parameters (Ndungo et al., 1988).

Besides these successful introgression efforts, several potential traits have been identified in exotic material but are yet to be introgressed into *G. hirsutum*. Several accessions of A-genome have been identified to possess disease and pest resistance traits, including resistance to nematodes (Carter, 1981; Yik and Birchfield, 1984), fungal and bacterial pathogens (Mathre and Otta, 1967; Bollenbacher and Fulton, 1971; Meredith, 1991), and insects (Benedict et al., 1987; Stanton et al., 1992; Uthamasamy,

1995). Other potential morphological traits, possessed by exotic species that could be introgressed into cotton breeding programs includes, caducous bracts from *G. armourianum*, *G. harknessii*, *G. turneri*, and G-genome species to potentially reduce trash content of harvested cotton, glandless seed/glanded plant trait from Australian species, double palisade layer from *G. armourianum*, *G. harknesii*, and *G. turneri* as a potential drought resistance mechanism and several other traits summarized by Stewart (1995).

Most previous work on introgression of wild diploid germplasm has focused on introgressing disease and pest resistances, and a few other traits as discussed above. The potential of these wild species in improving multigenic traits, such as fiber quality, and yield parameters has not been exploited intensively by breeders due to many factors, but mainly due to undesirable linkages (Miller and Rawlings, 1967; Meredith and Bridge, 1971), fertility issues, and time constraints (Ndungo et al., 1988). The most notable example of the wild, diploid germplasm utilization for fiber quality improvement is of introgressing fiber strength from *G. thurberi* into *G. hirsutum* (Miller and Rawlings, 1967; Meredith and Bridge, 1971; Harrell and Culp, 1979). Following this success, analogous diploid introgression efforts for fiber quality improvement were mounted in Europe (Ndungo et al., 1988).

Fiber quality evaluation of four allohexaploids between *G. hirsutum* and four diploid species (*G. australe*, *G. aridum*, *G. stocksii*, and *G. longicalyx*) by Demol et al., (1978) led them to conclude that *G. australe* hybrids had higher ginning outturn, *G. aridum* hybrids had higher fiber strength but decreased elongation and fiber length, *G. stocksii* hybrids had higher elongation, fiber strength and ginning outturn, and *G. longicalyx* hybrids had increased fiber fineness and strength when compared with standard cultivars. Evaluations of allohexaploids for drought resistance potential showed that *G. anomalum* hybrids had a high stomatal transpiration combined with the lowest cuticular transpiration and seemed the most promising for drought resistance (Demol et al., 1975). They also showed that the relative turgidity during drying in the detached leaves was higher for *G. anomalum*, *G. sturtianum*, and *G. longicalyx* than *G.*

hirsutum, and also concluded that *G. longicalyx* had the highest relative turgidity at wilting point, thus providing insight into the drought resistant potential of the wild diploids that could be exploited.

Concerns about the genetic vulnerability of major crop species due to lack of genetic variability in different crop species has been well documented (Anonymous, 1972), thus demanding the utilization of the exotic germplasm to broaden the genetic bases of crops. Since then, there has been some efforts towards utilization of the exotic germplasm in breeding programs, leading different research groups to develop methods of incorporating favorable genes controlling quantitative traits from exotic germplasm into adapted germplasm pools (Meredith and Bridge, 1971; Lawrence and Frey, 1975; Hallauer, 1978; Kenworthy, 1980; Hoffbeck et al., 1995). The optimum population for cultivar development is one with a high mean and a large genetic variance. While, increased genetic variation in breeding populations can be readily achieved from exotic germplasm introgression (Kenworthy, 1980), one of the biggest problems in these introgressed populations is low initial means, due to the lack of adaptability of the exotic germplasm (Bridges and Gardner, 1987). Field data (Hallauer, 1978; Kenworthy, 1980; Hoffbeck et al., 1995) and computer simulation studies (Bailey, 1977; Dudley, 1982; Ho and Comstock, 1980) suggest backcrossing as a possible solution to the problem of low initial means, and they also discuss the number of backcrosses that will be required to develop a foundation population for initiating the selection process to achieve means, higher than the best parent by accumulating favorable alleles from both exotic and adapted parents. Dudley (1982), and Bridges and Gardner (1980) suggest that at least one backcross to an adapted parent is needed prior to initiation of selection if the adapted parent has more loci with favorable alleles than the exotic parent, and the more diverse the parents in terms of loci with favorable alleles, the more generations they should be backcrossed to the best parent in order to develop the foundation population to initiate selection for a trait.

Linkage has been considered as one of the biggest concerns in introgressing traits from unadapted germplasm (Miller and Rawlings, 1967; Meredith and Bridge, 1971;

Nelson, 1973; Lonnquist, 1975). These authors recommend random-mating following adapted x exotic crosses as a way to break or reduce unfavorable linkages through genetic recombination by promoting crossing-over during meiosis. Fehr and Clark (1973) suggested at least one random-mating between the selections in a recurrent selection procedure to develop populations and cultivars with higher diversity and productivity. Since random-mating is expected to disrupt combinations of genes but not affect gene frequencies of the populations, changes in the mean performance of a population after random-mating may be due to epistatic gene action involving linkages, natural selection during the creation of successive random-mated generations, and different environments in which the seed for the populations was produced, while changes in the genetic variance would indicate a reduction of linkage disequilibrium (Miller and Rawlings, 1967; Meredith and Bridge, 1971; Hoffbeck et al., 1995).

In cotton, Miller and Rawlings (1967) and Meredith and Bridge (1971) studied the effects of random-mating in combining high fiber strength from strains derived from a tri-species hybrid (*G. thurberi* Tod. x *G. arboreum* L. x *G. hirsutum* L.) developed by Beasley in 1940 and high yield from tetraploid cotton, *G. hirsutum*. Both studies observed a decrease in negative genetic correlations between yield and fiber strength after random-mating. Miller and Rawlings (1967) further discuss that if loci affecting two traits are linked in the original parents, which probably would be the case in their wide cross introgression study, and if coupling and repulsion phases of such linkages are in disequilibrium, then one would expect that the correlation arising from such linkages would shift towards the equilibrium after random-mating. A similar pattern was observed in their study where the genetic correlation had shifted from -0.69 in the original population to -0.35 in the random-mated population and was observed to be shifting towards the average correlation of -0.20 (Miller et al., 1958) at equilibrium. This suggests that random-mating was successful in reducing the negative correlations and thus increasing the prospects of combining these traits in the future.

After comparing fiber quality and yield-related traits between the original and random-mated populations, Meredith and Bridge (1971) observed a reduction in genetic

variances in two (lint yield and seed index) of the seven traits studied, while Miller and Rawlings (1967) observed the reduction in six (lint yield, lint%, weight per boll, fiber strength, fiber elongation, and fiber fineness) of the seven traits studied. Meredith and Bridge (1971) attributed this difference in results to more extensive coupling phase linkages in the Miller and Rawlings (1967) study, due to higher differences between the two parents for the studied traits and also due to the fact that the donor parent, TH-131 in Miller and Rawlings (1967) study was more closely related to the original, tri-species hybrid in terms of the alien genome content since it had fewer backcrosses to *G. hirsutum* and had less selection for agronomic acceptability as compared to the Pee Dee-65 strain used by Meredith and Bridge (1971).

However, other studies in various crops found random-mating to be of very less value or not advantageous at all. In maize, Hoffbeck et al. (1995) observed no trend of changes in variances, means, and correlations between the traits after random-mating while comparing one, three and five random-matings within each of the three backcross generations (BC1, BC2, and BC3) created by crossing an adapted maize and a semi-adapted line derived from different land races of maize. In comparing the F2 generation with a random-mated generation (F2-syn8) from B73 x B84 cross of maize, Lamkey et al., (1995) observed no significant increase in the genetic variances of the traits after random-mating. In wheat, comparison of generations that had undergone one through four levels of random-mating with that of a non-random-mated generation created from adapted x adapted crosses showed change in the genetic variances in only two of the traits, while the other four traits had fluctuation in the genetic variances across the random-mated levels (Altman and Busch, 1984). Although change in variances of two of the six traits was observed suggesting the effect of random-mated in reducing the linkage disequilibrium for those traits, high standard errors associated with the genetic variances indicated lack of precision in the data, which led Altman and Busch (1984) conclude that random-mating did not enhance the recombination.

The potential of the wild diploid cotton, *G. longicalyx*, as an immune source for reniform nematode has been well documented (Yik and Birchfield, 1984; Stewart and

Robbins, 1996). Transfer of reniform nematode resistance from diploid *G. longicalyx* into tetraploid *G. hirsutum* was achieved through the creation of two tri-species hybrids, HLA (*G. hirsutum* + *G. longicalyx* + *G. armourianum*), and HHL (*G. hirsutum* + *G. herbaceum* + *G. longicalyx*), and then recurrent backcrossing of this tri species hybrids to *G. hirsutum* (Bell and Robinson, 2004; Robinson et al., 2007). While the resistance potential of *G. longicalyx* has been successfully introgressed into *G. hirsutum*, its fiber quality contribution to *G. hirsutum* remains to be evaluated. A few studies in the past have indicated that *G. longicalyx* has potential for improving fiber quality traits. Demol et al. (1978) reported that allohexaploids involving *G. longicalyx* and *G. hirsutum* exhibited high fiber strength and fiber fineness, and inferred that these fiber related traits could be contributed by *G. longicalyx*. Robinson et al., (2007), while comparing, homozygous, reniform-nematode resistant and susceptible $BC6S_2$ plants, derived from a single resistant family (HLA-A84) with commercial cultivar, DP-458 B/RR, observed significantly higher fiber strength in the resistant and susceptible plants, and significantly lower micronaire in the resistant plants, than in DP 458 B/RR. Though, these studies had pointed out some of the traits potentially contributed by *G. longicalyx*, they were either not analyzed at the tetraploid level (Demol et al., 1978) or were tested in an advanced backcross generation (Robinson et al., 2007) where there is less representation of the wild species genome.

Another wild, diploid used in the breeding scheme to develop HLA, tri-species hybrid described above is *G. armourianum* which is classified under the D-genome ($D_{2\text{-}1}$) of cotton (Fryxell, 1992). Though the fiber quality potential of *G. armourianum* has not been evaluated extensively in the past, Zhou et al. (2003) have documented its potential in the lines developed from an inter-specific hybrid between *G. armourianum* and *G. hirsutum*. They reported that the introgressed lines had fiber strength of 36.2 g tex^{-1}, fiber length of 33.5 mm, and micronaire value of 4.29 in the introgressed lines and concluded that the introgressed lines performed better than the elite Upland cotton used as a control in their study. Moreover, Jiang et al. (1998) with the use of quantitative trait locus (QTL) analysis reported that a majority of the loci affecting fiber quality and yield

in *G. hirsutum* are found in the D-subgenome of *G. hirsutum*. These lines of evidence indicate that beneficial alleles contributing to fiber quality traits could be present in *G. armourianum* but this possibility remains to be explored. The introgression of *G. armourianum* in the HLA tri-species hybrid would provide an opportunity to explore the potential of this wild D-genome species.

The main objectives of this study, which used HLA-tri species hybrid mentioned above (Fig. 1; Bell and Robinson, 2004) to create populations, are [1] to evaluate fiber quality traits and yield potential of the wild species-introgressed populations at BC1, BC2, and BC3 level generations by comparing with commercial cultivars, FM-832, PSC-355, Acala Nemx, and genetic standard, TM-1, and [2] to determine the effetcs of random-mating on the generation means and variances.

Materials and Methods

HLA Tri-species Hybrid

The breeding scheme used to create the HLA tri-species hybrid (Bell and Robinson, 2004) is explained in Fig. 1. *G. hirsutum* inbred 'TM-1' was used as the female parent in crosses with *G. longicalyx*. The product was a sterile triploid plant, which upon chromosome-doubling by colchicine treatment yielded a fertile hexaploid of the genomic composition $2[(AD)_1F_1]$, which was crossed with *G. armourianum* to obtain a tetraploid of the genomic composition $[(AD)_1F_1D_{2\text{-}1}]$. The balance between genomic groups in this hybrid, specifically AFDD, was expected to allow for high meiotic homology (A-F and D-D), recombination and fertility.

Development of the Research Material

Two sets of breeding materials, HLA-A and HLA-B (Robinson et al., 2007) that were developed asynchronously from backcrosses with the tri-species hybrid, HLA were used to derive generations used in this experiment. The diagrammatic representation of the research material development is outlined in Fig. 9. The major difference between the HLA-A and HLA-B sets lies in backcross parents used in deriving BC1F1 and BC2F1

plants. The agronomic male parents of the BC1F1 plants derived from HLA-A set included *G. hirsutum* cvs., Paymaster 1220 RR, Stoneville 373, Suregrow 125, and Tamcot Sphinx along with known root-knot nematode resistant *G. hirsutum* genotypes including Auburn M-315, Auburn 623 RNR, Acala NemX, and Stoneville LA887, while the male parent of the BC1F1 plants derived from HLA-B set was Acala NemX. The male parents of HLA-A derived BC2F1 plants included root-knot nematode resistant genotypes including Acala NemX, Auburn M-315, Auburn 623 RNR, Stoneville LA887, Pima S2, and several experimental breeding lines descended from sibling lines released by Jones et al. (1988), while HLA-B derived BC2F1 plants had TM-1 as their male parent.

Refer to Table 14 for the summary of the plant materials used in creating the introgressed generations used in this experiment. One hundred-nineteen BC2F1 and 25 BC1F2 plants from HLA-A set were used in creating 11 generations used in this experiment (Fig. 9). These 25 BC1F2 plants were random-mated with an average of three crosses per female (range 1-6) to create BC1F2R1 seed, while BC1F3 seed was created by harvesting open-pollinated seed from these plants. In 2004, seeds from 7 of the 25 BC1F2 plants were planted on the F&B field in College Station, which led to BC1F3 plants. Since natural outcrossing of plants on the F&B field in College Station is *ca*. 50% mainly due to honey bees, the open-pollinated seed harvested from the F&B field will be assigned a "U" letter, referring to uncontrolled pollinated generation than the normally assigned "F" (filial) letter. For e.g., the open-pollinated seed harvested from 39 BC1F3 plants grown on the F&B field will be called "B1F3U1" instead of "BC1F4". Five BC1F3 plants were random-mated with an average of one cross (range 1-3) per female to create BC1F3R1 seed. One hundred-nineteen BC2F1 plants were random-mated with an average of two crosses (range 1-6) per female to create BC2F1R1 seed, while BC2F2 seed was created by open-pollinating 66 BC2F1 plants. Seeds from 66 of these BC2F1 plants were planted on the F&B field to create BC2F2 population. These BC2F2 plants grown on the "F&B field" were allowed to open-pollinate to create "BC2F2U1" seed as well as random-mated using 160 plants with an average of one

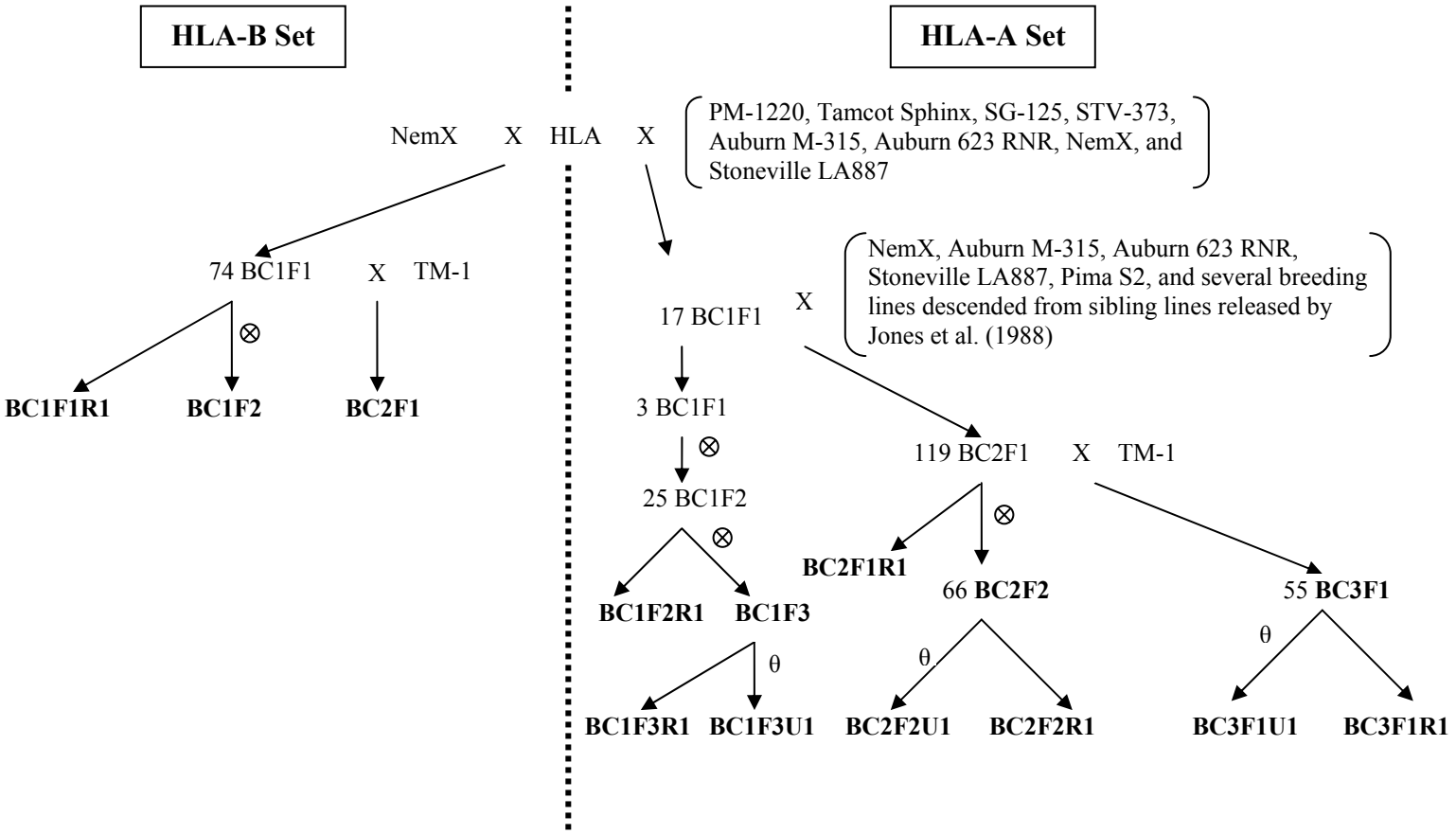

Figure 9. Breeding scheme used to create HLA-derived introgressed generations that were evaluated in the experiment. ⊗, open-pollinated seed harvested to create self generation. θ, uncontrolled pollination; Generations, highlighted in bold are the ones evaluated in this experiment. NemX, Acala NemX; PM, Paymaster; SG, Suregrow; STV; Stoneville variety; RNR, Root-knot resistant; TM, Texas marker. HLA, tri-species hybrid (*G. hirsutum* + *G. longicalyx* + *G. armourianum*).

Table 14. Summary of the plant material used in creating introgressed generations.

Generation	HLA Set	Number of Parents	Number of Crosses	Seeds Planted (Average)[†]	Seeds Planted (Range)[‡]
BC1F2R1	A	25	86	3	1-5
BC1F3[§]	A	14/25	NA	13/8	1-20/1-44
BC1F3R1	A	5 (7)[¶]	8	10	2-14
BC1F3U1	A	39 (7)	NA	5	1-6
BC2F1R1	A	119	356	1	NA
BC2F2	A	66	NA	3	1-5
BC2F2R1	A	160 (66)	178	1	1-2
BC2F2U1	A	226 (66)	NA	1	1-2
BC3F1	A	55	NA	3	1-4
BC3F1R1	A	227 (55)	225	1	NA
BC3F1U1	A	306 (55)	NA	1	NA
BC1F1R1	B	74	256	1	1-3
BC1F2[§]	B	10/31	NA	8/7	1-22/1-14
BC2F1	B	67	NA	2	1-4

[†]Average number of seeds planted per cross and per female parent for random-mated and non-random-mated generations, respectively.

[‡]Range of seeds planted per cross and per female parent for random-mated and non-random-mated generations, respectively.

[§]Variation in the number of parents, average seed per parent, and the range of seed planted in 2005/2006.

[¶]Number in parenthesis indicates number of parents used in creating the base population (number not in parenthesis) in 2004 that eventually was used for creating the introgressed generations used in this study.

NA, Not applicable.

cross per female (range 1-5) to create BC2F2R1 seed. Fifty-five of these BC2F1 plants were cross-pollinated with *G. hirsutum* cv. TM-1 to create BC3F1 seed, which was planted on F&B field in 2004. Two hundred twenty-seven of these BC3F1 plants grown on the "F&B field" were allowed to open-pollinate to create "BC3F1U1" seed, while 306 plants were random-mated with an average of one cross (range 1-5) per female to create BC3F1R1 seed. Seventy-four BC1F1 plants from HLA-B set were used to create three generations described below. BC1F1R1 seed was created by random-mating 74 BC1F1 plants with an average of three (range 1-12) crosses per female. Open-pollinated seed from some of these BC1F1 plants was used to create BC1F2 seed, while 67 BC1F1 plants were cross-pollinated with *G. hirsutum* cv. TM-1 to create BC2F1 seed. The three random-mated generations that were discussed are BC1F3R1, BC2F2R1, and BC3F1R1 because their respective generations of origin, BC1F3, BC2F2, and BC3F1 were included in the experiment (Fig. 9).

Seeds for BC1F1R1, BC1F2, $BC1F2R_1$, BC1F3, BC2F1, BC2F1R1, BC2F2, and BC3F1 were created in the greenhouse facilities at the Texas Agricultural Experiment Station in College Station, where crosses to create BCnF1, and BCnFnR1 generations (n = generation number) were made by hand-emasculating the flowers a day before anthesis and hand-pollinating the next day while selfed generations were developed by harvesting the greenhouse open-pollinated seed. Seeds for BC1F3R1, BC1F3U1, BC2F2R1, BC2F2U1, BC3F1R1, and BC3F1U1 were created on the F&B field at the Texas Agricultural Experiment Station in College Station, where crosses to create BCnF1, and BCnFnR1 generations (n = generation number) were done by hand-emasculating the flowers and covering the stigma with straw a day before anthesis and hand-pollinating the next day with flowers that were clipped a day before anthesis to avoid pollen contamination. After the flowers were hand-pollinated, the stigma was again covered with a straw to avoid any further open-pollination. The three open-pollinated generations harvested from the F&B field, which has high rates of cross-pollination, included BC1F3U1, BC2F2U1 and BC3F1U1.

Field Evaluation

Field evaluations were performed on the Agricultural Experiment Station farm near College Station, TX in 2005, and 2006. A total of 14 generations (Table 14) along with three commercial checks, *G. hirsutum* cvs., FM-832 (Fibermax), PSC-355 (Phytogen Seed Company) and Acala NemX, and a genetic standard, *G. hirsutum* cv. TM-1 (Texas Marker) were planted in a randomized complete block with four replications. Each entry in a replication was planted in two row plots of 13.1 x 1 m that included 25 plants spaced 45 cm apart, thus totaling 50 plants per entry in a replication with the exception of BC1F3R1, which was planted in one row plot that included only 25 plants per replication due to limited seed availability and poor seed germination. Plants within entries and entries within replications were randomly assigned. Due to low seed availability, and poor germination especially in the initial generations, seedlings were established in Jiffy® peat pellets in the greenhouse the last week of March and hand-transplanted in the field the first week of May. Over-seeding was used to compensate for expectedly low rates of (65-70%) germination. All cultural practices were consistent with commercial cotton production at College Station, including furrow irrigation when needed, chemical and mechanical weed control, pesticide application, and participation in the boll weevil eradication program, and chemical defoliation.

Both in 2005 and 2006, fiber samples from individual plants were hand harvested between first and the last week of October, and were ginned on a laboratory saw gin. Two different saw gins were used, but all the samples from each year were ginned on just one gin. Fiber quality analysis in both 2005 and 2006 was done at the fiber testing laboratory of Cotton Incorporated, located in Raleigh, NC. Lint samples weighing more than 10 grams were sent for HVI fiber quality analysis in 2005, however, due to changes in requirement for the HVI machines, lint samples weighing more than 12 grams were sent for the analysis in 2006. Since, none of the plants from the two early generations (BC1F1R1 and BC1F2) failed to produce the minimum quantity of lint required to do fiber analysis using high volume instrument (HVI), no data was available for those generations when dealing with fiber quality traits. Data were collected on total

seed cotton weight plant^{-1} (g), lint weight plant^{-1} (g), seed weight plant^{-1} (g). Total seed cotton weight which is the weight of unginned cotton was used as a measure of yield. Lint percentage was calculated by dividing lint weight with total seed cotton weight expressed in percentage. Samples weighing less than three grams of total seed cotton weight were not included in the lint percentage calculations because the scale used was not sensitive enough to determine the exact lint weight obtained after ginning, thus giving biased lint percent values. Fiber quality analysis was done by using High Volume Instrument (HVI) testing method (Zellweger Uster Inc.) that measures fiber length, fiber strength, fiber elongation, length uniformity, micronaire, and short fiber content (SFC).

Fiber length reported in both 100^{ths} and 32^{nds} of an inch is the average length of the longer one-half of the fibers (upper half mean length, UHML) and is measured by passing a beard of parallel fibers through a sensing point. Length uniformity is the ratio between the mean length and the upper half mean length and is expressed as a percentage. Fiber strength which is expressed as kN m kg^{-1} or grams/tex is the force in grams required to break a bundle of fibers one tex unit size where one tex unit is equal to weight in grams of 1,000 meters of fiber. Micronaire is a measure of resistance of the sample to the air flow and is considered an estimate of fiber fineness and/or maturity. Fiber elongation expressed in percentage is a degree of extension of the fibers before break occurs when measuring strength. Short fiber content is the percent by weight or number of cotton fibers in a sample that are less than 1.26 cm.

Table 15. Fixed and random effect significance from the mixed model analysis for the GMA experiment from 2005 and 2006.

	MIC[§]		ELO[§]		UI[§]		UHML[§]		STR[§]		SFC[§]		Yield[§]		Lint Percent[§]	
Fixed	F[†]		F[†]		F[†]		F[†]		F[†]		F[†]		F[†]		F[†]	
Generation	2.52	*	18.83	****	3.68	**	15.86	****	8.68	****	3.84	**	15.8	****	4.84	***
Random	G[‡]		G[‡]		G[‡]		G[‡]		G[‡]		G[‡]		G[‡]		G[‡]	
Year	0.2	NS	0.4	NS	1.1	NS	9.3	**	4.2	*	0	NS	1.6	NS	16.7	****
Gen*Year	199.8	****	10.9	NS	188	****	47.1	****	32.5	**	155.6	****	328.1	****	243.5	****
Rep (Year)	69.7	****	195.5	****	71.7	****	60.3	****	44	****	47.9	****	27.7	****	26.3	****

*, **, ***, **** significant at p values less than 0.05, 0.01, 0.001, and 0.0001, respectively.

NS, not significant at 5 % level.

GMA, generation mean analysis.

[§]MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%), Yield = seed cotton yield Yield (grams/plant), Lint Percent = lint percentage (%).

[†]F-test.

[‡]Likelihood ratio test.

Statistical Analysis

Data analysis was done on individual years and then combined across years after testing for homogeneity of error variances using Levene's test (Levene, 1960). In the individual data analysis, generations were treated as fixed factors while replications were treated random and the model used was: trait = $\beta_0 + \beta_1$replication + $\beta_3$generation + error. In the combined data analysis, generations were treated as fixed factors while years, generation by year, and replications within years were treated as random. The model for combined data analysis was: trait = $\beta_0 + \beta_1$generation + $\beta_2$year + $\beta_3$generation*year + $\beta_4$rep (year) + error.

Each trait was analyzed with SAS v9.1.3 (SAS Institute, Cary, NC) using mixed model analysis with PROC MIXED method REML (restricted maximum likelihood). LSMEANS which adjusts for other variables in the model was used to calculate means for generations. The multiple comparisons were tested for significance using Tukey-Kramer adjusted least significant difference (LSD) and the output was condensed into letter grouping using SAS macro, PDMIX800 (Saxton, 1998). Fixed effects were tested for significance using the F test, while the significance for the random effects was tested using likelihood ratio test as the difference between full model and the model excluding the random effect (Table 15).

To estimate variance for each generation in a year on an individual plant basis as well as computing 95% confidence interval, data sets were analyzed separately for each generation in a year using SAS PROC MIXED method REML with replications and individual plants within a generation, treated as random factors. Variances were considered significantly different if the confidence intervals for different generations did not overlap. The model used was: trait = $\beta_0 + \beta_1$replication + $\beta_2$plant + error.

Phenotypic correlations among the fiber quality and yield traits were based on individual plant data for each generation, combined across years. Phenotypic correlations were computed by Pearson's product-moment correlations method using PROC CORR procedure of SAS.

Table 16. Levene's test for homogeneity of error variances across years.

Trait	DF	F Value	P value	
Micronaire	1	1.06	0.3031	
Elongation	1	15.38	<.0001	*
Uniformity index	1	3.44	0.0638	
Upper half mean length	1	3.8	0.0513	
Fiber strength	1	20.16	<.0001	*
Short fiber content	1	110.89	<.0001	*
Yield	1	47.1	<.0001	*
Lint percentage	1	2.63	0.0482	*

*Error variances across years were significantly different at $P \leq 0.05$.

Results and Discussion

Generation Means

Micronaire (MIC)

Levene's test (Table 16) indicated that error variances for micronaire were homogeneous across years so data were combined. Means from the combined data indicated that all 12 introgressed generations had numerically lower micronaire readings than PSC-355, FM-832, and TM-1, while seven generations had numerically lower micronaire than Acala Nemx, which is known for its excellent fiber quality, including micronaire (Table 17; Fig. 10). Micronaire readings for Acala Nemx, FM-832, PSC-355, and TM-1 were 4, 4.28, 4.58, and 4.75 respectively, while the introgressed generations had a range from 3.53-4.27. Despite of the fact that the means for most the generations were numerically lower than the commercial checks, PSC-355, FM-832, and Acala Nemx, there were no significant differences observed between them ($P \leq 0.05$). Genetic standard, TM-1 (4.75), which was the last BC parent of four generations, BC2F1 (3.75), BC3F1 (3.9),

Table 17. Least square means by generations for HVI fiber quality traits for the combined data from 2005 and 2006.

	MIC[§]		ELO[§]		UI[§]		UHML[§]		STR[§]		SFC[§]	
BC1F2R1	3.53	B[†]	5.63	B	81.22	AB	26.86	ABCD	263.92	BCDE	10.12	AB
BC1F3	3.84	AB	5.40	BC	80.49	AB	26.15	BCDEF	260.59	BCDE	11.85	AB
BC1F3R1	4.27	AB	5.20	BCD	80.55	AB	26.03	CDEF	255.90	BCDE	11.77	AB
BC1F3U1	4.13	AB	5.04	BCD	80.58	AB	26.22	CDEF	258.27	CDE	11.31	AB
BC2F1	3.75	AB	5.34	BCD	82.29	AB	26.64	BCDE	285.61	ABCD	8.84	AB
BC2F1R1	4.01	AB	5.47	BC	80.78	AB	25.08	EF	271.07	BCDE	11.04	AB
BC2F2	3.83	AB	5.21	BCD	80.19	B	24.56	F	251.60	DE	12.60	A
BC2F2R1	4.10	AB	5.04	BCD	80.41	B	25.15	EF	266.58	BCDE	11.60	AB
BC2F2U1	4.11	AB	5.24	BCD	80.47	AB	25.46	DEF	265.96	BCDE	11.45	AB
BC3F1	3.90	AB	4.80	BCDE	81.53	AB	27.50	ABC	289.67	AB	9.74	AB
BC3F1R1	3.91	AB	4.74	CDE	81.58	AB	27.18	ABC	288.13	ABC	9.66	AB
BC3F1U1	3.78	AB	4.94	BCD	80.92	AB	26.96	BCD	278.51	ABCDE	10.54	AB
TM1	4.75	A	5.52	B	82.20	AB	27.10	ABC	251.72	E	8.77	AB
FM832	4.28	AB	4.17	E	81.92	AB	28.44	A	283.04	ABC	9.01	AB
PSC355	4.58	AB	6.70	A	82.70	AB	27.40	ABC	268.85	BCDE	8.54	AB
Acala Nem	4.00	AB	4.50	DE	83.01	A	27.81	AB	307.27	A	7.96	B

Significant differences between the least square means tested with Tukey-Kramer adjusted LSD.

[†]Means within coulmns followed by the same letter are not different at $p \leq 0.05$.

[§]MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%).

BC3F1R1 (3.91), and BC3F1U1 (3.78) was not different for 11 of the 12 generations, the only exception being BC1F2R1 (3.53), which had lower micronaire than TM-1. Micronaire readings for random-mated generations, BC1F3R1 (4.27), BC2F2R1 (4.1), and BC3F1R1 (3.91) were not different from their respective generations of origin, BC1F3 (3.84), BC2F2 (3.83), and BC3F1 (3.9).

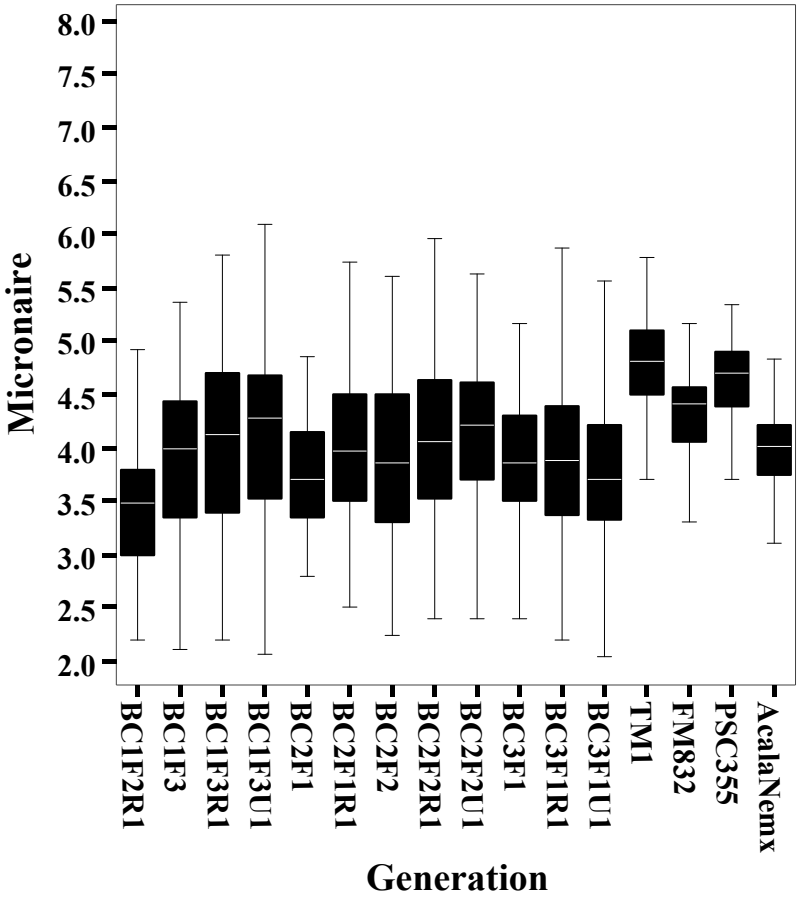

Figure 10. Boxplots displaying combined micronaire values for the introgressed generations and the checks.

Though, years had no significant effect on micronaire, there was a significant generation by year interaction (Table 15), with higher micronaire readings in 2006 for TM-1 and all the introgressed generations, while lower readings for PSC-355, FM-832, and Acala Nemx as compared to 2005 (Tables 18, 19; Fig 11). Due to significant genotype x year interaction, it will be legitimate to evaluate the data for individual years. All the introgressed generations had lower micronaire values than TM-1 in both 2005 and 2006 with an exception of BC1F3R1 in 2006, which was not different ($P \leq 0.05$; Tables 18, 19; Fig 11). In 2005, all the introgressed generations had lower micronaire values than FM-832; however in 2006, 10 of the 12 generations were not different from FM-832, the only exception being BC1F3R1, and BC3F1U1, which had higher and lower micronaire values, respectively. Micronaire values for all the generations were lower than PSC-355 in 2005, but lower only for BC1F2R1, BC2F2, and BC3F1U1 in 2006. Only BC1F2R1, BC1F3, BC2F1, and BC3F1R1 in 2005 and BC1F3R1 and BC2F2U1 in 2006 had lower micronaire values than Acala Nemx. None of the random-mated generations had changed the micronaire values in both 2005 and 2006 as compared to their respective generations of origin.

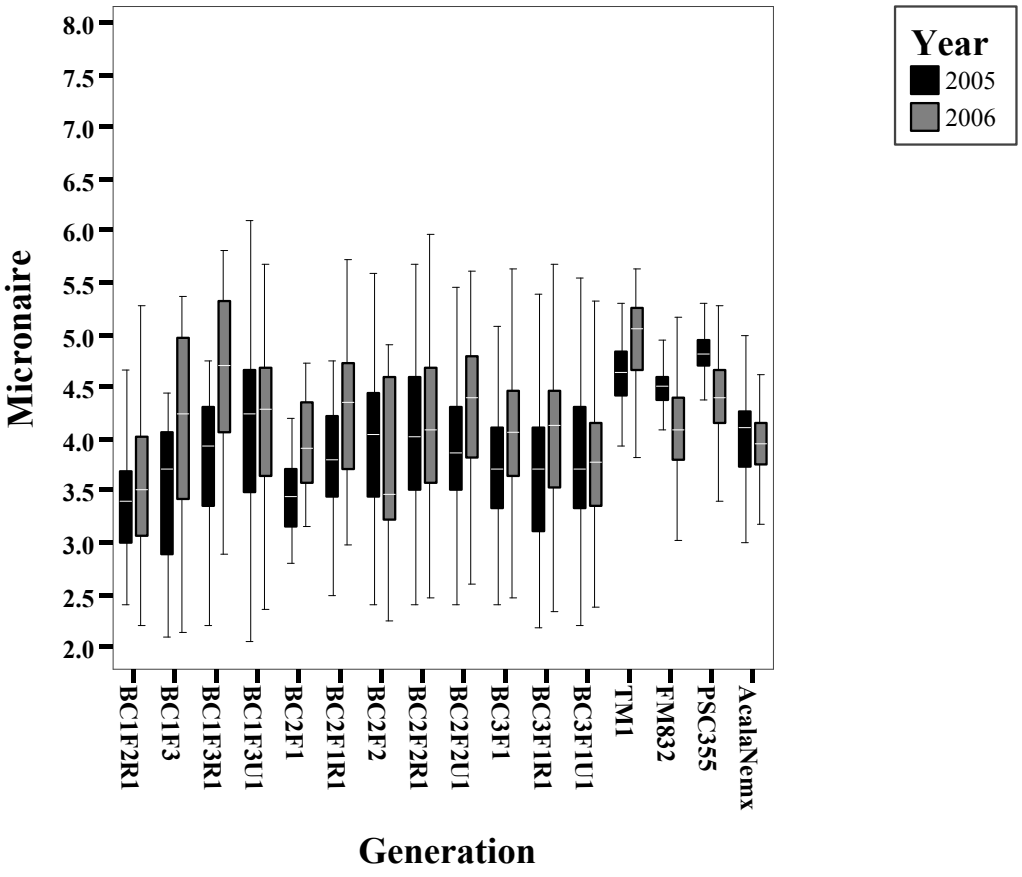

Figure 11. Boxplots displaying micronaire values for the introgressed generations and the checks in 2005 and 2006.

Table 18. Least square means by generations for HVI fiber quality traits for 2005.

	MIC[§]		ELO[§]		UI[§]		UHML[§]		STR[§]		SFC[§]	
BC1F2R1	3.41	F	6.16	B	81.60	CDEF	27.25	CDEF	273.11	EFG	10.68	BCDE
BC1F3	3.50	EF	5.20	CDEFG	80.01	FG	26.75	DEFG	253.29	FG	13.68	A
BC1F3R1	3.74	CDEF	5.54	BCDEF	80.63	CDEFG	26.71	EFG	271.46	DEFG	12.74	ABC
BC1F3U1	4.12	C	5.21	CDEF	80.71	DEFG	26.56	FG	261.31	FG	11.95	ABCD
BC2F1	3.50	EF	5.48	BCDEF	82.08	BCD	27.07	CDEFG	283.78	BCDE	9.19	EF
BC2F1R1	3.79	CDEF	5.84	BC	80.82	DEFG	25.82	GH	278.35	CDE	11.75	ABCD
BC2F2	3.97	CDE	5.58	BCD	80.19	G	24.76	H	269.38	EFG	13.26	A
BC2F2R1	4.03	CD	5.19	DEF	80.83	DEFG	26.09	G	275.61	E	11.68	ABCD
BC2F2U1	3.88	CDE	5.41	CDE	80.70	EFG	26.10	G	273.15	EF	12.16	AB
BC3F1	3.77	DEF	4.89	EFG	81.56	CDE	28.04	BCDE	293.09	BC	10.19	DE
BC3F1R1	3.74	EF	4.79	FG	81.89	C	27.97	BCDE	294.51	B	9.58	E
BC3F1U1	3.80	CDE	5.06	DEF	81.27	CDEF	27.75	BCDE	291.62	BCD	10.49	CDE
TM1	4.62	B	5.66	BC	82.76	B	28.01	BCD	257.93	G	8.23	F
FM832	4.49	B	4.18	H	83.27	B	29.47	A	292.09	B	8.00	F
PSC355	4.84	A	6.77	A	83.91	A	28.43	B	268.66	EF	7.53	F
AcalaNemx	4.02	CD	4.56	G	83.27	B	28.18	BC	311.97	A	7.62	F

Significant differences between the least square means tested with Tukey-Kramer adjusted LSD.

[†]Means within coulmns followed by the same letter are not different at $p \leq 0.05$.

[§]MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%).

Table 19. Least square means by generations for HVI fiber quality traits for 2006.

	MIC[§]		ELO[§]		UI[§]		UHML[§]		STR[§]		SFC[§]	
BC1F2R1	3.64	FG	5.06	BCD	80.83	CDEF	26.50	ABC	254.60	EFGI	9.53	CDEFGH
BC1F3	4.18	BCDEF	5.55	BC	80.93	BCDEF	25.53	CD	266.39	BCDEFG	10.13	ABCDEFG
BC1F3R1	4.84	AB	4.82	BCDEF	80.50	CDEF	25.33	CDE	238.68	GHI	10.70	ABCDEF
BC1F3U1	4.13	CDEF	4.87	D	80.45	EF	25.88	C	254.97	FGI	10.66	ABCD
BC2F1	3.98	CDEF	5.20	BCD	82.48	AB	26.18	BC	286.72	ABCD	8.51	GH
BC2F1R1	4.24	BCDE	5.08	BCD	80.75	CDEF	24.34	DE	263.62	CDEFH	10.31	BCDEF
BC2F2	3.68	EF	4.77	CDE	80.22	DEF	24.49	DE	231.20	I	11.87	AB
BC2F2R1	4.17	CDE	4.90	D	79.99	F	24.21	E	257.47	FG	11.51	A
BC2F2U1	4.34	BCD	5.07	BCD	80.24	F	24.81	DE	258.89	FG	10.75	ABC
BC3F1	4.03	CDEF	4.70	DE	81.50	BCD	26.96	AB	286.27	B	9.29	EFG
BC3F1R1	4.09	CDEF	4.68	DE	81.27	BCDE	26.39	BC	281.73	BC	9.74	DEFG
BC3F1U1	3.76	F	4.83	D	80.57	DEF	26.17	BC	265.32	DEFH	10.59	ABCD
TM1	4.89	A	5.39	B	81.65	BC	26.19	C	245.50	GI	9.31	FG
FM832	4.08	DEG	4.16	F	80.57	EF	27.41	A	273.95	BCDE	10.02	CDE
PSC355	4.31	BC	6.63	A	81.49	BC	26.38	BC	269.03	CDEF	9.54	EFG
AcalaNem	3.97	EF	4.45	E	82.76	A	27.44	A	302.57	A	8.30	H

Significant differences between the least square means tested with Tukey-Kramer adjusted LSD.

[†]Means within coulmns followed by the same letter are not different at $p \leq 0.05$.

[§]MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%).

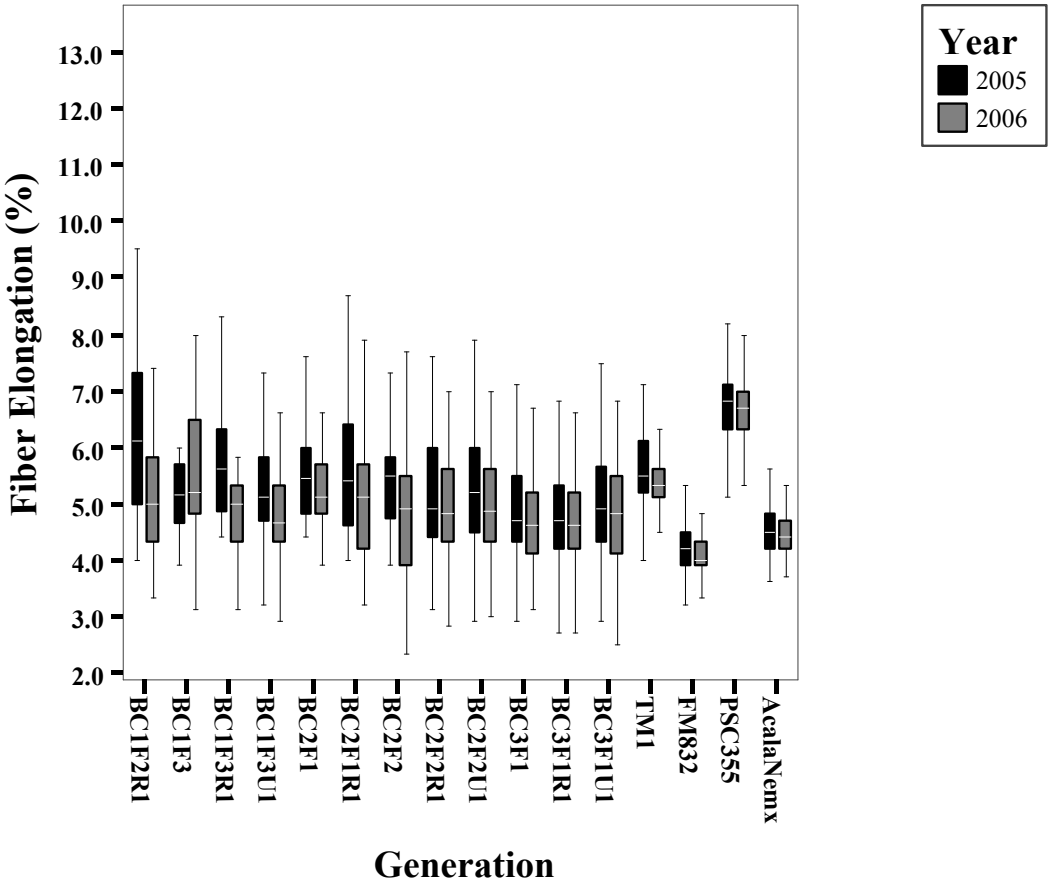

Figure 12. Boxplots displaying fiber elongation for the introgressed generations and the checks in 2005 and 2006.

Fiber Elongation (ELO)

Levene's test (Table 16) indicated that error variances were heterogeneous across years for fiber elongation (ELO), so data were analyzed separately for 2005 and 2006. The mean elongation for the introgressed generations ranged from 4.79 to 6.16 in 2005 and 4.68 to 5.55 in 2006 (Tables 18, 19; Fig. 12). In both years, PSC-355, which had the highest mean elongation of 6.77% and 6.63%, respectively had higher elongation than all 12 introgressed generations ($P \leq 0.05$; Tables 18, 19; Fig. 12). However, elongation values of all introgressed generations were higher than the commercial cultivar FM-832 for both years, the only exception being, BC1F3R1 in 2006, which was not different. For both, 2005 and 2006, three generations at BC3 level (BC3F1, BC3F1R1, and BC3F1U1) had lower elongation than their last BC parent, TM-1. Eight of the remaining nine generations in 2005 were not different from TM-1, the only exception being BC2F2R1, which had lower micronaire than TM-1. While in 2006, six of the remaining nine generations were not different from TM-1, the exceptions being BC1F4, BC2F2, and BC2F2R1, which had lower elongation. Except for three generations in 2005 and four generations in 2006, all other generations had higher elongation than Acala Nemx. There were no changes in elongation after random-mating the generations when compared to their respective generations of origin.

Uniformity Index (UI)

After determining that the homogeneity of error variances criteria was met using Levene's test (Table 16), the uniformity index value data were combined across years. Combined data analysis indicated that uniformity index (UI), for Acala Nemx, PSC-355, FM-832, and TM-1 were 83, 82.7, 81.9, and 82.2% respectively and were not different from each other ($P \leq 0.05$; Table 17; Fig. 13). The UI for the introgressed generations ranged from 80.2 to 82.3%. None of the introgressed generations were different from PSC-355, FM-832, TM-1, and Acala Nemx with the exception of BC2F2 and BC2F2R1 which had lower UI compared to Acala Nemx. Though there was a very slight

numerical increase in the means of all the random-mated generations compared to the generations of origin, the difference were not significant.

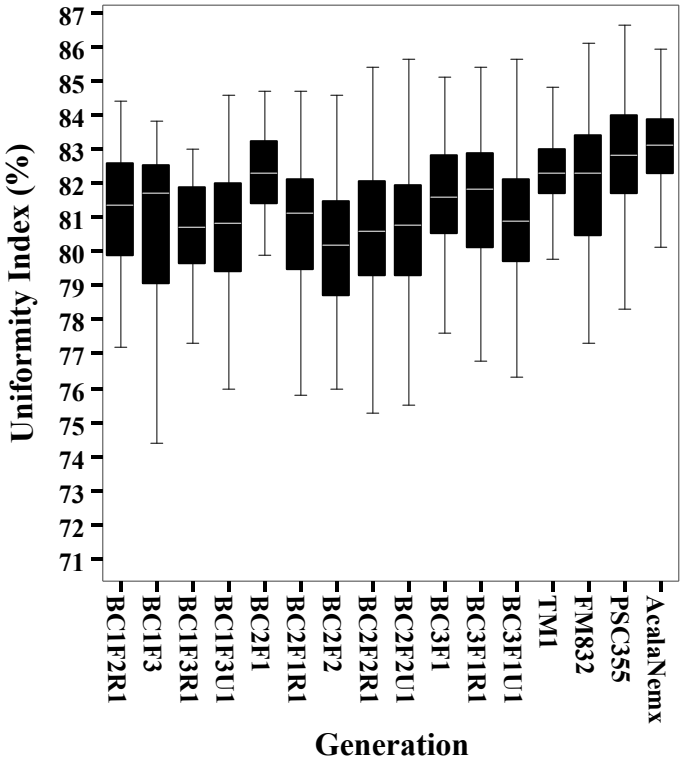

Figure 13. Boxplots displaying combined fiber uniformity index for the introgressed generations and the checks.

Due to significant generation x year interactions (Table 15), UI data were also evaluated for individual years. In 2005, all of the introgressed generations had lower UI values than TM-1 and PSC-355, the only exception being BC2F1, which had higher UI than TM-1 ($P \leq 0.05$; Table 18; Fig. 14). In 2006, however, 7 out of 12 generations had UI values not different from both TM-1 and PSC-355, while the remaining four generations (BC1F3U1, BC2F2, BC2F2R1, BC2F2U1, BC3F1U1) had lower UI values ($P \leq 0.05$; Table 19; Fig. 14). All but BC2F1 generation in 2005 had lower UI than FM-832, while BC2F1 was not different. However in 2006, 10 of the 12 generations were not different

from FM-832, while BC2F1 and BC3F1 had higher UI values than FM-832. With the exception of BC2F1 which had UI values not different from Acala Nemx in both years, all other generations had lower UI values than Acala Nemx. One generation of random-mating did not change the UI values in both years when compared to the generations of origin.

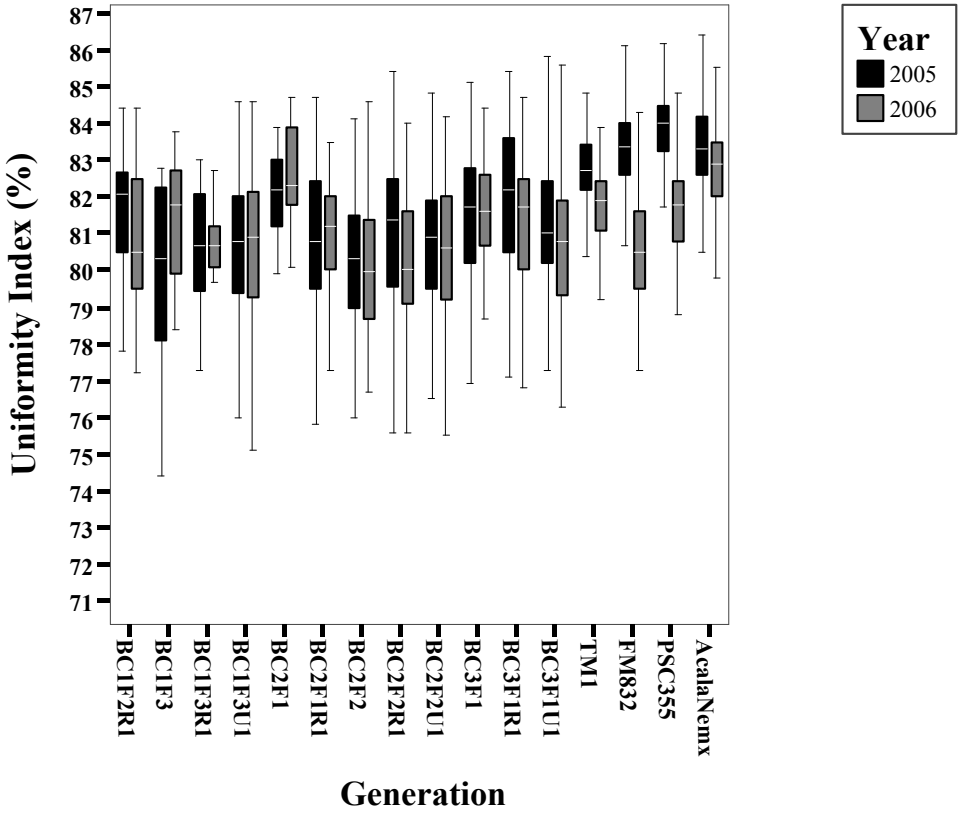

Figure 14. Boxplots displaying fiber uniformity index for the introgressed generations and the checks in 2005 and 2006.

Upper Half Mean Length (UHML)

Levene's test (Table 16) indicated that error variances were homogeneous across years so upper half mean length (UHML) data were combined. FM-832, which had the highest UHML (28.4 mm) in the combined analysis was not different from PSC-355

(27.4 mm), Acala Nemx (27.8 mm), TM-1 (27.1 mm) and three of the introgressed generations, BC1F2R1 (26.9 mm), BC3F1 (27.5 mm), and BC3F1R1 (27.2 mm) ($P \leq 0.05$; Table 17; Fig. 15). Though none of the generations had UHML higher than Acala Nemx (27.8 mm), there were six generations, BC1F2R1, BC1F3, BC2F1, BC3F1, BC3F1R1, and BC3F1U1 (range 26.2-27.5 mm) that were not different from it. After comparing generation means with TM-1 and PSC-355, it was observed that four BC2 level generations (BC2F1R1, BC2F2, BC2F2R1, and BC2F2U1) had lower UHML, while all other generations were not different. There was no change in the mean of any of the random-mated generations compared to their respective generations of origin.

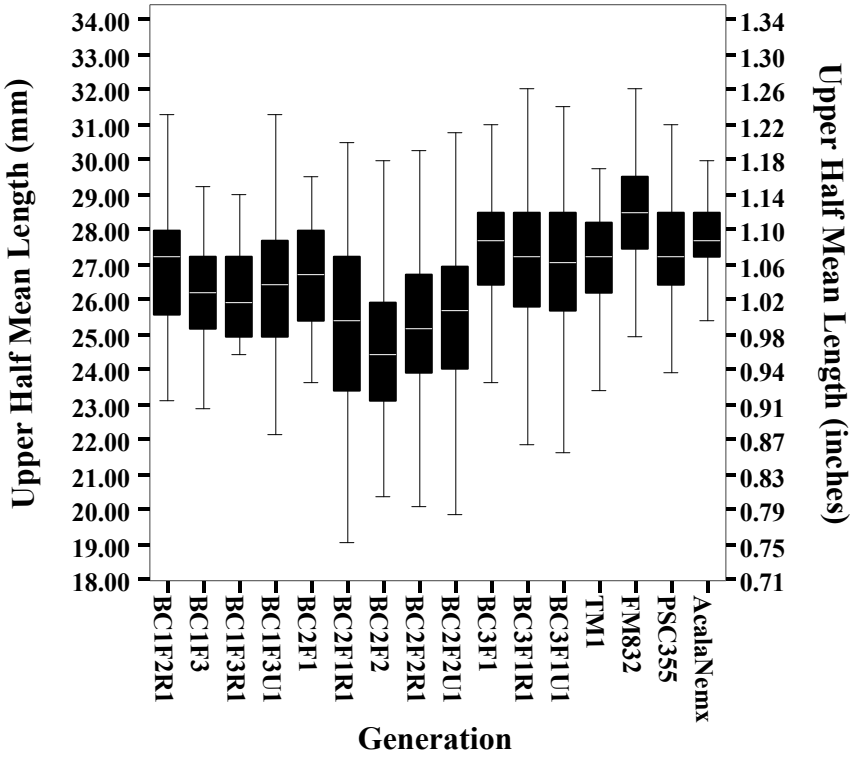

Figure 15. Boxplots displaying combined upper half mean length for the introgressed generations and the checks.

Due to significant generation x year interactions (Table 15), UHML data were also evaluated for individual years. In 2005, six introgressed-generations (BC1F2R1, BC1F3, BC2F1, BC3F1, BC3F1R1, and BC3F1U1) were not different from TM-1, while others had lower UHML ($P \leq 0.05$; Table 18; Fig. 16). In 2006, BC3F1 generation was the only generation which had higher UHML than TM-1, which was also its last backcross parent ($P \leq 0.05$; Table 19; Fig. 16). Out of remaining 11 generations, four BC2 level generations (BC2F1R1, BC2F2, BC2F2R1, and BC2F2U1) had lower UHML than TM-1, while others were not different. FM-832, which had the best UHML both years was higher than all the introgressed generations in 2005 and 2006, the only exceptions being BC1F2R1 and BC3F1 in 2006, which were not different from FM-832. In 2005, all BC3 level generations were not different from PSC-355, while others had lower UHML. In 2006, only BC2F1R1, BC2F2, BC2F2R1, and BC2F2U1 generations had lower UHML than PSC-355, while others were not different. Acala Nemx was not different from three BC3 level generations as well as from BC1F2R1 and BC2F1 in 2005; while in 2006 only BC1F2R1 and BC3F1 had UHML not different from Acala Nemx. Generation BC2F2R1 in 2005 was the only random-mated generation, which had higher UHML than its generation of origin, BC2F2.

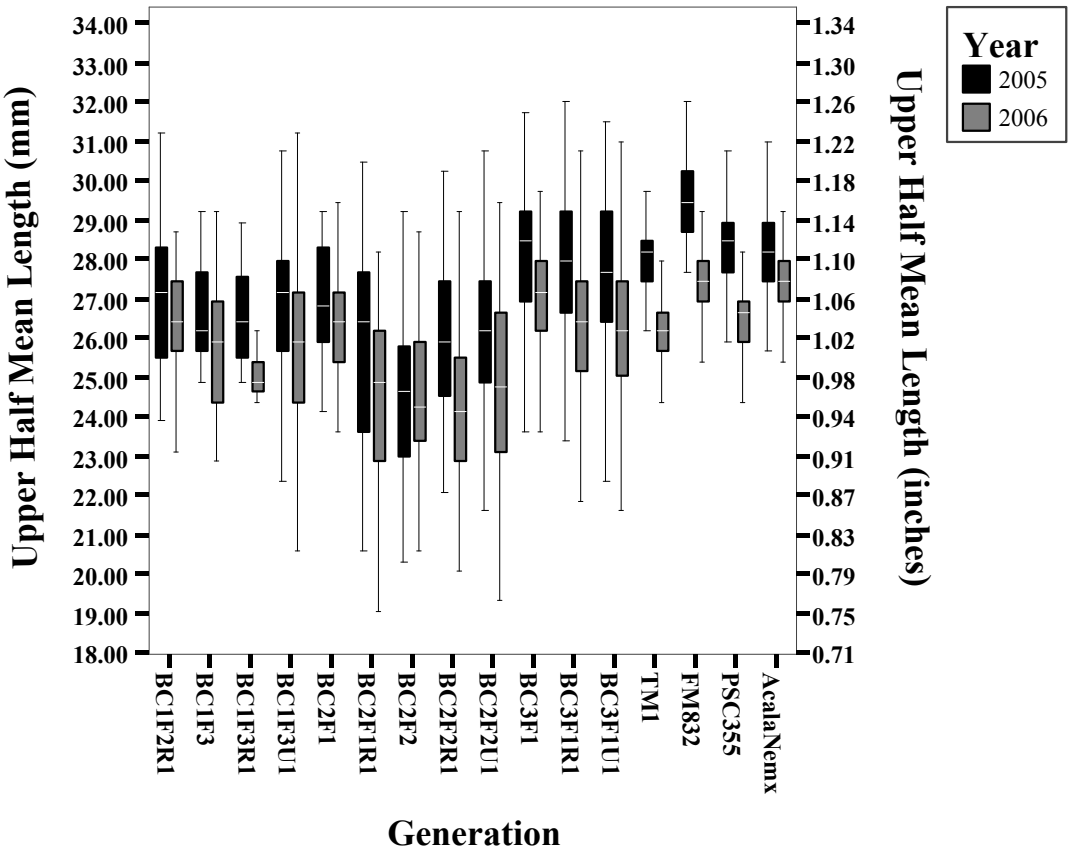

Figure 16. Boxplots displaying upper half mean length for the introgressed generations and the checks in 2005 and 2006.

Fiber Strength (STR)

Levene's test (Table 16) indicated that error variances were heterogeneous across years for fiber strength (STR), so data were analyzed separately for 2005 and 2006. The fiber strength for the introgressed generations ranged from 253 to 294 kN m kg^{-1} in 2005 and 231 to 287 kN m kg^{-1} in 2006 (Tables 18, 19; Fig. 17). Acala Nemx, which had the highest strength, both in 2005 (312 kN m kg^{-1}), and 2006 (302.6 kN m kg^{-1}) was different from all the generations, commercial checks, and TM-1 both in 2005 and 2006,

with the exception of BC2F1 (286.7 kN m kg^{-1}) in 2006, which was not different from Acala Nemx ($P \leq 0.05$; Tables 18, 19; Fig. 17).

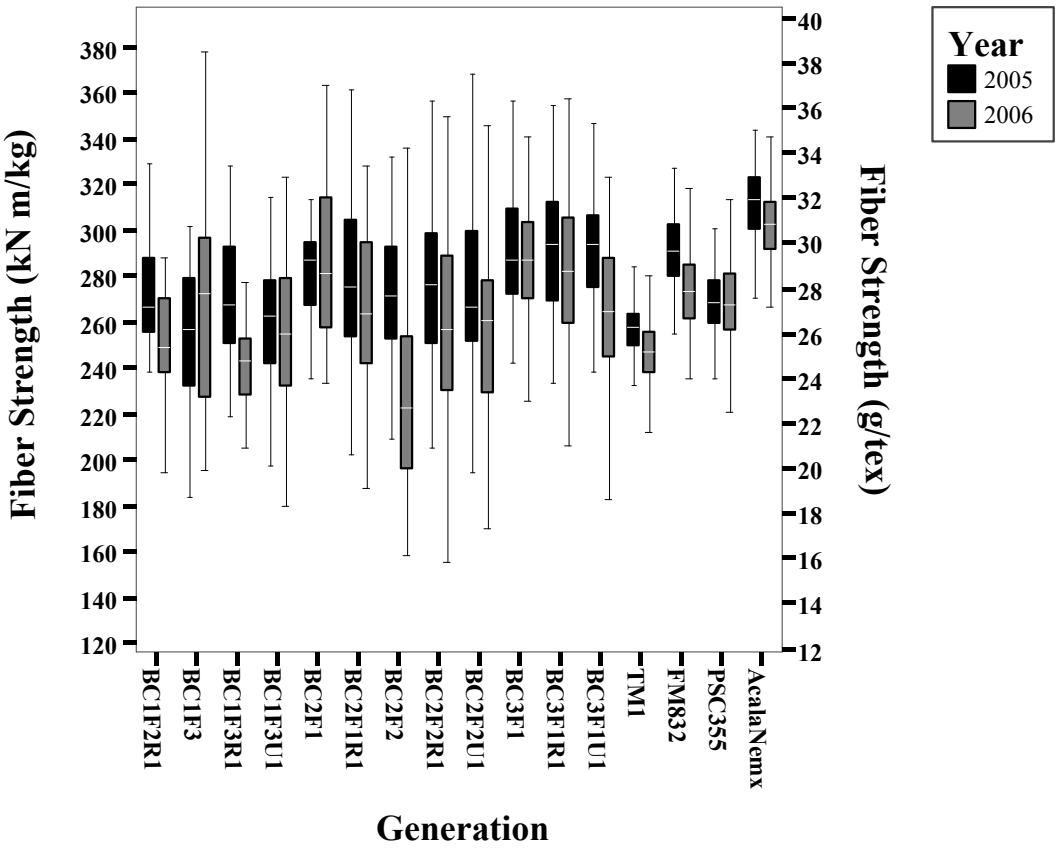

Figure 17. Boxplots displaying fiber strength for the introgressed generations and the checks in 2005 and 2006.

Fiber strength for four generations in 2005 (BC2F1, BC3F1, BC3F1R1, and BC3F1U1) and seven generations in 2006 (BC1F2R1, BC1F3, BC2F1, BC2F1R1, BC3F1, BC3F1R1, and BC3F1U1) was not different from FM-832, which is one of the best fiber strength cultivars currently cultivated. Eleven out of 12 introgressed-generations in 2005 and 10 out of 12 introgressed-generations in 2006 had numerically higher fiber strength than TM-1, but the differences were significant only for seven

generations (BC2F1, BC2F1R1, BC2F2R1, BC2F2U1, BC3F1, BC3F1R1, and BC3F1U1) in 2005 and five generations (BC2F1, BC2F1R1, BC3F1, BC3F1R1, and BC3F1U1) in 2006. In 2005, three BC3 level generations had higher STR than PSC-355, while none of the othet generations were different from PSC-355. In 2006, BC3F1 had higher strength than PSC-355, while nine generations were not different and two had lower STR than PSC-355. Amongst the random-mated generations, only BC2F2R1 in 2006 had higher strength than its generation of origin.

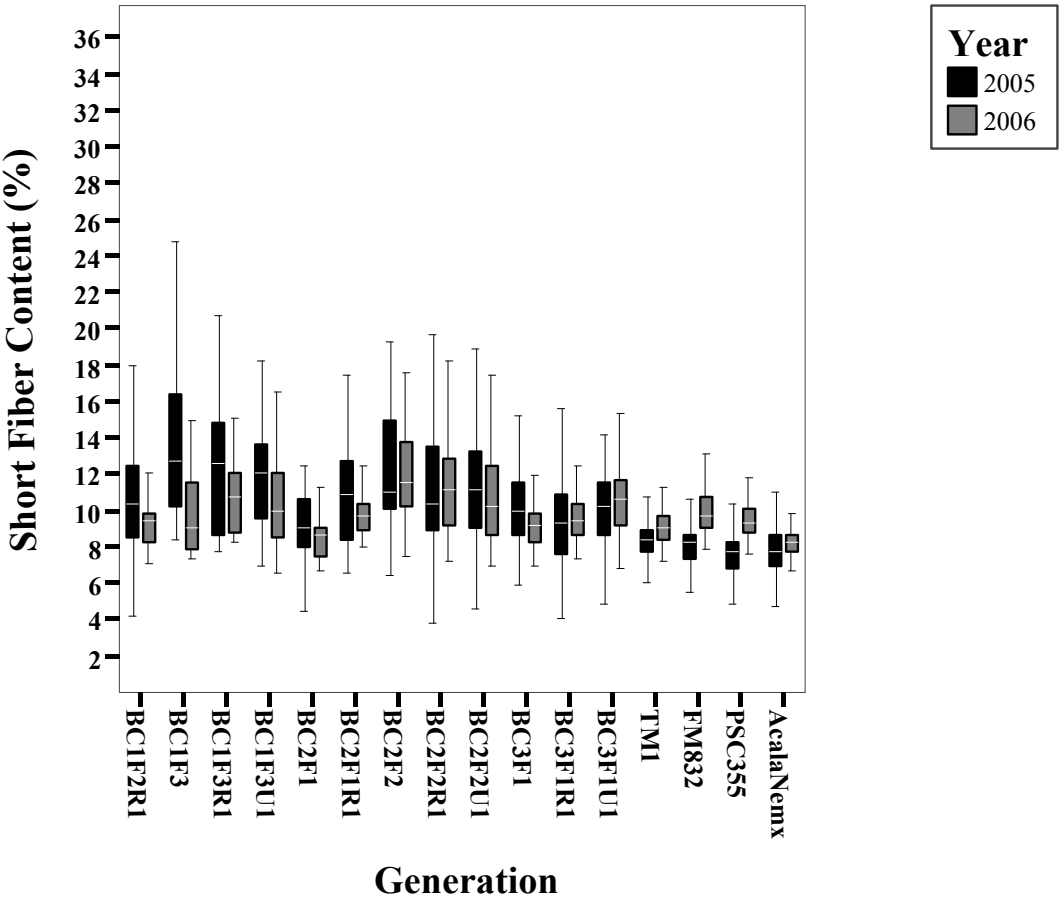

Figure 18. Boxplots displaying short fiber content for the introgressed generations and the checks in 2005 and 2006.

Short Fiber Content (SFC)

Levene's test indicated (Table 16) that error variances were heterogeneous across years for short fiber content (SFC), so data were analyzed separately for 2005 and 2006. In 2005 BC2F1 was the only generation that had the SFC not different from TM-1 and the three cultivars PSC-355, TM-1, FM-832, and Acala Nemx, while other generations had higher SFC than the four controls ($P \leq 0.05$; Table 18; Fig. 18). In 2006, BC2F1 and BC1F2R1 were not different from Acala Nemx, which had the lowest SFC ($P \leq 0.05$; Table 19; Fig. 18). In 2006, seven, nine, and seven generations were not different from TM-1, FM-832, and PSC-355, respectively, while remaining generations had higher SFC. None of the random-mated generations had different SFC than the generations of their origin.

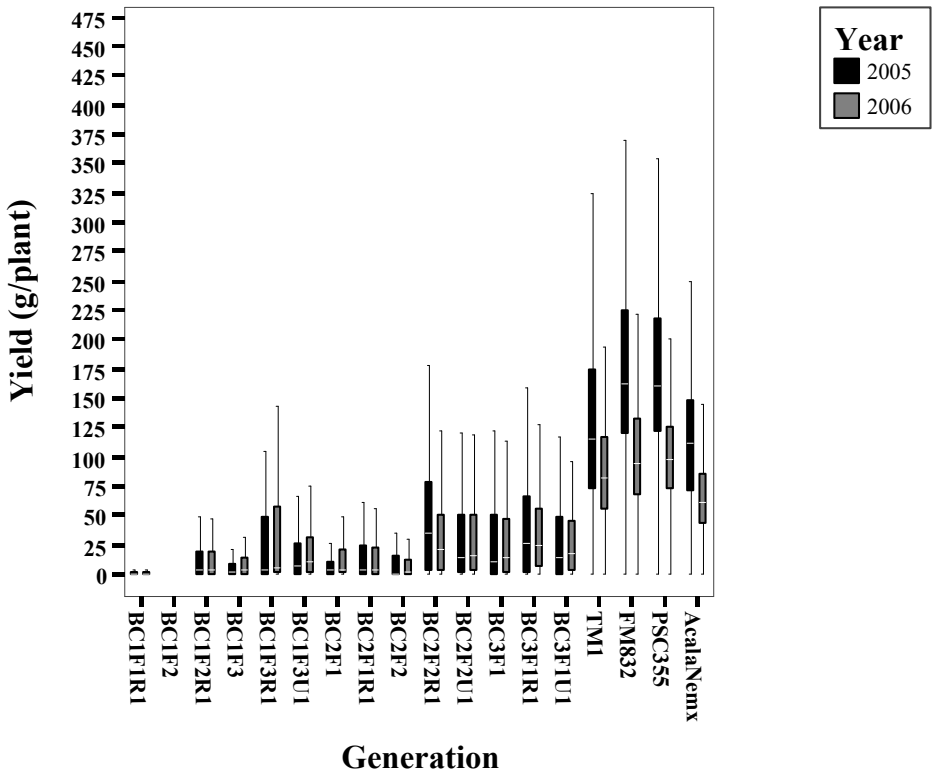

Figure 19. Boxplots displaying seed cotton yields for the introgressed generations and the checks in 2005 and 2006.

Seed Cotton Yield (Yield)

Levene's test (Table 16) indicated that error variances were heterogeneous across years for seed cotton yield (yield), so data were analyzed separately for 2005 and 2006. All the generations had lower yields than TM-1, PSC-355, FM-832, and Acala Nemx in both 2005 and 2006 ($P \leq 0.05$; Table 20; Fig. 19). Amongst the random-mated generations, BC2F2R1 had higher yields than its generation of origin, BC2F2 in both years, while BC1F3R1 had higher yield than its generation of origin, BC1F3 in 2005. BC2F2R1, which yielded 48 g plant^{-1} and 35 g plant^{-1} in 2005 and 2006 respectively, had 3- and 3.7-fold higher yields, respectively than its generation of origin, BC2F2, while BC1F3R1 had 3- and 3.2-fold increases in yields, compared to the generation of origin, BC1F3 in 2005 and 2006, respectively. Generation BC3F1R1, which had 1.4- and 1.1-fold increases in yields in 2005 and 2006, respectively, but the differences were not significant.

Similar results were obtained by Miller and Rawlings (1967) where they observed an increase in the yields after random-mating. They claimed that natural selection in the form of germination, seedling establishment, flowering time, amount of pollen produced by a plant etc., might have occurred during the random-mating generations, thus increasing the yields in the random-mated generations. The possibility of natural selection, especially, seed germination and seedling establishment, and others cannot be ignored to explain the increased yields in the random-mated generations in this study. The other possibilities that cannot be ignored are the presence of epistasis involving linkages in the original population, as well as the effects of different environments under which the random-mated and the original seed were created as pointed by Meredith and Bridge (1971). The generations that had the highest yields at BC1, BC2, and BC3 levels, respectively were BC1F3U1 (21 g plant^{-1}), BC2F2R1 (48 g plant^{-1}), and BC3F1R1 (43 g plant^{-1}) in 2005 and BC1F3R1 (36.7 g plant^{-1}), BC2F2R1 (34.9 g plant^{-1}), and BC3F1R1 (37.4 g plant^{-1}) in 2006; however, the differences were not significant in 2006, while in 2005, BC2F2R1, and BC3F1R1 had significantly higher yields than BC1F3U1.

Table 20. Least square means by generations for fiber yield and lint percentage for 2005 and 2006.

	2005				2006			
	Yield[§]		Lint Percent[§]		Yield[§]		Lint Percent[§]	
BC1F1R1	1.72	G[†]	25.43	GHI	1.92	HI[†]	33.49	FGH
BC1F2	2.49	G	22.02	I	1.53	I	36.36	BCDEFGH
BC1F2R1	15.32	EFG	25.42	HI	14.14	EFGHI	32.82	H
BC1F3	9.97	FG	24.16	I	11.45	FGHI	37.25	DEF
BC1F3R1	30.10	CDEF	28.80	DEFGHI	36.67	CDE	33.42	FGH
BC1F3U1	21.00	DEF	29.73	EFG	23.99	CDEF	37.53	CDEF
BC2F1	10.71	FG	24.82	I	15.37	DEFGH	33.65	GH
BC2F1R1	22.79	DEF	28.24	FGH	17.11	DEFG	38.61	BCDE
BC2F2	15.54	EFG	31.17	CDEF	9.55	GHI	41.85	AB
BC2F2R1	47.73	C	33.96	C	34.90	C	39.85	BCD
BC2F2U1	33.43	CD	31.81	CDE	34.04	C	39.37	BCD
BC3F1	31.00	CDE	31.94	CDE	33.13	C	40.21	BC
BC3F1R1	43.27	C	32.85	CD	37.43	C	39.74	BCD
BC3F1U1	35.34	CD	30.95	DEF	28.89	CD	39.07	BCD
TM1	126.68	B	33.15	CD	94.80	A	35.92	EFG
FM832	169.02	A	40.99	A	107.78	A	43.21	A
PSC355	171.77	A	40.75	AB	104.60	A	43.10	A
AcalaNem	112.70	B	38.40	B	73.12	B	38.53	CD

Significant differences between the least square means tested with Tukey-Kramer adjusted LSD.

[†]Means within coulmns followed by the same letter are not different at $p \leq 0.05$.

[§]Yield = seed cotton yield (grams/plant), Lint Percent = lint percentage (%).

Lint Percentage

Levene's test (Table 16) indicated that error variances were heterogeneous across years for lint percentage, so data were analyzed separately for 2005 and 2006. There was a significant year and generation x year interaction for lint percentage (Table 15). In 2006, lint percentages for the checks as well as the generations were higher than 2005. These differences could have been due to use of different gins in 2005 and 2006. Comparison of the introgressed generations with PSC-355, and FM-832, which had the highest lint percentages in both, 2005 and 2006 indicated that all the generations had significantly

lower lint percentages in both years, the only exception being BC2F2 in 2006, which was not significantly different ($P \leq 0.05$; Table 20; Fig. 20). Seven of the 12 generations in 2005 had lint percentages not different from TM-1, while other generations had lower lint percentages.

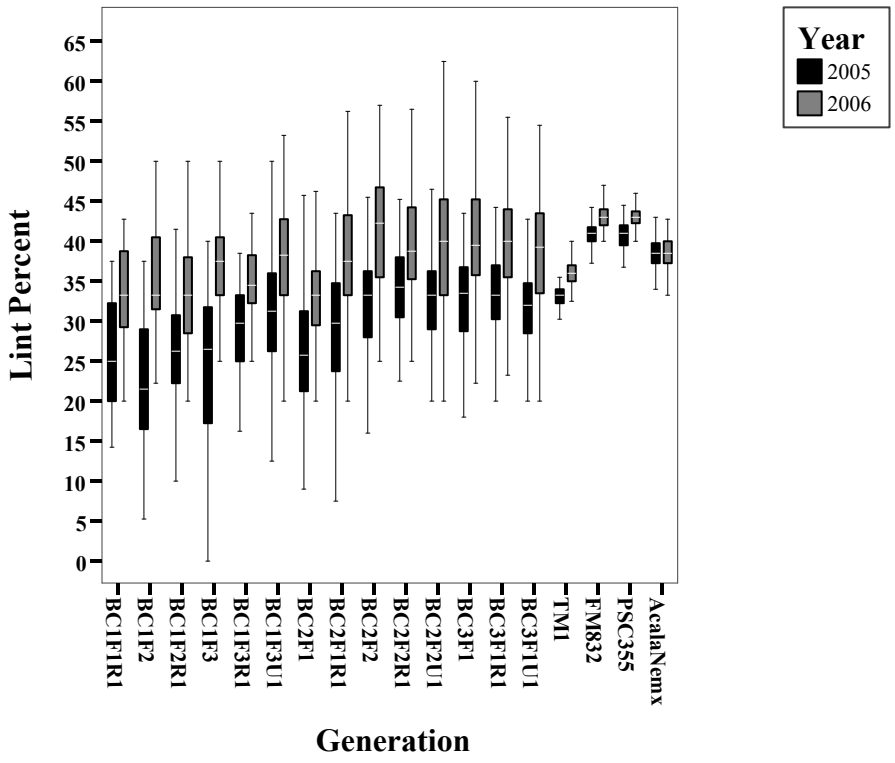

Figure 20. Boxplots displaying lint percentages for the introgressed generations and the checks in 2005 and 2006.

In 2006, six generations had higher lint percentages than TM-1, while seven generations were not different and one generation had lower lint percentage than TM-1. All introgressed generations had lower lint percentages than Acala Nemx in 2005; however in 2006 BC2F2 had higher lint percentage than Acala Nemx, while nine generations were not different from Acala Nemx and four generations had lower lint percentage. In both years, a trend of increase in lint percentage with every additional

backcross to *G. hirsutum* was observed, suggesting that the increase has been contributed by the *G. hirsutum* germplasm. None of the random-mated generations had different lint percentages than their generations of origin in both, 2005 and 2006.

Effect of Backcrossing on Fiber Quality and Yield Traits

Generations at BC2 and BC3 levels that were derived from the same set of BC2F1 plants were compared to determine the effect of a backcross on fiber quality, yield and lint percent (Fig. 9; Table 14). In 2005, BC2F1R1 had higher elongation than BC3F1R1, while BC3F1R1 had higher uniformity index, fiber length, fiber strength, yield and lint percentage, and lower short fiber content than BC2F1R1 (Tables 18, 20). In 2006, however, only two traits were different between BC2F1R1 and BC3F1R1, where BC3F1R1 had higher fiber length and yield than BC2F1R1 (Tables 19, 20). The BC2F1 generation was the only generation analyzed for fiber quality from HLA-B set (Fig. 9; Table 14). Comparison between BC2F1 and BC3F1 generations indicated that none of the fiber quality traits were different between the two generations; however yield and lint percent of BC2F1 was lower than BC3F1 in both 2005 and 2006.

Comparison of the BC3 level generations with their last backcross parent, TM-1, in 2005 showed that all three generations had lower micronaire, uniformity index, elongation and yield, and higher fiber strength and short fiber content than TM-1. In 2006, three BC3 level generations had lower micronaire, elongation and yield, and higher fiber length, strength and lint percent than TM-1. Micronaire and fiber strength were the two traits that were consistently better than TM-1 in both the years, other traits should be improved by additional backcrossing to Upland cotton cultivars that are high yielding and has good fiber quality. Major emphasis needs to be given to improve yield of the introgressed germplasm. Yields of all the introgressed generations were lower than TM-1 and all three commercial cultivars. With such a diverse genetic background between the backcross parent, which has been selected for yield, and the introgressed generations derived from two non-cultivated unyielding wild species, one would speculate that the differences in yield might be due to fewer loci with favorable alleles, and the existence of

unfavorable linkages in the introgressed generations. As suggested by Dudley (1982) and Bridges and Gardner (1980) more diverse parents differ at more loci, so more backcrosses are needed to develop a foundation population that contains an optimal number of favorable alleles from both the species. Random-mating, especially in the early generations would give more opportunities to break undesirable linkages through genetic recombination, thus creating genetic variability for effective selection. The selected plants then should go through seires of backcrossings to increase the yield of the introgressed germplasm. Initiation of selection for potential fiber quality trait during each backcross would allow successful introgression of the desirable trait(s) from the donor parent along with the recovery of desirable agronomic potential of the adapted parent.

Variances

One of the major advantages of random-mating is to break up linkage blocks through recombination in meiosis and thus generate genetic variability, which would presumably lead to high frequency of transgressive segregants (Lamkey et al., 1995). With studies involving germplasm introgression from primitive or exotic species into adapted cultivars, extreme linkages have often been shown to be prevalent (Al-Jibouri, et al., 1958; Miller and Rawlings, 1967; Meredith and Bridge, 1971). Miller and Rawlings (1967) while studying the role of random-mating in breaking linkages in an wild species-derived line of cotton x adapted cotton cultivar, points out that if the number of loci associated with a trait were linked in the original parents and if the coupling and repulsion-phase linkages were not in balance, genetic variances were expected to move towards linkage equilibrium after random-mating. In cases where coupling phase linkages between the loci associated with a trait were prevalent in the crosses, genetic variances are expected to decrease with random-mating, while in cases where repulsion phase linkages between the loci associated with a trait were prevalent, the genetic variance are expected to increase, assuming that the gene action is additive (Miller and Rawlings, 1967; Meredith and Bridge, 1971; Altman and Busch, 1984; Hoffbeck et al.,

1995). The introgressed germplasm analyzed in this experiment fits well with the above wild germplasm studies, since it is derived from a tri-species cross involving two wild *Gossypium* species, which are uncultivated and unyielding and an adapted Upland cotton. Thus the assumptions stated above are applied to this study in making inferences about the effects of random-mating on the variances of fiber quality and yield traits.

Variance for micronaire in 2005 was lower for BC1F3R1 and BC3F1R1, and higher in 2006 for BC1F3R1 when compared to their respective generations of origin (Tables 21, 22). BC1F3R1 was the only randomated generation that had lower variances for uniformity index in 2005, fiber length in 2005 and 2006, and short fiber content in 2006 as compared to its generation of origin, BC1F3. Variance for fiber strength was higher for BC3F1R1 in 2005 than its generations of origin, BC3F1. Higher variances were observed for yield in the BC1F3R1 and BC2F2R1 generations as compared to BC1F3 and BC2F2R1 generations, respectively, in 2005 and 2006 (Table 23). Lower lint percent variances were observed for BC2F2R1 when compared with BC2F2 in 2005 and 2006, while BC1F3R1 had lower variances than BC1F3 in 2005, and BC3F1R1 had lower variances than BC3F1 in 2006 (Table 23). These results suggest that the decrease in trait variances after random-mating could possibly be due to the pre-existence of coupling phase linkages between the loci associated with that trait in their respective, generations of origin, while the increased variances in the random-mated generations could be attributed to the pre-existence of repulsion phase linkage between the loci associated with that trait in their respective, generations of origin. The trend of change in the variances of the traits after random-mating was higher at the BC1 level generation than in the BC2 and BC3 level generations, with a tendency towards decreased variances for most of the traits, the only exception being yield, where the random-mated generation, BC1F3R1 had higher variance than BC1F3.

Meredith and Bridge (1971), while explaining the differences observed in decreased genetic variances after random-mating between their study and Miller and Rawlings study (1967), points out that since the donor parent, TH-131 in Miller and Rawlings (1967) study was more closely related to the original, tri-species hybrid in

terms of the alien genome content due to fewer backcrosses to *G. hirsutum* and less selection for agronomic acceptability as compared to the donor parent, Pee Dee-165 used in their study, the material used by Miller and Rawlings should be prevalent with the coupling phase linkages, which after random-mating would have a higher decrease in the genetic variance as compared to the study by Meredith and Bridge (1971). This explains the trend of a higher decrease in variances after random-mating in the early backcross generations as compared to the advanced backcross generations for most of the traits. However, for yield, there was an increase in the variances for BC1F3R1 and BC2F2R1, which indicate that the effects of repulsion linkages between the loci associated with yield had prevailing effects in the BC1F3 and BC2F2. The amount of plant-to-plant variability in TM-1 and other commercial checks for all fiber quality traits was less than the introgressed generations, and in some cases close to nil (Tables 21, 22). However, plant-to-plant variability for yield was higher in all the commercial cultivars and TM-1 as compared to the introgressed generations (Table 23). A trend of increased variances was observed in high yielding generations and the commercial checks. While part of this variation could be genetic, most of this variation can be explained by end-hill (end-of-row) effects where the first and last plants in a row yielded higher than other plants in that row, mainly due to more available resources including sunlight, light, etc., (data not shown). This becomes a major issue when yields are determined based on individual plant yields. Performing statistical analysis excluding the first and last plants in a row should help when dealing with this issue.

Table 21. Variance estimates and 95% confidence intervals by generations for fiber quality traits in 2005.

	MIC[§]		ELO[§]		UI[§]		UHML[§]		STR[§]		SFC[§]	
BC1F2R1	0.1	(0.0, 7.6)[¶]	1.4	(0.8, 3.8)	1.6	(0.9, 4.2)	3.2	(1.8, 7.1)	0	[‡]	9.0	(5.4, 17.7)
BC1F3	0.2	(0.0, 17.4)	1.4	(0.6, 5.7)	4.8	(2.3, 16.4)	1.3	(0.6, 5.7)	901	(472, 2361)	16.6	(8.3, 47.8)
BC1F3R1	0.0	[‡]	0.1	(0.0, 169x10$^{14)}$	0.0	[‡]	0.0	[‡]	781	416, 1972)	13.1	(6.8, 34.8)
BC1F3U1	0.3	(0.1, 1.5)	0.0	[‡]	3.7	(2.5, 5.9)	3.4	(2.2, 6.1)	779	(530, 1255)	7.4	4.9, 12.4)
BC2F1	0.0	[‡]	0.5	(0.2, 2.9)	0.0	[‡]	1.6	(0.7, 6.2)	767	(444, 1637)	3.4	(1.7, 10.7)
BC2F1R1	0.1	(0.02, 2.0)	1.7	(1.0, 3.5)	0.0	[‡]	5.8	(3.8, 10.0)	0	[‡]	16.3	(10.8, 27.4)
BC2F2	0.2	(0.1, 1.2)	1.9	(1.1, 4.2)	3.3	(2.0, 6.4)	4.7	(2.9, 8.8)	1180	(780, 1995)	26.8	17.5, 46.4)
BC2F2R1	0.2	(0.1, 0.5)	0.6	(0.4, 1.2)	3.9	(2.9, 5.6)	2.6	(1.9, 3.9)	1011	(786, 1348)	18.5	(14.2, 25.2)
BC2F2U1	0.2	(0.1, 0.6)	1.1	(0.7, 2.0)	3.7	(2.6, 5.8)	0.0	[‡]	1113	(823, 1590)	19.0	(13.8, 27.9)
BC3F1	0.1	(0.0, 0.8)	0.0	[‡]	2.2	(1.4, 3.8)	2.2	(1.4, 3.8)	462	(331, 691)	5.3	(3.6, 8.5)
BC3F1R1	0.0	[‡]	0.0	[‡]	3.0	(2.0, 4.8)	2.5	(1.7, 4.0)	1222	(899, 1755)	5.6	(3.9, 8.6)
BC3F1U1	0.2	(0.1, 0.6)	0.5	(0.3, 1.2)	0.0	[‡]	3.0	(2.0, 4.7)	920	(678, 1319)	8.9	(6.6, 12.6)
TM1	0.0	[‡]	0.0	[‡]	0.2	(0.1, 0.5)	0.0	[‡]	89	(73, 111)	0.8	(0.6, 1.3)
FM832	0.0	[‡]	0.0	[‡]	0.5	(0.3, 0.8)	0.0	[‡]	139	(114, 172)	1.0	(0.7, 1.4)
PSC355	0.0	[‡]	0.0	[‡]	0.0	[‡]	0.0	[‡]	130	(107, 161)	0.0	[‡]
AcalaNem	0.0	[‡]	0.0	[‡]	0.8	(0.5, 1.2)	0.7	(0.5, 1.0)	248	(203, 308)	0.9	(0.6, 1.5)

[§]MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%).

[¶]95% confidence intervals in parenthesis.

[‡]No confidence interval calculated due to zero estimate of variance.

Table 22. Variances estimates and 95% confidence intervals by generations for fiber quality traits in 2006.

	MIC[§]		ELO[§]		UI[§]		UHML[§]		STR[§]		SFC[§]	
BC1F2R1	0.2	(0.1, 2.2)	0.5	(0.2, 2.3)	0.0	[‡]	1.1	(0.5, 5.0)	965	(566, 2006)	0.0	[‡]
BC1F3	0.0	[‡]	0.0	[‡]	5.2	(2.7, 14.2)	2.3	(1.1, 7.4)	1562	(877, 3528)	6.5	(3.4, 17.1)
BC1F3R1	0.8	(0.3, 6.3)	0.0	[‡]	2.7	(1.0, 16.1)	0.0	[‡]	458	(235, 1248)	0.0	[‡]
BC1F3U1	0.2	(0.1, 0.9)	0.3	(0.1, 1.0)	6.1	(4.3, 9.3)	3.8	(2.5, 6.5)	1480	(1039, 2277)	7.0	(4.7, 11.4)
BC2F1	0.0	[‡]	0.4	(0.2, 2.6)	1.9	(1.0, 5.1)	2.9	(1.5, 7.5)	1324	(807, 2563)	1.3	(0.6, 3.8)
BC2F1R1	0.2	(0.1, 1.3)	0.6	(0.3, 1.7)	2.6	(1.6, 5.3)	0.0	[‡]	1372	(896, 2361)	4.6	(2.8, 8.6)
BC2F2	0.3	(0.1, 6.6)	1.0	(0.4, 3.4)	2.8	(1.5, 7.3)	4.9	(2.7, 11.6)	2283	(1351, 4664)	6.1	(3.4, 14.1)
BC2F2R1	0.3	(0.2, 0.7)	0.4	(0.2, 0.9)	3.3	(2.4, 5.1)	3.5	(2.5, 5.4)	2055	(1547, 2864)	7.5	(5.5, 10.9)
BC2F2U1	0.1	(0.0, 0.6)	1.0	(0.6, 1.7)	4.6	(3.2, 7.0)	4.3	(3.0, 6.5)	1645	(1222, 2335)	6.3	(4.5, 9.5)
BC3F1	0.2	(0.1, 0.6)	0.3	(0.2, 0.8)	2.3	(1.5, 3.7)	3.8	(2.8, 5.5)	883	(649, 1272)	1.5	(1.0, 2.7)
BC3F1R1	0.1	(0.1, 0.6)	0.3	(0.2, 0.7)	2.3	(1.6, 3.6)	2.8	(2.0, 4.3)	1501	(1133, 2083)	2.2	(1.5, 3.5)
BC3F1U1	0.2	(0.1, 0.8)	0.1	(0.0, 1455.6)	3.5	(2.4, 5.6)	2.9	(2.0, 4.6)	0	[‡]	3.5	(2.4, 5.5)
TM1	0.0	[‡]	0.0	[‡]	0.8	(0.6, 1.2)	0.1	(0.0, 2.4)	238	(195, 297)	0.9	(0.7, 1.3)
FM832	0.0	[‡]	0.0	[‡]	0.8	(0.6, 1.3)	0.0	[‡]	415	(339, 519)	0.4	(0.2, 0.8)
PSC355	0.0	[‡]	0.0	[‡]	0.7	(0.5, 1.2)	0.0	[‡]	298	(246, 369)	0.0	[‡]
AcalaNem	0.0	[‡]	0.0	[‡]	0.3	(0.1, 0.8)	0.0	[‡]	339	(276, 428)	0.0	[‡]

[§]MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%).

[¶]95% confidence intervals in parenthesis.

[‡]No confidence interval calculated due to zero estimate of variance.

Table 23. Variances estimates and 95% confidence intervals by generations for yield and lint percent in 2005 and 2006.

	2005				2006			
	Yield[§]		Lint Percent[§]		Yield[§]		Lint Percent[§]	
BC1F1R1	17	(14, 22)[¶]	53	(33, 100)	46	(38, 57)[¶]	38	(25, 69)
BC1F2	145	(118, 182)	72	(39, 174)	55	(45, 68)	66	(35, 165)
BC1F2R1	0	[‡]	74	(56, 101)	738	(612, 908)	0	[‡]
BC1F3	410	(336, 510)	77	57, 110)	388	(322, 478)	59	(45, 79)
BC1F3R1	2153	(1534, 3241)	26	(16, 51)	3446	(2303, 5719)	67	(6, 1.2×10^{32})
BC1F3U1	1132	(937, 1395)	77	(60, 101)	1239	(1028, 1524)	59	(47, 76)
BC2F1	336	(278, 414)	86	(65, 118)	651	(539, 800)	0	[‡]
BC2F1R1	1621	(1344, 1994)	92	(71, 125)	0	[‡]	76	(59, 103)
BC2F2	894	(740, 1102)	84	(63, 117)	367	(304, 452)	88	(65, 125)
BC2F2R1	2796	(2315, 3444)	0	[‡]	1835	(1521, 2256)	44	(36, 57)
BC2F2U1	2291	(1896, 2824)	61	(49, 80)	1949	(1614, 2401)	0	[‡]
BC3F1	2371	(1963, 2921)	56	(44, 73)	1900	(1576, 2337)	68	(54, 88)
BC3F1R1	2282	(1842, 2901)	31	(24, 42)	1711	(1417, 2108)	46	(37, 58)
BC3F1U1	2836	(2348, 3493)	50	(40, 64)	1128	(932, 1394)	46	(37, 60)
TM-1	4325	(3581, 5327)	1	(0, 1)	3154	(2616, 3879)	3	(3, 4)
FM-832	7751	(6419, 9548)	1	(1, 2)	3757	(3111, 4628)	2	(2, 3)
PSC-355	6878	(5693, 8477)	1	(1, 2)	2410	(1996, 2968)	1	(1, 2)
AcalaNemx	3630	(3006, 4472)	6	(4, 7)	3231	(2679, 3973)	2	(2, 3)

[§]Yield = seed cotton yield (grams/plant), Lint Percent = lint percentage (%).

[¶]95% confidence intervals in parenthesis.

[‡]No confidence interval calculated due to zero estimate of variance.

Correlations

While there existed 11 different significant trait correlations at BC1, BC2, and BC3 levels, only three were consistent across the three backcross levels. These include a positive correlation between strength and uniformity index, and negative correlations between short fiber content and strength, and short fiber content and uniformity index (Tables 24, 25, 26). While other correlations are equally important, more emphasis has been given to see if there exist any correlation trends between yield and fiber quality traits. There was no trend of significant positive correlation that existed between yield

and the fiber quality traits in BC1 level generations. However, 4 of the 5 BC2 level generations and one of the 3 BC3 level generations had significant positive correlation between yield and fiber strength.

Other Challenges and Potential

Most of the plants in the BC1 level generations had severe fertility issues, but this problem decreased in subsequent backcrosses. This can be in part explained by the cytogenetic constitution of HLA-derived backcross generations, in that early generations included more hypo- and hyper-aneuploids, and high frequencies of univalents in the meiotic metaphase-I stage of meiosis than advanced backcross generations (Chapter II). Cytological analysis also suggested that with every backcross, the percentage of plants with euploid chromosomal complement and normal chromosomal pairing in meiosis increased and leads to more normal and fertile plants in the advanced generations (Chapter II). While, this germplasm needs to be evaluated for the potential insect and pest resistance/susceptibility, preliminary observations in the greenhouse indicate increased spider-mite susceptibility in some of this germplasm (Bell, personnel communication). Though no obvious linkage of this spider-mite susceptibility to any economical traits has been determined, further studies are needed to address this issue. While, *G. armourianum* has various xeromorphic adaptations, including a double palisade layer in the leaves, *G. longicalyx* is mesophytically adapted, thus offering the potential to select for phenotypes adapted to extreme weather conditions (Fryxell, 1984, p. 189; Bell, personnel communication). Selection for caducous bracts from *G. armourianum* should potentially reduce trash content of harvested cotton (Fryxell, 1984, p. 117-118; Stewart, 1995). There still remains large amount of genetic potential in this germplasm that remains to be evaluated.

Table 24. Phenotypic correlations calculated as Pearson's product-moment correlations among the fiber quality and yield traits averaged across years for the BC1 level generations.

		MIC																	
ELO†	BC1F2R1	0.23																	
	BC1F3	0.37	*																
	BC1F3R1	0.06																	
	BC1F3U1	0.24	*	ELO															
UI	BC1F2R1	0.38	*	0.12															
	BC1F3	0.56	*	0.12															
	BC1F3R1	0.44	*	0.13															
	BC1F3U1	0.36	*	0.26	*	UI													
UHML	BC1F2R1	0.30	*	-0.19		0.28	*												
	BC1F3	-0.27		-0.27		0.30													
	BC1F3R1	-0.13		-0.02		0.28													
	BC1F3U1	-0.07		0.04		0.57	*	UHML											
STR	BC1F2R1	0.12		-0.15		0.61	*	0.45	*										
	BC1F3	0.47	*	0.07		0.77	*	0.34	*										
	BC1F3R1	-0.08		0.27		0.63	*	0.52	*										
	BC1F3U1	0.25	*	0.28	*	0.74	*	0.54	*	STR									
SFC	BC1F2R1	-0.31	*	0.17		-0.70	*	-0.31	*	-0.48	*								
	BC1F3	-0.61	*	-0.04		-0.83	*	-0.21		-0.67	*								
	BC1F3R1	-0.54	*	0.06		-0.84	*	-0.23		-0.46	*								
	BC1F3U1	-0.41	*	-0.14		-0.81	*	-0.51	*	-0.71	*	SFC							
Yield	BC1F2R1	0.38	*	0.02		0.29	*	0.03		0.24		-0.2							
	BC1F3	-0.15		0.00		-0.16		0.12		-0.10		0.34	*						
	BC1F3R1	0.20		-0.08		-0.08		-0.12		-0.10		0.07							
	BC1F3U1	0.10		0.05		0.19		0.08		0.15		-0.2		Yield					
Lint%	BC1F2R1	0.21		0.22		-0.01		-0.33	*	-0.16		-0.2		0.29	*				
	BC1F3	0.44	*	0.46	*	0.21		-0.34	*	0.08		-0.4	*	0.18	*				
	BC1F3R1	0.72	*	0.15		0.35	*	-0.40	*	-0.11		-0.4	*	0.25					
	BC1F3U1	0.34	*	0.13		0.18		-0.12		0.09		-0.3	*	0.25	*				
		MIC		ELO		UI		UHML		STR		SFC		Yield					

*Significantly different from zero at the 0.05 probability level.

†MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%), Lint%, lint percentage; Yield, seed cotton yield (grams/plant).

Table 25. Phenotypic correlations calculated as Pearson's product-moment correlations among the fiber quality and yield traits averaged across years for the BC2 level generations.

		MIC		ELO		UI		UHML		STR		SFC		Yield	
ELO†	BC2F1	0.07													
	BC2F1R1	0.01													
	BC2F2	0.38	*												
	BC2F2R1	0.05													
	BC2F2U1	0.23	*												
UI	BC2F1	0.01		-0.03											
	BC2F1R1	0.03		-0.09											
	BC2F2	-0.22		-0.32	*										
	BC2F2R1	0.20	*	-0.04											
	BC2F2U1	0.04		-0.13											
UHML	BC2F1	-0.40	*	-0.32	*	0.54	*								
	BC2F1R1	-0.27	*	-0.17		0.58	*								
	BC2F2	-0.39	*	-0.34	*	0.71	*								
	BC2F2R1	0.10		-0.18	*	0.53	*								
	BC2F2U1	-0.06		-0.20	*	0.54	*								
STR	BC2F1	0.05		-0.16		0.43	*	0.15							
	BC2F1R1	-0.05		-0.21		0.62	*	0.49	*						
	BC2F2	0.09		-0.12		0.60	*	0.47	*						
	BC2F2R1	0.05		-0.02		0.72	*	0.48	*						
	BC2F2U1	-0.04		-0.20	*	0.56	*	0.45	*						
SFC	BC2F1	-0.03		-0.14		-0.77	*	-0.36	*	-0.33	*				
	BC2F1R1	-0.18		0.19		-0.80	*	-0.47	*	-0.49	*				
	BC2F2	0.14		0.48	*	-0.84	*	-0.55	*	-0.56	*				
	BC2F2R1	-0.30	*	0.17	*	-0.79	*	-0.47	*	-0.63	*				
	BC2F2U1	-0.16	*	0.16		-0.77	*	-0.49	*	-0.49	*				
Yield	BC2F1	0.01		-0.15		0.03		0.02		0.21		-0.1			
	BC2F1R1	0.07		-0.01		0.14		0.09		0.24	*	-0.1			
	BC2F2	0.17		0.11		0.21		0.11		0.34	*	-0.2			
	BC2F2R1	0.06		0.01		0.07		0.01		0.19	*	0.02			
	BC2F2U1	0.06		-0.07		0.32	*	0.01		0.25	*	-0.2	*		
Lint%	BC2F1	0.43	*	-0.20		0.20		-0.15		-0.16		-0.2		0.27	*
	BC2F1R1	0.34	*	0.01		-0.03		-0.31	*	-0.22	*	-0.1		0.23	*
	BC2F2	0.08		-0.15		0.01		-0.01		-0.11		-0.2		0.12	
	BC2F2R1	0.03		-0.05		-0.22	*	-0.21	*	-0.27	*	0.08		0.09	
	BC2F2U1	0.30	*	-0.18	*	-0.09		-0.24	*	-0.25	*	-0.1		0.24	*
		MIC		ELO		UI		UHML		STR		SFC		Yield	

*Significantly different from zero at the 0.05 probability level.

†MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%), Lint%, lint percentage; Yield, seed cotton yield (grams/plant).

Table 26. Phenotypic correlations calculated as Pearson's product-moment correlations among the fiber quality and yield traits averaged across years for the BC3 level generations.

		MIC																	
ELO[†]	BC3F1	0.13																	
	BC3F1R1	0.07																	
	BC3F1U1	0.19	*	ELO															
UI	BC3F1	0.15		0.03															
	BC3F1R1	0.15		0.12															
	BC3F1U1	0.23	*	0.00		UI													
UHML	BC3F1	-0.35	*	-0.13		0.36	*												
	BC3F1R1	0.22		-0.17	*	0.49	*												
	BC3F1U1	-0.17	*	-0.06		0.43	*	UHML											
STR	BC3F1	0.02		-0.02		0.71	*	0.28	*										
	BC3F1R1	-0.05		-0.04		0.67	*	0.56	*										
	BC3F1U1	0.07		-0.12		0.65	*	0.51	*	STR									
SFC	BC3F1	-0.21	*	-0.09		-0.78	*	-0.32	*	-0.61	*								
	BC3F1R1	-0.23	*	-0.11		-0.86	*	-0.43	*	-0.68	*								
	BC3F1U1	-0.18	*	0.13		-0.81	*	-0.43	*	-0.64	*	SFC							
Yield	BC3F1	0.16		0.03		0.11		0.06		0.09		-0.10							
	BC3F1R1	0.28	*	-0.03		0.07		0.00		0.04		-0.14							
	BC3F1U1	0.23	*	0.10		0.21	*	0.15		0.24	*	-0.17	*	Yield					
Lint%	BC3F1	0.23	*	-0.03		0.05		-0.23	*	-0.20	*	-0.16		0.22	*				
	BC3F1R1	0.24	*	0.05		-0.10		-0.19	*	-0.27	*	0.03		0.08					
	BC3F1U1	0.32	*	0.03		-0.01		-0.24	*	-0.28	*	-0.08		0.12	*				
		MIC		ELO		UI		UHML		STR		SFC		Yield					

*Significantly different from zero at the 0.05 probability level.

[†]MIC = micronaire, ELO = elongation (%), UI = uniformity index (%), UHML = upper half mean length (mm), STR = strength (kN m kg^{-1}), SFC= short fiber content (%), Lint%, lint percentage; Yield, seed cotton yield (grams/plant).

Conclusion

Low micronaire readings and high fiber strength were the two most promising traits identified in the HLA-derived backcross generations. Micronaire and fiber strength are amongst the few important fiber quality traits that affect the market price of cotton in domestic and international markets. Tronstad et al. (2000) reported that there has been an average micronaire increase in the cultivars, grown from 1975-2000 in the US cotton belt region with an increment of 0.0092 micronaire per year. An ideal range for micronaire to receive premium price is from 3.7-4.2 and price discount begins with readings less than 3.5 and more than 4.9 (Bradow and Davidonis, 2000; Perkins et al., 1984). This germplasm, which had a micronaire range from 3.53 to 4.27, will provide valuable genetic resources to achieve optimum micronaire readings in the current cultivars that fail to achieve that goal. The fiber strength for the introgressed generations ranged from 253 to 294 kN m kg^{-1} (25.8-30 g tex^{-1}) in 2005 and 231 to 287 kN m kg^{-1} (23.5-29.3 g tex^{-1}) in 2006. Most of introgressed generations had higher strength than TM-1, while the generations at BC2 and BC3 level had higher or non-significantly different strength than FM-832, which is considered as one of the best fiber strength cultivars. Choices for selection procedures depend on how heritable the trait is. Heritabilities for the traits in this germplasm were not estimated, but different studies have predicted different heritabilities for micronaire, ranging from 8% (Al-Rawi and Kohel, 1969) to 68% (Al-Jibouri et al., 1958), thus indicating low to moderate levels of heritability for micronaire. Thus, selection for micronaire should be based on progeny testing of the selected individuals. For traits that are not greatly influenced by environment, such as strength, which has high heritability ranging from 52% to 90% (Baker and Verhalen, 1975; Al-Jibouri et al., 1958), selections can be effectively done based on individual plant data.

Detailed analysis of the germplasm may help identify some of the high-impact genes and their origin. Though the genetic source(s) for these traits was not addressed in this work, earlier studies by Demol et al. (1978) revealed finer and stronger fiber in the *G. hirsutum* x *G. longicalyx* allohexaploids, which suggests that these attributes of HLA-

derived germplasm may be due to introgression from *G. longicalyx.* However, molecular marker tools should be used to determine the respective contributions of *G. armourianum* versus *G. longicalyx* to these valuable traits. Furthermore, some of the good fiber characteristics in this germplasm could trace to Acala Nemx, which has good fiber quality including low micronaire and high fiber strength, and was used as one of the parents in the early backcrossing efforts (Fig. 9). In addition to this, *G. armourianum* introgressed-germplasm has been reported to have excellent fiber strength, fiber length and micronaire values (Zhou et al., 2003), so even if they are of alien origin, the beneficial novel alleles contributing to high fiber strength and low micronaire values in this germplasm may or may not have originated from *G. longicalyx*.

While, the origin of these beneficial alleles contributing to low micronaire readings and high fiber strength remains to be evaluated, we speculate that the majority of novel alleles could have been contributed by the exotic germplasm. Though other fiber quality traits were not as promising as that of micronaire and fiber strength in the introgressed generations, it is quite likely that the exotic germplasm might have contributed some novel beneficial alleles for these other fiber-quality traits. Moreover, most of the introgressed generations had higher plant-to-plant variability for fiber quality traits than the checks, thus providing more opportunities for selection.

Most of the BC3 level generations had significantly higher yields and lint percentage than BC2 level generations, indicating either accumulation of favorable alleles for these traits, or reduction of alien germplasm predominantly associated with undesirable linkages, or both. Random-mating did not significantly change the generation means for lint percent or any fiber quality traits, with the exception of BC2F2R1 in 2006, which had significantly higher means than the original BC2F2 generation. There was an increase in the yield for all the random-mated generations; however the differences were significant only for BC2F2R1 in both years and BC1F3R1 in 2005. This increase in mean is more likely to occur due to natural selection, but other factors, including epistasis involving linkages in the original population cannot be discounted.

Random-mating lowered the fiber quality variances for the BC1F3R1 than BC2F2R1 and BC3F1R1, indicating the existence of the higher coupling phase linkages between the loci associated with those traits in BC1F3 generation than BC2F2 and BC3F1 generations. For yield, however, BC1F3R1 and BC2F2R1 had higher variances, while BC3F1R1 had decreased variance. There was a positive correlation between yield and fiber strength for all the BC2 and BC3 level generations, which would allow simultaneous selection for both traits. However, there were no other significant correlations between yield and other traits that were consistent across all three backcross generations. All the introgressed generations had significantly lower yields than TM-1 and the 3 commercial checks (FM-832, PSC-355, and Acala Nemx) and need more backcrosses to high yielding cultivars to achieve higher yields. Greenhouse data suggest that atleast five to six backcrosses are required to boost the yields of this HLA-derived germplasm (Bell, personnel communication). Based on our findings and findings from a parallel backcross breeding effort to introgress resistance from one of the wild species used in creating the HLA tri-species hybrid used to create this germplasm (Bell et al., 2007; Robinson et al., 2007), we recommend random-mating at early generations to break unfavorable linkage blocks as well as generate variability, and then backcrossing the identified transgressive segregants for five to six generations as a breeding methodology for this germplasm.

CHAPTER IV

CONCLUSION

Reniform nematode has become a serious nematode pest of cotton in the United States causing heavy economic losses to US farmers. Due to lack of high reniform nematode-resistance in the *G. hirsutum* germplasm and cultivars, reniform nematode control is mainly done by the application of nematicides, use of crop rotations, and planting of tolerant breeding lines. However, planting resistant cultivars remains the optimum solution to this important problem. Extensive screening of the *Gossypium* germplasm led to the identification of a few resistant sources, including diploid cotton *G. longicalyx*, which was classified as immune to this pest. To transfer resistance from the diploid species, *G. longicalyx* into tetraploid *G. hirsutum*, a pseudophyletic breeding scheme was followed that involved creation of a sterile triploid by crossing tetraploid *G. hirsutum* with diploid *G. longicalyx*. The obtained triploid was doubled by colchicine application to create a fertile hexaploid which was then crossed with a D-genome bridging species, *G. armourianum* to create a 52 chromosome hybrid, designated as HLA (*G. hirsutum*|*G. longicalyx*||*G. armourianum*) (Bell and Robinson, 2004). The 52-chromosome HLA tri-species hybrid was backcrossed to *G. hirsutum* with reniform nematode screening and cytogenetic evaluation at each generation to transfer the reniform nematode-resistance locus into *G. hirsutum* (Robinson et al., 2007) and the resistant germplasm was released at BC7S1 generation (Bell et al., 2007).

In concert with backcross breeding to introgress resistance from *G. longicalyx* into *G. hirsutum,* plants were cytologically analyzed by meiotic metaphase I analysis. Chromosomal numbers and chromosome configurations were determined for the HLA tri-species hybrid, and its backcross-derived plants from BC1F1, BC1S1, BC2F1, BC3F1, BC4F1, and BC5F1 generations. The results helped guide the breeding process in terms of selection and by providing important insights into the chromosome number, composition, the inheritance of resistance, and recombination rates between A and F

homeologs. Progress towards the euploid state (26II) was evident in early generations. Highly resistant plants with 52 chromosomes and euploid chromosomal complement (26II) were identified at BC2F1, BC3F1, BC4F1, and BC5F1 generations, and were included in the backcross breeding scheme to introgress reniform nematode-resistance into *G. hirsutum*. A trend of increase in the percentage of plants with modal chromosomal configuration of normal 26II pairing was observed with every backcrossing, where BC1F1 had 0%, while BC5F1 had 100% of the plants with 26II. To estimate crude rate of recombination between the *G. longicalyx*-chromosome, carrying the resistance gene and the *G. hirsutum* chromosome, resistant BC2F1 plants created by crossing resistant BC1F1 plants with *G. hirsutum* pollen were analyzed for the metaphase I chromosomal configurations. Two of the 17 highly resistant BC2F1 plants had 26II configuration in 100% of their PMCs, indicating a proximal recombination event in *ca*. 12% of megasporocytes of BC1F1 resistant plants.

To enable breeders to transfer reniform nematode resistance from the introgressed tetraploid-germplasm into different cultivars without having to conduct nematode resistance evaluations in pots, led to the second goal of this project of identifying flanking markers that are tightly linked to the reniform nematode resistance loci. Preliminary screening of 58 and four SSR markers from the A- and D-subgenomes of *G. hirsutum*, respectively, led to the identification of three putatively-linked co-dominant markers, BNL3279_114, BNL1066_156, and BNL836_215. In addition to these three linked molecular markers, green-colored fuzz (Fzg^{lon}), a maternal trait expressed in the seed coat was also observed to be co-segregating with the reniform nematode-resistance locus. Extension of the association analysis and linkage estimation to 16 susceptible self progeny (BC1S1, BC3S1 and BC6S1) and 374 susceptible backcross hybrids (BC2-BC8) mapped the resistance locus to chromosome 11 of cotton with BNL3279_114 on one side and Fzg^{lon} on the other at 0.8 and 2.8 cM, respectively. Use of the flanking markers will enable breeders to transfer the resistance locus into different breeding lines and cultivars with ease and precision.

Though the two wild diploid species, *G. longicalyx* and *G. armourianum* used in the creation of HLA tri-species hybrid produce only small amounts of cotton lint, they could possess some favorable alleles for fiber quality and yield-related traits that can be introgressed into *G. hirsutum*. The HLA-derived backcrossed generations provided an excellent opportunity to evaluate fiber-related traits introgressed from these wild species into *G. hirsutum*. Micronaire readings of the introgressed generations ranged from 3.41 to 4.12 in 2005, and 3.64 to 4.84 in 2006, while the checks, TM-1, FM-832, PSC-355, and Acala Nemx had micronaire readings of 4.62, 4.49, 4.84, and 4.02, respectively, in 2005, and 4.89, 4.08, 4.31, and 3.97, respectively, in 2006. Since the ideal range for micronaire is from 3.7-4.2, the HLA-derived germplasm provides additional genetic resources to lower the micronaire values of the US cotton. The other fiber quality trait this germplasm could offer is fiber strength. Fiber strength for the introgressed generations ranged from 253 to 294 kN m kg^{-1} in 2005 and 231 to 287 kN m kg^{-1} in 2006, while the strength for the checks, TM-1, FM-832, PSC-355, and Acala Nemx was 258, 292, 269, and 312 kN m kg^{-1}, respectively, in 2005, and 246, 274, 269, and 303 kN m kg^{-1}, respectively, in 2006.

Random-mating at BC1, BC2 and BC3 level generations did not change the generation means for lint percent and all fiber quality traits, the only exceptions being increased fiber length in 2005 and increased fiber strength in 2006 in the BC2F2R1 generation compared to its generation of origin, BC2F2. Yield of the BC2F2R1 generation was consistently higher than BC2F2 for both years, while BC1F3R1 had higher yield than BC1F3 in 2006. This increased mean in the random-mated generations could be attributed to epistasis or natural selection during the creation of random-mated generations, or both. Random-mated generation, BC1F3R1 had lower variances for most of the fiber quality traits, compared to its generation of origin. All three random-mated generations had lower variances for lint percent, compared to their, respective, generations of origin, while BC1F3R1 and BC2F2R1 had higher variances for yield, compared to BC1F3, and BC2F2, respectively. The lower variances in the random-mated generations could be attributed to the existence of predominant coupling-phase

linkages between the loci associated with the trait in the original generations, while increased variances could be attributed to the existence of predominant repulsion-phase linkages between the loci associated with the trait in the original generations.

The three positive correlations that were consistent across BC1, BC2, and BC3 levels include positive correlation between strength and uniformity index, negative correlation between short fiber content and uniformity index, and negative correlation between short fiber content and strength. Significant positive correlation between yield and fiber strength were observed in 4 out of 5 BC2 level generations and one out of 3 BC3 level generations.

All the introgressed generations had lower yields than TM-1 and the three commercial checks (FM-832, PSC-355, and Acala Nemx). With the inclusion of two wild species in this novel introgressed germplasm, one would expect lower yields during the early phases of breeding because of the fact that these exotic species were never selected for high yields. Though these species hardly yield any fiber, they could possess some beneficial alleles that can be introgressed in the cultivated cotton germplasm, and thus would broaden the genetic base of cotton and provide more opportunities for selection. Use of backcrossing along with random-mating would provide more opportunities to select for transgressive segregants. While, use of molecular markers in identifying and introgressing these potential chromosomal regions is one of the ways we can capture the exotic potential, new methodology to capture beneficial alleles from these wild species needs to be addressed.

REFERENCES

Abdalla, A.M., O.U.K. Reddy, K.M. El-Zik, and A.E. Pepper. 2001. Genetic diversity and relationships of diploid and tetraploid cottons revealed using AFLP. Theor. Appl. Genet. 102:222-229.

Agudelo, P.A., R.T. Robbins, K.S. Kim, and J.M. Stewart. 2005. Histological changes in *Gossypium hirsutum* associated with reduced reproduction of *Rotylenchulus reniformis*. J. Nematol. 37:185-189.

Al-Jibouri, H.A., P.A. Miller, and H.F. Robinson. 1958. Genotypic and environmental variances and covariances in an Upland cotton cross of interspecific origin. Agron. J. 50:623-636.

Al-Rawi, K.M., and R.J. Kohel. 1969. Diallel analysis of yield and other agronomic characters in *Gossypium hirsutum* L. Crop Sci. 9:779-783.

Altman, D.W., and R.H. Busch. 1984. Random intermating before selection in spring wheat. Crop Sci. 24:1085-1089.

Anonymous. 1972. Genetic vulnerability of major crops. Reports of committee on genetic vulnerability of major crops. Agri. Board Div. of Biol. and Agric., NRC NAS, Washington, DC. p. 307.

Arondel, V., I. Lemieux, Gibson, N.H. Goodman, and C.R. Sommerville. 1992. Map-based cloning of a gene controlling Omega-3 fatty acid desaturatuion in *Arabidopsis*. Science 258:1353-1355.

Bailey, T.B. 1977. Selection limits in self-fertilizing populations following the cross of homozygous lines. p. 399-412. *In* E. Pollack, et al., (ed.) Proc. Int. Conf. Quant. Genet. Iowa State University Press, Ames, IA.

Baker, J.L., and L.M. Verhalen. 1975. Heterosis and combining ability for several agronomic and fiber properties among selected lines of Upland cotton. Cott. Grow. Res. 52:209-223.

Beasley, J.O. 1940. The origin of American tetraploid *Gossypium* species. Amer. Nat. 74:285-286.

Beasley, J.O. 1942. Meiotic chromosome behavior in species, species hybrids, haploids, and induced polyploids of *Gossypium*. Genetics 27:25-54.

Bell, A.A., 1984. Morphology, chemistry, and genetics of *Gossypium* adaptations to pests. p. 197–230. *In* B.N. Timmermann, C. Steelink and F.A. Loewus (ed.) Recent Advances in Phytochemistry. Plenum Press, New York.

Bell, A. A., and A.F. Robinson. 2004. Development and characteristics of tri-species hybrids used to transfer reniform nematode resistance from *Gossypium longicalyx* to *Gossypium hirsutum*. p. 422-426. *In* Proc. Beltwide Cotton Prod. Res. Conf., San Antonio, TX. 5-9 Jan. 2004. Natl. Cotton Counc. Am. Memphis, TN.

Bell, A.A., R.D. Stipanovic, G.W. Elzen, and H.J. Williams. 1987. Structural and genetic variation of natural pesticides in pigment glands of cotton (*Gossypium*). p. 477–490. *In* G.R. Waller (ed.) Allelochemicals: Role in agriculture and forestry. American Chemical Society, Washington, DC.

Bell, A.A., R.D. Stipanovic, M.E. Mace, and R.J. Kohel. 1994. Genetic manipulation of terpenoid phytoalexins in *Gossypium*: Effects on disease resistance. p. 231–249. *In* B.E. Ellis, G.W. Kuroki and H.A. Stafford (ed.) Genetic Engineering of Plant Secondary Metabolism. Plenum Press, New York.

Bell, A.A., A.F. Robinson, N.D. Dighe, M.M. Menz, R. Nichols, and D.M. Stelly. 2007. Release of two reniform nematode resistant Upland cotton germplasm lines. (Germplasm release).

Benedict, J.H., M.F. Treacy, D,W. Altman, and K.M. Schmidt. 1987. Preference of boll weevils and tobacco budworms for five species of *Gossypium*. p. 92. *In* Proc. Beltwide Cotton Conf., Dallas, TX. 5-9 Jan. 1987. Natl. Cotton Counc. Am. Memphis, TN.

Birchfield, W. 1962. Host-parasitic relations of *Rotylenchulus reniformis* on *Gossypium hirsutum*. Phytopathol. 52:862-865.

Birchfield, W., and J.E. Jones. 1961. Distribution of the reniform nematode in relation to crop failure of cotton in Louisiana. Plant Dis. Rept. 45:671-673.

Birchfield, W., and L.R. Brister. 1962. New hosts and non-hosts of reniform nematode. Plant Dis. Rep. 46:683-685.

Birchfield, W., and W.J. Martin. 1967. Reniform nematode survival in air-dried soil. Phytopathol. 57:804 (Abstract).

Bird, A.F. 1983. Growth and molting in nematodes: Changes in the dimensions and morphology of *Rotylenchulus reniformis* from start to finish of molting. Int. J. Parasitol. 13:201-206.

Bird, A.F. 1984. Growth and molting and development of the hatched larvae of *Rotylenchulus reniformis*. Parasitol. 89:107-119.

Blasingame, D. 2006. Cotton disease loss estimate committee report p. 155-157. *In* Proc. Beltwide Cotton Prod. Res. Conf., San Antonio, TX 3-6 Jan. 2006. Natl. Cotton Counc. Am. Memphis, TN.

Blenda, A., J. Scheffler, B. Scheffler, M. Palmer, J-M. Lacape, J.Z. Yu, C. Jesudurai, S. Jung, S. Muthukumar, P. Yellambalase, S. Ficklin, M. Staton, R. Eshelman, M. Ulloa, S. Saha, B. Burr, S. Liu, T. Zhang, D. Fang, A. Pepper, S. Kumpatla, J. Jacobs, J. Tomkins, R. Cantrell, and D. Main. 2006. CMD: A cotton microsatellite database resource for *Gossypium* genomics. BMC Genomics. 7:132

Bollenbacher, K., and N.D. Fulton. 1971. Susceptibility of *Gossypium* species and varieties to seedling anthracnose. Plant Dis. Rept. 55:879-882.

Bowman, D.T., O.L. May, and D.S. Calhoun. 1996. Genetic base of Upland cotton cultivars released between 1970 and 1990. Crop Sci. 36:577-581.

Bradow, J.M., and G.H. Davidonis. 2000. Quantitation of fiber quality and the cotton production-processing interface: A physiologist's perspective. J. Cotton Sci. 4:34-64.

Bridges, W.C., and C.O. Gardner. 1987. Foundation populations for adapted by exotic crosses. Crop Sci. 27:501-506.

Brown, M.S. 1951. The spontaneous occurrence of amphiploidy in species hybrids of *Gossypium*. Evol. 5:25-41.

Brown, M.S. 1954. A comparison of pachytene and metaphase pairing in species hybrids of *Gossypium*. Genetics 39:962-963

Brown, M.S. 1958. The implications of pacytene and metaphase pairing for species differentiation. X. Int. Congress of Genetics Proc. 2:36.

Brown, M.S. 1961. Chromosome differentiation in *Gossypium*. Am. J. Bot. 48:532.

Brown, M.S., and M.Y. Menzel. 1952. The cytology and crossing behavior of *Gossypium gossypioides*. Bull. Torrey Bot. Club 79:110-125.

Brubaker, C.L., and J.F. Wendel. 1994. Reevaluating the origin of domesticated cotton (*Gossypium hirsutum*; Malvaceae) using nuclear restriction fragment length polymorphisms (RFLPs). Am. J. Bot. 81:1309-1326.

Brubaker, C.L., A.H.D. Brown, J.M. Stewart, M.J. Kilby, and J.P. Grace. 1999. Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. Euphytica 108:199-213.

Burr, P., J. Johnson, H. Fazel, K. Scearce, and H. Waters. 2006. World cotton supply, use and trade, 2001/02-2006-07. Cotton: World markets and trade. USDA, FAS, Series FC-07-06. http://www.fas.usda.gov/cotton/circular/2006/07/toc.htm

Carter, W.W. 1981. Resistance and resistance reaction of *Gossypium arboreum* to the reniform nematode, *Rotylenchulus reniformis*. J. Nematol. 13:369-374.

Chang, C., S.F. Kwok, A.B. Bleecker, and E.M. Meyerowitz. 1993. *Arabidopsis* ethylene-responsive gene ERT1: Similarity of product to two-component regulators. Science 262:539-544.

Cook, C. G., and A. F. Robinson. 2005. Registration of RN96425, RN96527, and RN96625-1 nematode-resistant cotton germplasm lines. Crop Sci. 45:1667-1668.

Cook, R., and K. Evans. 1987. Resistance and tolerance. p. 179-231. *In* R.H. Brown and B.R. Kerry (ed.) Principles and practice of nematode control in crops. Academic Press. Sydney.

Cook, R.J., C.J. Gabriel, A. Kelman, S. Tolin, and A.K. Vidaver. 1995. Research on plant disease and pest management is essential to sustainable agriculture. BioScience 45:354-357.

Davis, R.F., S.R. Koenning, R.C. Kemerait, T.D. Cummings, and W.D. Shurley. 2003. *Rotylenchulus reniformis* management in cotton with crop rotation. J. Nematol. 35:58-64.

Demol, J., and R. Marechal. 1975. Contribution to the study of drought resistance in some allohexaploid cottons. (In French, with English abstract.) Bulletin des Recherches Agronomiques de Gembloux. 1975. 10:151-162.

Demol, J., L. Verschraege, and R. Marechal. 1978. Use of wild species in cotton breeding: Observations on the technological characters of new allohexaploid forms. (In French, with English abstract.) Coton Fibres Trop. 1978. 33:327-333.

Dudley, J.W. 1982. Theory for transfer of alleles. Crop Sci. 22:631-637.

Ehou, M. K. 1983. Attempt at utilization of the wild diploid species *G. longicalyx* for the improvement of the cultivated tetraploid species *G. hirsutum* by the method of addition and substitution lines. (In French.) Universite Pierre et Mari Curie, Paris.

Emdrizzi, J.E. 1957. The cytology of two hybrids of *Gossypium*. J. Hered. 48:221-226.

Endrizzi, J.E. 1959. Cytogenetics of four sets of twins in cotton. J. Hered. 50:222-226.

Endrizzi, J.E. 1962. The diploid-like cytological behavior of tetraploid cotton. Evol. 16:325-329.

Endrizzi, J.E., and L.L. Phillips. 1960. A hybrid between *Gossypium arboreum* L. and *G. raimondii* Ulb. Can. J. Genet. Cytol. 2:311-319.

Endrizzi, J.E., E.L. Turcotte, and R.J. Kohel. 1985. Genetics, cytology and evolution of *Gossypium*. Adv. Genet. 23:271-375.

Eshad, Y., and D. Zamir. 1994. Introgressions from *Lycopersicon pennellii* can improve the soluble-solids yield of tomato hybrids. Theor. Appl. Genet. 88:891-897.

Fassuliotis, G. 1979. Plant breeding for root-knot nematode resistance. p. 425-453. *In* F. Lamberti, and C.E. Taylor (ed.) Root-knot nematodes (*Meloidogyne* species): Systematics, biology and control. Academic Press, New York.

Fehr, W.R., and R.C. Clark. 1973. Registration of five soybean germplasm populations. Crop Sci. 13:778.

Frelichowski, J.E., M. Palmer, D. Main, J.P. Tomkins, R.G. Cantrell, D.M. Stelly, J. Yu, R.J. Kohel, and M. Ulloa. 2006. Cotton genome mapping with new microsatellites from Acala 'Maxxa' BAC-ends. Mol. Gen. Genomics 275:479-491.

Frerich, S.J. 1995. Evaluation of *Gossypium longicalyx* monosomic addition lines of *Gossypium hirsutum* for resistance to *Rotylenchulus reniformis* nematode. M.S. thesis. Texas A&M Univ., College Station.

Fryxell, P.A. 1976. Germplasm utilization: *Gossypium*, a case history. USDA Rep. ARS-S-137.

Fryxell, P.A. 1979. The natural history of the cotton tribe (Malvaceae, tribe Gossypieae). Texas A&M Univ. Press, College Station.

Fryxell, P.A. 1984. Taxonomy and germplasm resources. *In* R.J. Kohel and C.F. Lewis (ed.) Cotton, Agronomy Monograph No. 24. Crop Science Society of America, Madison, WI.

Fryxell, P.A. 1992. A revised taxonomic interpretation of *Gossypium* L. (Malvaceae). Rheedea 2:108-165.

Gao, W., Z.J. Chen, J.Z. Yu, D. Raska, R.J. Kohel, J.E. Womack, and D.M. Stelly. 2004. Wide-cross whole-genome radiation hybrid (WWRH) mapping of cotton (*Gossypium hirsutum* L.). Genetics 167:1317-1329.

Gao, W., Z.J. Chen, J.Z. Yu, R.J. Kohel, J.E. Womack, D.M. Stelly. 2006. Wide-cross whole-genome radiation hybrid mapping of the cotton (*Gossypium barbadense* L.) genome. Mol. Gen. Genom. 275:105-113.

Gaur, H.S., and R.N. Perry. 1991. The biology and control of plant parasitic nematode *Rotylenchulus reniformis*. Agric. Zool. Rev. 4:177-212.

Gazaway, W. S., J. R. Akridge, and K. McLean. 2000. Impact of various crop rotations and various winter cover crops on reniform nematode in cotton. p. 162-163. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 4-8 Jan. 2000. Natl. Cotton Counc. Am., Memphis, TN.

Gazaway, W. S., J. R. Akridge, and R. Rodríguez-Kábana. 1998. Management of reniform nematode in cotton using various rotation schemes. p. 141-142. *In* Proc.

Beltwide Cotton Conf., San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.

Gerstel, D.U. 1953. Chromosome translocations in interspecific hybrids of the genus *Gossypium*. Evol. 7:234-244.

Gerstel, D.U. 1954. A new lethal combination in interspecific cotton hybrids. Genetics 39:628-639.

Giraudat, J., B.N. Hauge, C. Valon, J. Smalle, F. Parcy, and H.M. Goodman. 1992. Isolation of the *Arabidopsis AB-13* gene by positional cloning. Plant Cell 4:1251-1261.

Givry, S., M. Bouchez, P. Chabrier, D. Milan, and T. Schiex. 2005. Carthagene: multipopulation integrated genetic and radiated hybrid mapping. Bioinformatics 21:1703-1704.

Gupta, PK., and S. Rustgi. 2004. Molecular markers from the transcribed/expressed region of the genome in higher plants. Funct. Integr. Genom. 4:139-162.

Haldane, J.B.S. 1919. The combination of linkage values, and the calculation of distance between the loci of linked factors. J. Genet. 8:299-309.

Hallauer, A.R. 1978. Potential of exotic germplasm for maize improvement. p. 229-247. *In* D.B. Walden (ed.) Maize breeding and genetics. John Wiley & Sons, New York.

Harlan, J.R., and J.M.J. deWet. 1971. Towards a rationale classification of cultivated plants. Taxon. 20:509-517.

Harrell, S.C., and T.W. Culp. 1979. Registration of Pee Dee 0259 and Pee Dee 2165 germplasm lines of cotton (Reg. No. GP39 and GP40). Crop Sci. 19:418.

Ho, Y.T., and R.E. Comstock. 1980. Combining superior alleles from two homozygous populations in cross-fertilizing species. Genet. Res. 36:277-287.

Hoffbeck, M.D., S.J. Openshaw, J.L. Geadelmann, R.H. Pterson, and D.D. Stuthman. 1995. Backcrossing and intermating in an exotic x adapted cross of maize. Crop Sci. 35:1359-1364.

Hutchinson, J. 1959. The application of genetics to cotton improvement. Cambridge University Press, Cambridge, UK.

Hutchinson, J.B., and B.J.S. Lee. 1958. A new species of *Gossypium* from central Tanganyika. Notes from East Africa herbarium: IX. Kew Bulletin p. 221-222.

Hutchinson, J.B., R.A. Silow, and S.G. Stephens. 1947. The evolution of *Gossypium*. Oxford Univ. Press, London and New York.

Hutchinson, J.B., S.G. Stephens, and K.S. Dodd. 1945. The seed hairs of *Gossypium*. Ann. Bot. 9:361-367.

Iqbal, J., O.U.K. Reddy, K.M. El-Zik, A.E. Pepper. 2001. A genetic bottleneck in the 'evolution under domestication' of Upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. Theor. Appl. Genet. 103:547–554.

Islam-Faridi, M.N., K.L Childs, P.E. Klein, G. Hodnett, M.M. Menz, R.R. Klein, W.L. Rooney, J.E. Mullet, D.M. Stelly, and H.J. Price. 2002. A molecular cytogenetic map of sorghum chromosome *1*: Fluorescence *in situ* hybridization analysis with mapped bacterial artificial chromosomes. Genetics 161:345-353.

Jiang, C., R.J. Wright, K.M. El-Zik, and A.H. Paterson. 1998. Polyploid formation created unique avenues for response to selection in *Gossypium* (cotton). Proc. Natl. Acad. Sci. USA 95:4419-4424.

Jones, J.E, J.P. Beasley, J.I. Dickson, and W.D. Caldwell. 1988. Registration of four cotton germplasm lines with resistance to reniform and root-knot nematodes. Crop Sci. 28:199-200.

Kammacher, P. 1966. Study of the genetic and caryologic relations between genomes close to the Gossypium kind. (In French, with English conclusion.) *Coton et Fibres Tropicales* 21:263-290.

Kebede, H., G. Burow, R.G. Dani, and R.D. Allen. 2007. A-genome cotton as a source of genetic variability for Upland cotton (*Gossypium hirsutum*). Genet. Resour. Crop Evol. 54:885-895.

Kenworthy, W.J. 1980. Strategies for introgressing exotic germplasm in breeding programs. p. 217-223. *In* F.T. Corbin (ed.) Proc. World Soybean Res. Conf. II. Westview Press, Boulder, CO.

Khan, R.M., and M.W. Khan. 1985. Histopathology of cauliflower roots infected with reniform nematode. Pakistan J. Nematol. 3:73-82.

Khan, S.A., D. Hussain, E. Askari, J.M. Stewart, K.A. Malik, and Y. Zafar. 2000. Molecular phylogeny of *Gossypium* species by DNA fingerprinting. Theor. Appl. Genet. 101:931-938.

Kim, J.S., K.L Childs, M.N. Islam-Faridi, M.M. Menz, R.R. Klein, P.E. Klein, H.J. Price, J.E. Mullet, and D.M. Stelly. 2002. Integrated karyotyping of sorghum by *in situ* hybridization of landed BACs. Genome 45:402-412.

Kim, J.S., P.E. Klein, R.R. Klein, H.J. Price, J.E. Mullet, and D.M. Stelly. 2005. Molecular cytogenetic maps of sorghum linkage groups 2 and 8. Genetics 169:955-965.

Kimber, G. 1961. Basis of the diploid-like meiotic behavior of polyploidy cotton. Nature 191:98-100.

Kinloch, R. A., and J. R. Rich. 2001. Management of root-knot and reniform nematodes in ultra-narrow row cotton with 1,3-dichloropropene. J. Nematol. 33:311-313.

Koenning, S. R., K. R. Barker, and D. T. Bowman. 2000. Tolerance of selected cotton lines to *Rotylenchulus reniformis*. J. Nematol. 32:519-523.

Koenning, S.R., T.L. Kirkpatrick, J.L. Starr, J.A. Wrather, N.R. Walker, and J.D. Mueller. 2004. Plant-parasitic nematodes attacking cotton in the United States: Old and emerging production challenges. Plant Dis. 88:100-113.

Konan, O.N., A.D. Hont, J.P. Baudoin, and G. Mergeai. 2007. Cytogenetics of a new trispecies hybrid in cotton: [(*Gossypium hirsutum* L. x *G. thurberi* Tod.)^2 x G. *longicalyx* Hutch. & Lee.]. Plant Breeding 126:176-181.

Lacape, J.M., T.B. Nguyen, S. Thibivilliers, B. Bojinov, B. Courtois, R.G. Cantrell, B. Burr, and B. Hau. 2003. A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* x *Gossypium barbadense* backcross population. Genome 46:612-626.

Lacape, J.M., T.B. Nguyen, B. Courtois, J.L Belot, M. Giband, J.P. Gourlot, G. Ggawryziak, S. Roques, and B. Hau. 2005. QTL analysis of cotton fiber quality using multiple *Gossypium hirsutum* x *Gossypium barbadense* backcross generations. Crop Sci. 45:123-140.

Lacape, J.M., D. Dessauw, M. Rajab, J.L. Noyer, and B. Hau. 2007. Microsatellite diversity in tetraploid *Gossypium* germplasm: Assembling a highly informative genotyping set of cotton SSRs. Mol. Breeding 19:45-58.

Lamkey, K.R., B.J. Schnicker, and A.E. Melchinger. 1995. Epistasis in an elite maize hybrid and choice of generation for inbred development. Crop Sci. 35:1272-1281.

Lawrence, G.W. 1999. Managing the reniform nematodes with nematicides. p. 100-101. *In* Proc. Beltwide Cotton Conf., Orlando, FL 6-10 Jan. 1999. Natl. Cotton Counc. Am. Memphis, TN.

Lawrence, G.W., and K.S. McLean. 1995. Distribution of nematodes. p. 196. *In* Proc. Beltwide Cotton Prod. Res. Conf., San Antonio, TX 4-7 Jan. 1995. Natl. Cotton Counc. Am. Memphis, TN.

Lawrence, G.W., and K.S. McLean. 2001. The reniform nematode. p. 42-44. *In* T.L. Kirkpatrick, and C.S. Rothrock (ed.) Compendium of cotton diseases. APS Press, St. Paul, MN.

Lawrence, G. W., K. S. McLean, W. E. Batson, D. Miller, and J. C. Borbon. 1990. Response of *Rotylenchulus reniformis* to nematicide applications on cotton. J. Nematol. 22:707-711.

Lawrence, P.K., and K.J. Frey. 1975. Backcross variability for grain yield in oat species crosses (*Avena sativa* L. x *A. sterilis* L.). Euphytica 24:77-85.

Lee, H.K., G.W. Lawrence, A.T. Kelley, and K.S. McLean. 2002. Horizontal and vertical distribution of *Rotylenchulus reniformis* in a Mississippi cotton field. 2002. *In* Proc. Beltwide Cotton Prod. Res. Conf., Atlanta, GA 8-12 Jan. 2002. Natl. Cotton Counc. Am. Memphis, TN.

Lee, J.A. 1984. Cotton as a world crop. p. 1-25 *In* R.J. Kohel and C.L. Lewis (ed.) Cotton, agronomy monograph no. 24. CSSA, Madison, WI.

Levene, H. 1960. Robust tests for the equality of variance. p. 278-292. *In* I. Olkin (ed.) Contributions to probability and statistics. Stanford University Press, Palo Alto, CA.

Lewis, H. 2001. A review of yield and fiber quality trends and components in American Upland cotton. p. 1447-1453. *In* Proc. Beltwide Cotton Conf., Anaheim, CA. 9-13 Jan. 2001. Natl. Cotton Council Am., Memphis, TN.

Linford, M.B., J.M. Oliveira. 1940. *Rotylenchulus reniformis*, nov. gen. sp., a nematode parasite of roots. Proceeding of the Helminthological Society of Washington. 7:35-42.

Lonnquist, J.H. 1975. Consideration and experiences with recombination of exotic and corn belt maize germplasms. p. 102-117. In H.D. Loden and D. Wilkinson (ed.) Rep. Annu. Corn Sorghum Res. Conf., 30th, Chicago, IL. 10-12 Dec. 1975. Am. Seed Trade Assoc., Washington, DC.

Maréchal, R. 1974. Analyses de la conjugaison méoitique chez les hybrides triploïdes entre *Gossypium hirsutum* L. et des espèces sauvages australiennes. ***Bull. Rech. Agron. Gemboux*** 9**:**193-204.

Martin, G.B., S.H. Brommonschenkel, J. Chunwongse, A. Frary, M.W, Ganal, R. Spivey, T. Wu, E.D. Earle, and S.D. 1993. Tanksley. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432-1436.

Mathre, D.E., and J.D. Otta. 1967. Sources of resistance in the genus *Gossypium* to several soil borne pathogens. Plant Dis. Rept. 51:864-867

McLean, K.S., and G.W. Lawrence. 2000. A survey of plant-parasitic nematodes associated with cotton in Northwestern Louisiana. Suppl. J. Nematol. 32:508-512.

Medina-Filho, H.P. 1980. Linkage of APS-1, Mi and other markers on chromosome 6. Rep. Tomato Genet. Coop. 30:26-28.

Medina-Filho, H.P., and S.D. Tanksley. 1983. Breeding for nematode resistance. p. 904-923. *In* D.A. Evans, W.R. Sharp, P.V. Ammirato, and Y. Yamada (ed.) Handbook of plant cell culture, Vol. 1. MacMillan Press, New York.

Mei, M., N.H. Syed, W. Gao, P.M. Thaxton, C.W. Smith, D.M. Stelly and Z.J. Chen. 2004. Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). Theor. Appl. Genet. 108:280–291.

Menzel, M.Y., and M.S. Brown. 1954. The significance of multivalent formation in three-species *Gossypium* hybrids. Genetics 39:546-557.

Meredith, W.R. 1991. Contributions of introductions to cotton improvement. p. 127-146. *In* H.L. Shands and L.E. Wiesner (ed.) Use of plant introductions in cultivar development, Part I. CSSA Spec. Publ. 17. ASA-CSSA-SSSA, Madison, WI.

Meredith, W.R., and R.R. Bridge. 1971. Breakup of linkage blocks in cotton, *Gossypium hirsutum* L. Crop Sci. 11:695-698.

Meredith, W.R., Jr. 2000. Cotton yield progress- Why has it reached a plateau? Better Crops 84:6-9.

Mergeai, G., 2004. Forty years of genetic improvement of cotton through interspecific hybridization at Gembloux Agricultural University: Achievement and prospects. p. 120-133. *In* A. Swanepoel (ed). Proc. World Cotton Research Conference-3. Cotton production for the new millennium. Cape Town, South Africa. 9-13 March, 2003. Agricultural Research Council, Institute for Industrial crops, Pretoria, South Africa.

Meyer, J.R. 1957. Origin and inheritance of D2 smoothness in Upland cotton. Crop Sci. 48:249-250.

Meyer, V.G. 1975. Male sterility from *Gossypium harknesii* [Cotton]. Crop Sci. 66:23-27.

Miller, P.A., and J.O. Rawlings. 1967. Breakup of initial linkage blocks through intermating in a cotton breeding program. Crop Sci. 7:199-204.

Miller, P.A., J.C. Williams, Jr., H.F. Robinson, and R.E. Comstock. 1958. Estimates of genotypic and environmental variances and covariances in Upland cotton and their implications in selection. Agron. J. 50:126-131.

Mursal, I. El. J., and J.E. Endrizzi. 1976. A reexamination of the diploid-like meiotic behavior of polyploidy cotton. Theor. Appl. Genet. 47:171-178.

Nakasona, K. 1966. An instance of male and young female development of the reniform nematode (*Rotylenchulus* sp.) within the egg-shell (Tylenchida: Hoplolaimidae). Appl. Ento. Zoo. 1:49-50.

Nakasona, K. 1977. Sexual attraction of *Rotylenchulus reniformis* females and some differences in the attractiveness between amphimictic and parthenogenetic populations (Nematoda: Nacobbidae). Appl. Ento. Zoo. 12:154-161.

Nakasona, K. 1983. Studies on morphological and physio-ecological variations of the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940 with an emphasis on differential geographical distribution of amphimictic and parthenogenetic populations in Japan. Bull. Natl. Inst. Agric. Sci., Japan 38:1-67.

Ndungo, V., J. Demol, R. Marechal. 1988. Improvement of the cotton *Gossypium hirsutum* L. by interspecific hybridization. 1. Phylogeny and speciation of the genus *Gossypium*. (In French, with English abstract.) Publications agricoles No. 23. Faculte des Sciences Agronomiques de l'Etat, Gembloux, Belgium.

Nelson, H.G. 1973. The use of exotic germplasm in practical corn breeding programs. p. 115-118. *In* D. Wilinson (ed.) Rep. Annu. Corn Sorghum Res. Conf., 28th, Chicago, IL. 4-6 Dec. 1973. Am. Seed Trade Assoc., Washington, DC.

Newman, M.A., and T.C. Stebbins. 2002. Recovery of reniform nematodes at various soil depths in cotton. p. 254-255. *In* Proc. Beltwide Cotton Prod. Res. Conf., San Antonio, TX. 8-13 Jan. 2002. Natl. Cotton Counc. Am. Memphis, TN.

Nguyen T.B., M. Giband, P. Brottier, A.M. Resterucci, and J.M. Lacape. 2004. Wide coverage of the tetraploid cotton genome using newly developed microsatellite markers. Theor. Appl. Genet. 109:167-175.

Overstreet, C. 1999. Reniform nematode- an introduction. p. 100. *In* Proc. Beltwide Cotton Conf., Orlando, FL 6-10 Jan 1999. Natl. Cotton Counc. Am. Memphis, TN.

Overstreet, C., and T. L. Erwin. 2003. The use of Telone in cotton production in Louisiana. p. 277-278. *In* Proc. Beltwide Cotton Conf., Nashville, TN. 6-10 Jan. 2003. Natl. Cotton Counc. Am., Memphis, TN.

Overstreet, C., and E.C. McGawley. 1997. Reniform nematode and its influence on the cotton industry in the United States. p. 92. *In* Proc. Beltwide Cotton Conf., New Orleans, LA 6-10 Jan. 1997. Natl. Cotton Counc. Am. Memphis, TN.

Peacock, F.C. 1956. The reniform nematode in the Gold Coast. Nematologica 1:307-310.

Perkins, H.H., D.E. Ethridge, and C.K. Bragg. 1984. Fiber. p. 447-448. *In* R.J. Kohel and C.L. Lewis (ed.) Cotton, agronomy monograph no. 24. CSSA, Madison, WI.

Phillips, L.L. 1962. The cytogenetics of speciation in Asiatic cotton. Genetics 46:77-83.

Phillips, L.L. 1963. The cytogenetics of *Gossypium* and the origin of New World cottons. Evol. 17:460-469.

Phillips, L.L. 1966. The cytology and phylogenetics of the diploid species of *Gossypium*. Amer. J. Bot. 53:328-335.

Phillips, L.L., and M.A. Strickland. 1966. The cytology of a hybrid between *Gossypium hirsutum* and *G. longicalyx*. Can. J. Genet. Cytol. 8:91-95.

Pillay, M., and G.O. Myers. 1999. Genetic diversity in cotton assessed by variation in ribosomal RNA genes and AFLP markers. Crop Sci. 39:1881-1886.

Razak, A.R., and A.A.F. Evans. 1976. An intracellular tube associated with feeding by *Rotylenchulus reniformis* on cowpea root. Nematologica 22:182-189.

Rebois, R.V. 1973. Effect of soil water on infectivity and development of *Rotylenchulus reniformis* on soybean, *Glycine max*. J. Nematol. 5:246-249.

Rebois, R.V., J.M. Epps, and E.E. Hartwig. 1970. Correlation of resistance in soybeans to *Heterodera glycines* and *Rotylenchulus reniformis*. Phytopathol. 60:695-700.

Rebois, R.V., P.A. Madden, and B.J. Eldrige. 1975. Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by *Rotylenchulus reniformis*. J. Nematol. 7:122-139.

Reinisch, A., J. Dong, C.L. Brubaker, D.M. Stelly, J.F. Wendel, and A.H. Paterson. 1994. A detailed RFLP map of cotton, *G. hirsutum* x *G. barbadense*: Chromosome organization and evolution in a disomic polyploidy genome. Genetics 138:829-847.

Reiter, R. 2001. PCR-based marker systems. p. 9-29. *In* R.L. Phillips and I.K. Vasil (ed.) DNA-based markers in plants. Vol.6. 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rhyne, C.L. 1957. Duplicate linkage groups in cotton. J. Hered. 48:59-62.

Rick, C.M. and J.A. Fobes. 1974. Association of an allozyme with nematode resistance. Rep. Tomato Genet. Coop. 24:25.

Roberts, P.A., D. May, and W.C. Matthews. 1986. Root-knot nematode resistance in processing tomatoes. Calif. Agric. 40:24-26.

Robinson, A.F. 1999. Cotton nematodes. p. 595-616. *In* C.W. Smith and J.T. Cothren (ed.) Cotton: Origin, history, technology, and production. John Wiley & Sons, New York.

Robinson, A.F., and C.C. Orr. 1980. Histopathology of *Rotylenchulus reniformis* on sunflower. J. Nematol. 12:84-85.

Robinson, A.F., and A.E. Percival. 1997. Resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in wild accessions of *Gossypium hirsutum and G. barbadense* from Mexico. Suppl. J. Nematol. 29:746-755.

Robinson, A.F., R.N. Inserra, F.P. Caswell-Chen, N.Volvas, and A. Troccoli. 1997. *Rotylenchulus* species: Identification, distribution, host ranges, and crop plant resistance. Nematropica 27:127-180.

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 Sci. 39:850-858.

Robinson, A.F., A.C. Bridges, and A.E. Percival. 2004. New sources of resistance to the reniform (*Rotylenchulus reniformis* Linford and Oliveira) and root-knot (*Meloidogyne incognita* (Kofoid & White) Chitwood) nematode in Upland (*Gossypium hirsutum* L.) and Sea Island (*G. barbadense* L.) cotton. J. Cotton Sci. 8:191-197.

Robinson, A.F., J.R. Akridge, J.M. Bradford. C.G. Cook, E.C. McGawley, J.L. Starr, L.D. Young. 2006. Suppression of *Rotylenchulus reniformis* 122 cm deep endorses resistance introgression in *Gossypium*. J. Nematol. 38:195-209.

Robinson, A.F., A.A. Bell, N.D. Dighe, M.A. Menz, R.L. Nichols, and D.M. Stelly. 2007. Introgression of resistance to nematode *Rotylenchulus reniformis* into Upland cotton (*Gossypium hirsutum*) from *Gossypium longicalyx*. Crop Sci. 47:1865-1877.

Rommens, J.M., M.C. Jannuzzi, B. Kerem, M.L. Drumm, G. Melmer, M. Dean, R. Rozmahel, J.L. Cole, D. Kennedy, N. Hidaka, M. Zsiga, M. Buchwald, J.R. Riordan, L.C. Tsui, and F.C. Collins. 1989. Identification of cystic fibrosis gene: Chromosome walking and jumping. Science 245:1059-1065.

Rong, J., C. Abbey, J.E. Bowers, C.L. Brubaker, C. Chang, P.W. Chee, T.A. Delmonte, X. Ding, J.J. Garza, B.S. Marler, C. Park, G.J. Pierce, K.M. Rainey, V.K. Rastogi, S.R. Schulze, N.L. Trolinder, J.F. Wendel, T.A. Wilkins, T.D. William-Coplin, R.A. Wing, R.J. Wright, X. Zhao, L. Zhu, and A.H. Paterson. 2004. A 3347-locus genetic recombination map of sequence-tagged sites reveals features of genome organization, transmission and evolution of cotton (*Gossypium*). Genetics 166:389-417.

Rungis, D., D. Llewellyn, E.S., Dennis, and B.R. Lyon. 2005. Simple sequence repeat (SSR) markers reveal low levels of polymorphism between cotton (*Gossypium hirsutum* L.) cultivars. Aus. J. Agr. Res. 56:301–307.

Sastry, G.R.K., and M.S. Swaminathan. 1960. Chromosome association in haploid *Gossypium barbadense*. Current Sci. 29:398-400.

Saunders, J.H. 1961. The wild species of *Gossypium* and their evolutionary history. Oxford University Press, London, UK.

Saxton, A. M. 1998. A macro for converting mean separation output to letter groupings in PROC MIXED. p. 1243-1246. *In* Proc. 23rd SAS User's Group International. SAS Institue, Cary, NC.

Shappley, Z.W., J.N. Jenkins, W.R. Meredith, and J.C. McCarty. 1998. An RFLP linkage map of Upland cotton, *Gossypium hirsutum* L. Theor. Appl. Genet. 97:756-761.

Shepherd, R.L. 1988. Resistance of cotton cultivars and elite breeding lines to root-knot nematodes. Mississippi Agri. and Forestry Expt. Station Tech. Bull. I-58.

Sivakumar, C.V., and A.R. Seshadri. 1971. Life history of the reniform nematode *Rotylenchulus reniformis* Linford and Oliveira, 1940. Indian J. Nematol. 1:7-20.

Skovsted, A. 1934. Cytological studies in cotton. II. Two interspecific hybrids between Asiatic cottons and New World cottons. J. Genet. 28:407-424.

Skovsted, A. 1937. Cytological studies in cotton. IV. Chromosome conjugation in interspecific hybrids. J. Genet. 34:97-134.

Small, R.L., and J.F. Wendel. 2000. Phylogeny, duplication, and intraspecific variation of *Adh* sequences in New World diploid cottons (*Gossypium* L., Malvaceae). Molecular Phylogenetics and Evolution. 16:73-84.

Smith, A.L. 1940. Distribution and relation of meadow nematode, *Pratylenchulus pratensis*, to fusarium wilt of cotton in Georgia. Phytopathol. 30:710.

Smith, C.W. 1999. Production statistics. p. 435-449. *In* C.W. Smith and J.T. Cothren (ed.) Cotton: Origin, history, technology, and production. John Wiley & Sons, New York.

Stanton, M.A., J.M. Stewart, A.E. and N.P. Tugwell. 1992. Evaluation of *Gossypium arboreum* L. for resistance to thrips. Gen. Resour. Crop Evol. 39:89-95.

Stephens, S.G. 1944a. Phenogenetic evidence for amphidiploid origin of New World cottons. Nature 153:53-54.

Stephens, S.G. 1944b. The genetic organization of leaf-shape development in the genus *Gossypium*. J. Genet. 46:28-51.

Stewart, J.M. 1992. A new cytoplasmic male sterile and restorer for cotton. Proc. 1992 Cotton Res. Meeting. Ark. Ag. Expt. Sta. Special report No. 158. p. 50-53.

Stewart, J.M., and R.T. Robbins. 1995. Evaluation of Asiatic cottons for resistance to reniform nematode. p. 165-168. *In* D.M. Oosterhius (ed.) Proc. 1994 Cotton Research Meeting, Special Report 166. Arkansas Ag. Expt. Sta., Fayetteville, AR.

Stewart, J.M., and R.T. Robbins. 1996. Identification and enhancement of resistance to reniform nematode in cotton germplasm. p. 255. *In* Proc. Beltwide Cotton Conf., Nashville, TN. 9-12 Jan. 1996. Natl. Cotton Counc. Am., Memphis, TN.

Stewart, J.McD., 1995. Potential for crop improvement with exotic germplasm and genetic engineering. p. 313–327. *In* G.A. Constable & N.W. Forrester (ed.) Challenging the Future: Proceedings of the World Cotton Research Conference-1, Brisbane Australia. 14-17 Feb. 1994. CSIRO, Melbourne, Australia.

Taha, A.H., S.A. Sultan. 1979. Cellular response of grape seedlings to *Rotylenchulus reniformis* and *Tylenchulus semipenetrans* infections. Nematologia Mediterranea 7:45-50.

Tanksley, S.D., and J.C. Nelson. 1996. Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92:191-203.

Triantaphyllou, A.C., and H. Hirschmann. 1964. Reproduction in plant and soil nematodes. Annual Rev. Phytopathol. 2:57-80.

Tronstad, R., J.C. Silvertooth, and S. Husman. 2003. Irrigation termination of cotton: An economic analysis of yield, quality, and market factors. J. Cotton Sci. 7: 86-94.

Ulloa, M., and W.R. Meredith. 2000. Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an intraspecific population. J. Cotton Sci. 4:161-170.

Ulloa, M., S. Saha, J.N. Jenkins, W.R. Jr. Meredith, J.C. McCarty, and D.M. Stelly. 2005. Chromosomal assignment of RFLP linkage groups harboring important QTLs on an intraspecific cotton (*Gossypium hirsutum* L.) joinmap. J. Hered. 96:132-144.

Uthamasamy, S. 1995. Host resistance to the leafhopper, *Amarasca devastans* (Distant) in cotton, *Gossypium* spp. p. 494-498. *In* G.A. Constable and N.W. Forrester (ed.)

Challenging the Future: Proceedings of the World Cotton Research Conference-1, Brisbane Australia. 14-17 Feb. 1994. CSIRO, Melbourne, Australia.

Van Esbroeck, G.A., D.T. Bowman, D.S. Calhoun, and O.L. May. 1998. Changes in the genetic diversity of cotton in the USA from 1970 to 1995. Crop Sci. 38:33-37.

Varaprasad, K.S. 1986. *Rotylenchulus reniformis* Linford and Oliveira, 1940-A comparative account of systematics, biology and management. p. 194-210. *In* G. Swarup and D.R. Dasgupta (ed.) Plant parasitic nematodes of India, problems and progress. Indian Agric. Res. Inst., New Delhi, India.

Vroh, Bi, I., J.P. Baudoin, and G. Mergeai. 1998. Cytogenetics of cotton plants used to introgress the "glandless-seed and glanded-plant" trait from *Gossypium sturtianum* Willis into cultivated Upland cotton (*Gossypium hirsutum* L.). Plant Breeding 117:235-241.

Wang, C., M. Ulloa, and P.A. Roberts. 2006a. Identification and mapping of microsatellite markers linked to a root-knot nematode resistance gene (*rkn1*) in Acala NemX cotton (*Gossypium hirsutum* L.). Theor. Appl. Genet. 112:770-777.

Wang K., X. Song, Z. Han, W. Guo, J.Z. Yu, J. Sun, J. Pan, R.J. Kohel, T. Zhang. 2006b. Complete assignment of the chromosomes of *Gossypium hirsutum* L. by translocation and fluorescence in situ hybridization mapping. Theor. Appl. Genetics 113:73-80.

Wendel J.F., C.L. Brubaker, and A.E. Percival. 1992 Genetic diversity in *Gossypium hirsutum* and the origin of Upland cotton. Am. J. Bot. 79:1291-1310.

Wrather, J.A., W.E. Stevens, T.L. Kirkpatrick, and N.R. Kitchen. 2002. Effects of site-specific application of aldicarb on cotton in a *Meloidogyne incognita*-infested field. J. Nematol. 34:115-119.

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

Zhang, H-B., G.B. Martin, S.D. Tanksley, and R.A. Wing. 1994. Map-based cloning in crop plants: Tomato as a model system II. Isolation and characterization of a set of

overlapping yeast artificial chromosomes *encompassing* the *jointless* locus. Mol. Gen. Gent. 244:613-621.

Zhang, J., and J.McD. Stewart. 2000. Economical and rapid method for extracting cotton genomic DNA. J. Cotton Sci. 4:193-201.

Zhang, J., W. Guo, and T. Zhang. 2002. Molecular linkage map of allotetraploid cotton (*G. hirsutum* L. x *G. barbadense* L.) with a haploid population. Theor. Appl. Genet. 105:1166-1174.

Zhong, X.B., J. Bodeau, P.F. Fransz, V.M. Williamson, A. Kammen, J.H. de Jong, and P. Zabel. 1999. FISH to meiotic pachytene chromosomes of tomato locates the root-knot nematode resistance gene *Mi-1* and the acid phosphotase gene *APS-1* near the junction of euchromatin and pericentromeric heterochromatin of chromosome arms 6S and 6L, respectively. Theor. Appl. Genet. 98:365-370.

Zhou, B., S. Chen, X. Shen, X. Zhang, and Z. Zhang. 2003. Construction of gene pools with superior fiber properties in Upland cotton through interspecific hybridization between *Gossypium hirsutum* and *Gossypium* wild species. (In Chinese, with English abstract.) Acta Agronomica Sinicia. Science press, Beijing, China.

Zimet, D., J.R. Rich, A. LaColla, and R.A. Kinloch. 1999. Economic analysis of Telone II (1,3-D) and Temik 15G (aldicarb) to manage reniform nematode (*Rotylenchulus reniformis*) in cotton. p. 111-112. *In* Proc. Beltwide Cotton Conf., Orlando, FL. 3-7 Jan. 1999. Natl. Cotton Counc. Am., Memphis, TN.

Zwick, M.S., M.N. Islam-Faridi, D.G. Czeschin Jr., R.A. Wing, G.A. Hart, D.M. Stelly, and H.J. Price. 1998. Physical mapping of the liguleless linkage group in *Sorghum bicolor* using rice RFLP-selected sorgum BACs. Genet. 148:1983-1992.

APPENDIX

A-1. Average and modal meiotic-chromosomal configurations in the individual plants of successive generations produced by repeated backcrossing of the reniform nematode-resistant plants with *G. hirsutum*.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC1F1	unknown	20031744.16	NA	1	52	6	6.33	22.83	0.00	0.00	0.00	0.00	6	23	0	0	0	0	3	50
BC1F1	unknown	20031746.05	NA	2	52	14	3.07	23.21	0.07	0.57	0.00	0.00	4	24	0	0	0	0	5	36
BC1F1	unknown	20031841.06	NA	3	52	5	13.20	19.40	0.00	0.00	0.00	0.00	14	19	0	0	0	0	2	40
BC1F1	unknown	20031841.09	NA	4	52	5	5.20	23.40	0.00	0.00	0.00	0.00	6	23	0	0	0	0	4	80
BC1F1	unknown	20031841.12	NA	5	52	4	4.50	23.75	0.00	0.00	0.00	0.00	4	24	0	0	0	0	3	75
BC1F1	unknown	20031842.05	NA	6	52	6	7.33	22.33	0.00	0.00	0.00	0.00	6	23	0	0	0	0	3	50
BC1F1	unknown	20031842.06	NA	7	53	2	3.00	25.00	0.00	0.00	0.00	0.00	1/5[‡‡]	26/24	0/0	0/0	0/0	0/0	2	100
BC1F1	unknown	20031843.05	NA	8	52	12	2.33	24.83	0.00	0.00	0.00	0.00	2	25	0	0	0	0	10	83
BC1F1	unknown	20031844.01	NA	9	52	7	7.71	22.14	0.00	0.00	0.00	0.00	10	21	0	0	0	0	3	43
BC1F1	unknown	20031844.04	NA	10	52	8	6.50	22.25	0.00	0.25	0.00	0.00	4	24	0	0	0	0	3	38
BC1F1	unknown	20031845.02	NA	11	54	4	4.00	24.50	0.00	0.25	0.00	0.00	4	25	0	0	0	0	3	75
BC1F1	unknown	20031845.09	NA	12	52	3	5.33	22.33	0.67	0.00	0.00	0.00	3	23	1	0	0	0	2	67
BC1F1	unknown	20031846.17	NA	13	52	8	9.25	21.38	0.00	0.00	0.00	0.00	10	21	0	0	0	0	4	50
BC1F1	unknown	20031911.03	NA	14	52	3	4.00	24.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	2	67
BC1F1	unknown	20031921.06	NA	15	52	2	8.00	22.00	0.00	0.00	0.00	0.00	8	22	0	0	0	0	2	100
BC1F1	unknown	20031921.20	NA	16	52	13	2.54	24.62	0.08	0.00	0.00	0.00	2	25	0	0	0	0	4	31
BC1F1	unknown	20031923.19	NA	17	52	2	6.00	23.00	0.00	0.00	0.00	0.00	6	23	0	0	0	0	2	100
BC1F1	unknown	20031931.13	NA	18	52	4	8.00	22.00	0.00	0.00	0.00	0.00	8	22	0	0	0	0	2	50
BC1F1	unknown	20031943.04	NA	19	52	9	1.22	24.56	0.11	0.33	0.00	0.00	2	25	0	0	0	0	3	33
BC1F1	unknown	20031943.15	NA	20	52	4	4.50	23.75	0.00	0.00	0.00	0.00	4	24	0	0	0	0	3	75
BC1F1	HLA-A103	HLA-A103	NA	21	54	3	3.33	25.33	0.00	0.00	0.00	0.00	4	25	0	0	0	0	2	67
BC1F1	HLA-A83	HLA-A83	NA	22	51	8	3.00	24.00	0.00	0.00	0.00	0.00	3	24	0	0	0	0	8	100
BC1F1	HLA-A88	HLA-A88	NA	23	52	4	10.00	21.00	0.00	0.00	0.00	0.00	6/8/12/14	23/22/20/19	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	4	100

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC1F1	HLA-B100	HLA-B100	NA	24	52	10	3.00	23.70	0.40	0.10	0.00	0.00	2	25	0	0	0	0	3	30
BC1F1	HLA-B15	HLA-B15	NA	25	52	5	4.00	23.20	0.00	0.40	0.00	0.00	4	24	0	0	0	0	2	40
BC1F1	HLA-B102	HLA-B102	NA	26	52	3	6.67	22.67	0.00	0.00	0.00	0.00	2/8/10	25/22/21	0/0/0	0/0/0	0/0/0	0/0/0	3	100
BC1F1	HLA-B17	HLA-B17	NA	27	52	4	7.50	22.25	0.00	0.00	0.00	0.00	8	22	0	0	0	0	2	50
BC1F1	HLA-B18	HLA-B18	0	28	53	20	3.20	24.65	0.10	0.05	0.00	0.00	3	25	0	0	0	0	10	50
BC1F1	HLA-B19	HLA-B19	NA	29	52	5	1.40	25.00	0.20	0.00	0.00	0.00	2	25	0	0	0	0	3	60
BC1F1	HLA-B30	HLA-B30	NA	30	52	6	1.67	24.00	0.33	0.33	0.00	0.00	2	25	0	0	0	0	3	50
BC1F1	HLA-B32	HLA-B32	NA	31	52	5	10.00	21.00	0.00	0.00	0.00	0.00	10	21	0	0	0	0	5	100
BC1F1	HLA-B40	HLA-B40	NA	32	52	12	5.33	23.33	0.00	0.00	0.00	0.00	4	24	0	0	0	0	5	42
BC1F1	HLA-B43	HLA-B43	NA	33	52	4	4.00	24.00	0.00	0.00	0.00	0.00	4	24	0	0	0	0	4	100
BC1F1	HLA-B49	HLA-B49	NA	34	52	7	4.86	23.57	0.00	0.00	0.00	0.00	4	24	0	0	0	0	4	57
BC1F1	HLA-B51	HLA-B51	NA	35	54	6	5.00	24.00	0.33	0.00	0.00	0.00	4	25	0	0	0	0	2	33
BC1F1	HLA-B52	HLA-B52	NA	36	54	6	2.33	25.50	0.00	0.17	0.00	0.00	2	26	0	0	0	0	3	50
BC1F1	HLA-B53	HLA-B53	NA	37	52	7	2.86	24.57	0.00	0.00	0.00	0.00	2	25	0	0	0	0	4	57
BC1F1	HLA-B55	HLA-B55	NA	38	54	8	4.25	24.63	0.00	0.13	0.00	0.00	4	25	0	0	0	0	5	63
BC1F1	HLA-B74	HLA-B74	NA	39	54	7	3.71	24.86	0.00	0.14	0.00	0.00	4	25	0	0	0	0	4	57
BC1F1	HLA-B77	HLA-B77	1	40	53	10	1.40	24.80	0.00	0.50	0.00	0.00	1/1	24/26	0/0	1/0	0/0	0/0	8	80
BC1F1	HLA-B79	HLA-B79	NA	41	53	11	6.36	22.82	0.09	0.18	0.00	0.00	7	23	0	0	0	0	5	45
BC1F1	HLA-B9	HLA-B9	NA	42	53	3	2.33	23.33	0.00	1.00	0.00	0.00	3	23	0	1	0	0	2	67
BC1F1	HLA-B91	HLA-B91	2	43	52	13	3.00	23.31	0.08	0.54	0.00	0.00	2	25	0	0	0	0	4	31
BC1F1	HLA-B93	HLA-B93	NA	44	52	4	5.50	23.25	0.00	0.00	0.00	0.00	6	23	0	0	0	0	2	50
BC1F1	HLA-B97	HLA-B97	NA	45	52	9	4.67	23.44	0.00	0.11	0.00	0.00	4	24	0	0	0	0	4	44
Total						301													166	55
Min.					51	2	1.22	19.40	0.00	0.00	0.00	0.00	0	19	0	0	0	0		30
Max.					54	20	13.20	25.50	0.67	1.00	0.00	0.00	14	26	1	1	0	0		100
Mean						7														

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC1S1	HLA-A85	30	1	1	52	8	1.63	24.63	0.38	0.00	0.00	0.00	1	24	1	0	0	0	3	38
BC1S1	HLA-A88	33	77	2	52	8	2.50	24.75	0.00	0.00	0.00	0.00	2	25	0	0	0	0	3	38
BC1S1	HLA-A103	44	41	3	53	4	3.00	25.00	0.00	0.00	0.00	0.00	3	25	0	0	0	0	2	50
BC1S1	HLA-A103	87	3	4	53	9	1.00	25.33	0.00	0.33	0.00	0.00	1	26	0	0	0	0	6	67
BC1S1	HLA-A103	102	1	5	52	12	1.08	25.33	0.08	0.00	0.00	0.00	0	26	0	0	0	0	7	58
BC1S1	HLA-A103	110	0	6	52	2	1.00	23.50	0.00	1.00	0.00	0.00	0/2	24/23	0/0	1/1	0/0	0/0	2	100
BC1S1	HLA-A103	115	0	7	53	7	1.29	25.86	0.00	0.00	0.00	0.00	1	26	0	0	0	0	6	86
BC1S1	HLA-A103	122	1	8	54	8	1.75	26.13	0.00	0.00	0.00	0.00	2	26	0	0	0	0	5	63
BC1S1	HLA-A84	84-2-12	1	9	52	12	2.67	24.67	0.00	0.00	0.00	0.00	2	25	0	0	0	0	8	67
BC1S1	HLA-A84	84-2-16	2	10	52	11	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	7	64
BC1S1	HLA-A84	84-3-9	2	11	53	9	1.00	26.00	0.00	0.00	0.00	0.00	1	26	0	0	0	0	9	100
BC1S1	HLA-A84	84-3-13	4	12	52	9	0.44	25.78	0.00	0.00	0.00	0.00	0	26	0	0	0	0	7	78
BC1S1	HLA-A84	84-3-16	2	13	52	10	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	6	60
BC1S1	HLA-A84	84-3-20	3	14	52	9	0.44	25.78	0.00	0.00	0.00	0.00	0	26	0	0	0	0	8	89
BC1S1	HLA-A84	84-4-20	1	15	53	5	1.80	25.60	0.00	0.00	0.00	0.00	1	26	0	0	0	0	4	80
BC1S1	HLA-A84	84-4-19	2	16	52	5	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	5	100
BC1S1	HLA-A84	84-4-9	0	17	52	10	1.40	25.30	0.00	0.00	0.00	0.00	2	25	0	0	0	0	7	70
BC1S1	HLA-A84	84-4-8	1	18	53	4	1.50	25.75	0.00	0.00	0.00	0.00	1	26	0	0	0	0	3	75
BC1S1	HLA-A84	84-1-7	8	19	52	10	1.80	25.10	0.00	0.00	0.00	0.00	2	25	0	0	0	0	9	90
BC1S1	HLA-A84	84-2-14	59	20	52	9	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	9	100
BC1S1	HLA-A84	84-1-11	2	21	53	11	1.09	25.09	0.09	0.36	0.00	0.00	1/1	24/26	0/0	1/0	0/0	0/0	9	82
BC1S1	HLA-A84	84-4-12	19	22	52	4	2.50	23.75	0.00	0.50	0.00	0.00	2	25	0	0	0	0	2	50
BC1S1	HLA-A84	84-4-18	6	23	52	8	2.25	24.88	0.00	0.00	0.00	0.00	2	25	0	0	0	0	7	88
BC1S1	HLA-A84	84-1-12	1	24	52	10	2.60	24.50	0.00	0.10	0.00	0.00	2	25	0	0	0	0	7	70
BC1S1	HLA-A84	84-2-1	1	25	52	18	1.22	25.39	0.00	0.00	0.00	0.00	0/2	26/25	0/0	0/0	0/0	0/0	17	94
BC1S1	HLA-A84	84-1-19	0	26	52	17	1.94	24.82	0.06	0.06	0.00	0.00	2	25	0	0	0	0	14	82

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
Total						229													172	75
Min.					52	2	0.44	23.50	0.00	0.00	0.00	0.00	0	23	0	0	0	0		38
Max.					54	18	3.00	26.13	0.38	1.00	0.00	0.00	3	26	1	1	0	0		100
Mean						9														
BC2F1	HLA-A85	70	0	20	52	6	3.17	23.50	0.17	0.33	0.00	0.00	0/6	24/23	0/1	1/0	0/0	0/0	4	67
BC2F1	HLA-A85	129	0	46	52	37	1.84	24.89	0.05	0.05	0.00	0.00	2	25	0	0	0	0	25	68
BC2F1	HLA-A84	84-3-4	0	73	52	37	1.16	24.62	0.03	0.38	0.00	0.00	0	26	0	0	0	0	13	35
BC2F1	HLA-A122	22	1	5	53	4	3.50	24.00	0.50	0.00	0.00	0.00	3/4	25/23	0/1	0/0	0/0	0/0	4	100
BC2F1	HLA-A85	101	1	33	51	3	3.00	24.00	0.00	0.00	0.00	0.00	3	24	0	0	0	0	3	100
BC2F1	HLA-A85	176	1	66	52	21	0.57	25.05	0.19	0.19	0.00	0.00	0	26	0	0	0	0	9	43
BC2F1	HLA-A122	43	2	10	53	2	8.00	22.50	0.00	0.00	0.00	0.00	7/9	23/22	0/0	0/0	0/0	0/0	2	100
BC2F1	HLA-A99	140	2	51	52	15	4.00	23.33	0.13	0.13	0.00	0.07	4	24	0	0	0	0	6	40
BC2F1	HLA-A123	146	2	54	51	8	1.50	24.75	0.00	0.00	0.00	0.00	1	25	0	0	0	0	6	75
BC2F1	HLA-A132	111	3	37	53	10	5.40	23.60	0.00	0.10	0.00	0.00	5	24	0	0	0	0	5	50
BC2F1	HLA-A132	178	3	67	52	3	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	3	100
BC2F1	HLA-A103	38	4	8	53	14	1.43	25.79	0.00	0.00	0.00	0.00	1	26	0	0	0	0	11	79
BC2F1	HLA-A122	18	5	4	52	13	4.31	23.85	0.00	0.00	0.00	0.00	6	23	0	0	0	0	6	46
BC2F1	HLA-A103	49	5	12	52	13	0.31	24.31	0.00	0.77	0.00	0.00	0	24	0	1	0	0	9	69
BC2F1	HLA-A77	112	5	38	52	13	1.92	24.92	0.08	0.00	0.00	0.00	2	25	0	0	0	0	8	62
BC2F1	HLA-A4	139	5	50	52	8	4.00	24.00	0.00	0.00	0.00	0.00	2/6	25/23	0/0	0/0	0/0	0/0	6	75
BC2F1	HLA-A77	167	5	61	52	10	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	10	100
BC2F1	HLA-A85	181	5	68	52	6	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	6	100
BC2F1	HLA-A4	158	6	58	52	11	5.09	23.45	0.00	0.00	0.00	0.00	4	24	0	0	0	0	4	36
BC2F1	HLA-A85	121	7	42	52	7	2.29	24.57	0.00	0.14	0.00	0.00	2	25	0	0	0	0	5	71
BC2F1	HLA-A84	84-4-7	7	75	52	6	0.33	25.83	0.00	0.00	0.00	0.00	0	26	0	0	0	0	5	83
BC2F1	HLA-A123	118	8	40	51	5	1.80	24.60	0.00	0.00	0.00	0.00	1	25	0	0	0	0	3	60

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC2F1	HLA-A4	1	9	1	53	3	1.00	26.00	0.00	0.00	0.00	0.00	1	26	0	0	0	0	3	100
BC2F1	HLA-A77	13	9	3	51	4	3.00	24.00	0.00	0.00	0.00	0.00	3	24	0	0	0	0	4	100
BC2F1	HLA-A4	42	9	9	52	2	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	2	100
BC2F1	HLA-A99	98	9	32	52	5	5.60	22.80	0.00	0.20	0.00	0.00	8	22	0	0	0	0	3	60
BC2F1	HLA-A123	154	12	56	52	2	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	2	100
BC2F1	HLA-A123	88	13	26	51	10	2.40	24.30	0.00	0.00	0.00	0.00	3	24	0	0	0	0	7	70
BC2F1	HLA-A85	136	13	48	51	5	1.40	24.80	0.00	0.00	0.00	0.00	1	25	0	0	0	0	4	80
BC2F1	HLA-A4	169	13	63	52	5	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	5	100
BC2F1	HLA-A123	168	24	62	51	11	2.45	24.27	0.00	0.00	0.00	0.00	3	24	0	0	0	0	8	73
BC2F1	HLA-A85	23	26	6	52	12	3.33	23.83	0.33	0.00	0.00	0.00	4	24	0	0	0	0	7	58
BC2F1	HLA-A85	84	26	25	52	10	4.70	23.50	0.10	0.00	0.00	0.00	6	23	0	0	0	0	5	50
BC2F1	HLA-A123	63	28	18	51	8	2.25	24.38	0.00	0.00	0.00	0.00	3	24	0	0	0	0	5	63
BC2F1	HLA-A123	172	28	64	52	2	9.00	21.50	0.00	0.00	0.00	0.00	8/10	22/21	0/0	0/0	0/0	0/0	2	100
BC2F1	HLA-A123	11	31	2	52	6	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	6	100
BC2F1	HLA-A123	128	34	45	51	8	1.75	24.13	0.00	0.25	0.00	0.00	1/3	25/24	0/0	0/0	0/0	0/0	6	75
BC2F1	HLA-A99	141	35	52	52	2	5.00	23.50	0.00	0.00	0.00	0.00	2/8	25/22	0/0	0/0	0/0	0/0	2	100
BC2F1	HLA-A4	29	37	7	52	12	1.00	25.50	0.00	0.00	0.00	0.00	0/2	26/25	0/0	0/0	0/0	0/0	12	100
BC2F1	HLA-A85	61	37	17	52	12	0.42	25.33	0.08	0.17	0.00	0.00	0	26	0	0	0	0	8	67
BC2F1	HLA-A123	104	38	35	51	10	2.30	24.20	0.10	0.00	0.00	0.00	3	24	0	0	0	0	7	70
BC2F1	HLA-A123	159	38	59	52	4	1.00	25.50	0.00	0.00	0.00	0.00	0/2	26/25	0/0	0/0	0/0	0/0	4	100
BC2F1	HLA-A84	84-1-1	41	70	53	4	2.00	25.50	0.00	0.00	0.00	0.00	1/3	26/25	0/0	0/0	0/0	0/0	4	100
BC2F1	HLA-A84	84-4-1	41	74	52	10	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	10	100
BC2F1	HLA-A123	66	45	19	51	10	3.20	23.70	0.00	0.10	0.00	0.00	3	24	0	0	0	0	7	70
BC2F1	HLA-A84	84-3-18	46	72	52	18	3.22	24.39	0.00	0.00	0.00	0.00	2	25	0	0	0	0	9	50
BC2F1	HLA-A99	73	47	21	52	10	1.40	25.30	0.00	0.00	0.00	0.00	0	26	0	0	0	0	5	50
BC2F1	HLA-A110	166	47	60	53	4	3.00	25.00	0.00	0.00	0.00	0.00	3	25	0	0	0	0	4	100

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC2F1	HLA-A4	103	49	34	52	7	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	7	100
BC2F1	HLA-A123	183	50	69	52	16	1.13	25.44	0.00	0.00	0.00	0.00	0/2	26/25	0/0	0/0	0/0	0/0	15	94
BC2F1	HLA-A84	84-1-13	50	71	52	15	2.67	23.93	0.13	0.27	0.00	0.00	4	24	0	0	0	0	6	40
BC2F1	HLA-A123	96	51	31	52	13	3.23	24.38	0.00	0.00	0.00	0.00	2/4	25/24	0/0	0/0	0/0	0/0	12	92
BC2F1	HLA-A99	50	53	13	52	6	1.33	25.33	0.00	0.00	0.00	0.00	0	26	0	0	0	0	3	50
BC2F1	HLA-A123	133	54	47	52	2	1.00	25.50	0.00	0.00	0.00	0.00	0/2	26/25	0/0	0/0	0/0	0/0	2	100
BC2F1	HLA-A85	156	57	57	52	5	4.40	23.80	0.00	0.00	0.00	0.00	4	24	0	0	0	0	4	80
BC2F1	HLA-A85	46	60	11	52	19	2.32	24.26	0.11	0.21	0.00	0.00	2	25	0	0	0	0	8	42
BC2F1	HLA-A122	90	60	27	52	6	2.67	24.67	0.00	0.00	0.00	0.00	4	24	0	0	0	0	3	50
BC2F1	HLA-A123	173	61	65	52	10	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	10	100
BC2F1	HLA-A123	138	65	49	52	2	2.00	25.00	0.00	0.00	0.00	0.00	0/4	26/24	0/0	0/0	0/0	0/0	2	100
BC2F1	HLA-A123	78	66	23	52	2	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	2	100
BC2F1	HLA-A4	113	69	39	53	14	2.36	25.21	0.07	0.00	0.00	0.00	3	25	0	0	0	0	7	50
BC2F1	HLA-A85	105	82	36	52	13	3.46	24.15	0.08	0.00	0.00	0.00	4	24	0	0	0	0	7	54
BC2F1	HLA-A77	95	88	30	52	6	0.67	25.67	0.00	0.00	0.00	0.00	0	26	0	0	0	0	4	67
BC2F1	HLA-A103	60	89	16	52	7	2.29	24.86	0.00	0.00	0.00	0.00	2	25	0	0	0	0	4	57
BC2F1	HLA-A123	93	95	29	52	8	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	8	100
BC2F1	HLA-A4	75	105	22	52	10	0.20	25.90	0.00	0.00	0.00	0.00	0	26	0	0	0	0	9	90
BC2F1	HLA-A123	143	112	53	52	7	1.43	25.29	0.00	0.00	0.00	0.00	2	25	0	0	0	0	5	71
BC2F1	HLA-A4	55	135	15	51	2	2.00	24.50	0.00	0.00	0.00	0.00	1/3	25/24	0/0	0/0	0/0	0/0	2	100
BC2F1	HLA-A123	151	136	55	52	6	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	6	100
BC2F1	HLA-A4	126	142	44	52	4	1.50	25.25	0.00	0.00	0.00	0.00	2	25	0	0	0	0	3	75
BC2F1	HLA-A110	119	145	41	51	14	1.14	24.93	0.00	0.00	0.00	0.00	1	25	0	0	0	0	13	93
BC2F1	HLA-A122	125	146	43	52	18	2.67	24.50	0.11	0.00	0.00	0.00	0	26	0	0	0	0	6	33
BC2F1	HLA-A123	91	179	28	51	16	1.00	25.00	0.00	0.00	0.00	0.00	1	25	0	0	0	0	16	100
BC2F1	HLA-A110	80	189	24	52	2	2.00	25.00	0.00	0.00	0.00	0.00	0/4	26/24	0/0	0/0	0/0	0/0	2	100

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC2F1	HLA-A118	52 8 14 52	7		2.57	24.71	0.00	0.00	0.00	0.00	2	25	0	0	0	0	5	71		
BC2F1	HLA-A103	BC2-15-11 (04)	3	76	50	2	0.00	25.00	0.00	0.00	0.00	0.00	0	25	0	0	0	0	2	100
BC2F1	HLA-A103	BC2-15-8 (04)	1	77	52	5	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	5	100
BC2F1	HLA-A103	BC2-2-3 (03)	1	78	52	22	1.09	25.45	0.00	0.00	0.00	0.00	0/2	26/25	0/0	0/0	0/0	0/0	21	95
BC2F1	HLA-B4	BC2-34-1 (04)	1	79	52	5	1.20	25.40	0.00	0.00	0.00	0.00	2	25	0	0	0	0	3	60
BC2F1	HLA-B45	BC2-39-2 (04)	2	80	52	2	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	2	100
BC2F1	HLA-B48	BC2-40-4 (04)	6	81	53	2	1.00	26.00	0.00	0.00	0.00	0.00	1	26	0	0	0	0	2	100
BC2F1	HLA-A4	BC2-4-19 (03)	152	82	52	10	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	10	100
BC2F1	HLA-B91	BC2-43-5 (04)	3	83	52	2	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	2	100
BC2F1	HLA-A83	BC2-5-4 (04)	1	84	51	9	2.56	24.22	0.00	0.00	0.00	0.00	3	24	0	0	0	0	5	56
BC2F1	HLA-A77	BC2-6-18 (03)	1	85	52	8	1.50	25.25	0.00	0.00	0.00	0.00	2	25	0	0	0	0	6	75
BC2F1	HLA-A77	BC2-6-19 (03)	4	86	51	4	1.50	24.75	0.00	0.00	0.00	0.00	1	25	0	0	0	0	3	75
BC2F1	HLA-A77	BC2-6-5 (03)	129	87	52	10	0.20	25.90	0.00	0.00	0.00	0.00	0	26	0	0	0	0	9	90
BC2F1	HLA-A77	BC2-6-6 (03)	1	88	52	7	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	7	100
BC2F1	HLA-A83	BC2-7-4 (03)	0	89	53	7	2.00	24.43	0.14	0.43	0.00	0.00	1/3	24/25	0/0	1/0	0/0	0/0	4	57
Total						773													546	71
Min.					50	2	0.00	21.50	0.00	0.00	0	0.00	0	21	0	0	0	0		33
Max.					53	37	9.00	26.00	0.50	0.77	0	0.07	10	26	1	1	0	0		100
Mean						9														
BC3F1	HLA-A85	BC3-10-9 (04)	4	1	52	2	1.00	25.50	0.00	0.00	0.00	0.00	2/0	25/26	0/0	0/0	0/0	0/0	2	100
BC3F1	HLA-A85	BC3-11-10 (04)	0	2	52	7	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	7	100
BC3F1	HLA-A77	BC3-1-13 (03)	2	3	52	4	1.00	25.50	0.00	0.00	0.00	0.00	0	26	0	0	0	0	3	75
BC3F1	HLA-A77	BC3-1-14 (03)	1	4	52	12	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	12	100
BC3F1	HLA-A85	BC3-12-5 (04)	0	5	52	4	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	4	100
BC3F1	HLA-A85	BC3-13-9 (04)	5	6	52	5	0.40	25.80	0.00	0.00	0.00	0.00	0	26	0	0	0	0	4	80
BC3F1	HLA-A85	BC3-14-4 (04)	2	7	52	5	0.00	24.00	0.00	1.00	0.00	0.00	0	24	0	1	0	0	5	100

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC3F1	HLA-A77	BC3-1-6 (03)	1	8	52	14	0.43	25.79	0.00	0.00	0.00	0.00	0	26	0	0	0	0	11	79
BC3F1	HLA-A2	BC3-1-9 (04)	1	9	52	2	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	2	100
BC3F1	HLA-A77	BC3-1-9 (03)	0	10	52	9	0.22	25.67	0.00	0.11	0.00	0.00	0	26	0	0	0	0	7	78
BC3F1	HLA-A77	BC3-26-4 (04)	2	11	52	3	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	3	100
BC3F1	HLA-A85	BC3-32-10 (04)	3	12	52	3	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	3	100
BC3F1	HLA-A84	BC3-3-22 (03)	3	13	52	8	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	8	100
BC3F1	HLA-A85	BC3-32-2 (04)	1	14	52	6	0.00	25.00	0.00	0.50	0.00	0.00	0/0	24/26	0/0	1/0	0/0	0/0	6	100
BC3F1	HLA-A84	BC3-3-25 (03)	0	15	52	3	0.00	24.67	0.00	0.67	0.00	0.00	0	24	0	1	0	0	2	67
BC3F1	HLA-A84	BC3-3-27 (03)	2	16	52	4	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	4	100
BC3F1	HLA-A103	BC3-33-2 (04)	1	17	52	6	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	6	100
BC3F1	HLA-A84	BC3-3-5 (03)	1	18	52	7	0.00	25.71	0.00	0.14	0.00	0.00	0	26	0	0	0	0	6	86
BC3F1	HLA-A84	BC3-7-10 (04)	0	19	52	3	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	3	100
BC3F1	HLA-A84	BC3-8-3 (04)	6	20	52	5	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	5	100
BC3F1	HLA-A84	BC3-9-1 (04)	4	21	52	2	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	2	100
BC3F1	HLA-A84	BC3-9-4 (04)	5	22	52	5	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	5	100
Total						119													110	92
Min.					52	2	0.00	24.00	0.00	0.00	0.00	0.00	0	24	0	0	0	0		67
Max.						14	2.00	26.00	0.00	1.00	0.00	0.00	2	26	0	1	0	0		100
Mean						5														
BC4F1	HLA-A84	BC4-30-3 (04)	3	1	52	4	1.50	25.25	0.00	0.00	0.00	0.00	2	25	0	0	0	0	3	75
BC4F1	HLA-A77	BC4-47-10 (04)	1	2	52	6	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	6	100
BC4F1	HLA-A77	BC4-48-3 (04)	2	3	52	7	0.29	25.86	0.00	0.00	0.00	0.00	0	26	0	0	0	0	6	86
BC4F1	HLA-A77	BC4-51-7 (04)	1	4	52	9	0.00	25.56	0.00	0.22	0.00	0.00	0	26	0	0	0	0	7	78
BC4F1	HLA-A84	BC4-78-2 (04)	0	5	52	5	0.80	25.60	0.00	0.00	0.00	0.00	0	26	0	0	0	0	4	80
BC4F1	HLA-A84	BC4-78-3 (04)	1	6	52	4	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	4	100
BC4F1	HLA-A84	BC4-78-4 (04)	0	7	52	9	0.22	25.89	0.00	0.00	0.00	0.00	0	26	0	0	0	0	8	89

A-1. Continued.

Gen	Family	Plant ID	%DP-16[††]	Plt No.	Chro[‡]	Cells	AVERAGE						MODE							
							I[†]	II	III	IV	V	VI	I	II	III	IV	V	VI	Mode[§]	% Cells[¶]
BC4F1	HLA-A84	BC4-78-6 (04)	1	8	52	9	0.67	24.56	0.00	0.56	0.00	0.00	0	24	0	1	0	0	4	44
BC4F1	HLA-A84	BC4-78-8 (04)	1	9	52	2	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	2	100
BC4F1	HLA-A84	BC4-79-5 (04)	1	10	52	3	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	3	100
BC4F1	HLA-A84	BC4-80-10 (04)	0	11	52	5	2.00	25.00	0.00	0.00	0.00	0.00	2	25	0	0	0	0	5	100
BC4F1	HLA-A84	BC4-80-3 (04)	0	12	52	6	1.67	25.17	0.00	0.00	0.00	0.00	2	25	0	0	0	0	5	83
BC4F1	HLA-A84	BC4-80-4 (04)	1	13	52	2	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	2	100
BC4F1	HLA-A84	BC4-81-4 (04)	1	14	52	6	0.67	25.67	0.00	0.00	0.00	0.00	0	26	0	0	0	0	4	67
BC4F1	HLA-A84	BC4-81-6 (04)	0	15	52	10	0.60	25.70	0.00	0.00	0.00	0.00	0	26	0	0	0	0	7	70
Total						87													70	80
Min.					52	2	0.00	24.56	0.00	0.00	0.00	0.00	0	24	0	0	0	0		44
Max.						10	2.00	26.00	0.00	0.56	0.00	0.00	2	26	0	1	0	0		100
Mean						6														
BC5F1	HLA-A84	BC5-76-4 (04)	1	1	52	9	0.00	26.00	0.00	0.00	0.00	0.00	0	26	0	0	0	0	9	100
BC5F1	HLA-A84	BC5-76-7 (04)	1	2	52	11	0.00	25.45	0.00	0.27	0.00	0.00	0	26	0	0	0	0	8	73
Total						20													17	85
Min.					52	9	0.00	25.45	0.00	0.00	0.00	0.00	0	26	0	0	0	0		73
Max.						11	0.00	26.00	0.00	0.27	0.00	0.00	0	26	0	0	0	0		100
Mean						10														

[†]I, II, III, IV, V, VI represents univalent, bivalent trivalent, quadrivalent, pentavalent, and hexavalent chromosomal configurations respectively.
[††]% Deltapine-16.
[‡]Chromosome number.
[§]Modal value for the respective chromosomal configuration.
[¶]% cells with modal chromosomal configuration.

[‡‡]Represents two or more type of modal chromosomal configurations. For example, 1/5I, 26/24II, 0/0III, 0/0IV, 0/0V, and 0/0VI represent 1I+26II+0III+0IV+0V+0VI and 5I+24II+0III+0IV+0V+0VI as the modal types.

VITA

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Education

Doctor of Philosophy- Texas A&M University, College Station, TX

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Bachelor of Science- Mahatma Phule Agricultural University, Rahuri, India

Awards and Honors

Recipient of the IMC Endowed Assistantship, 2007

First place in the student oral presentation competition at the 2007 Beltwide Cotton Conference meeting held in New Orleans, LA

Best Agriculture Student of the Year, 2000

Indian Council of Agricultural Research Fellowship, 1996-2000

Sports Authority of India Scholar, 1988-1994

Publications

Robinson, A.F., A.A. Bell, N.D. Dighe, M.A. Menz, R.L. Nichols, and D.M. Stelly. 2007. Introgression of resistance to nematode *Rotylenchulus reniformis* into Upland Cotton (*Gossypium hirsutum*) from *Gossypium longicalyx*. Crop Sci. 47:1865-1877.

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