

## BREEDING AND GENETICS

### Major Leaf Shapes of Cotton: Genetics and Agronomic Effects in Crop Production

Ryan J. Andres, Daryl T. Bowman, Don C. Jones, and Vasu Kuraparthi\*

#### ABSTRACT

There exist four major leaf shape alleles in tetraploid cotton: normal, sub-okra/Sea-Island, okra, and super-okra. This allelic series has long served as a model genetic locus both in cotton and the broader leaf development research community. Over the years, numerous studies have attributed various production advantages to specific leaf shapes. The objective of this study was to provide a comprehensive review of this literature in order to provide a definitive report on the true benefits of these leaf shapes. In addition, a history of the genetic dissection of the major leaf shape locus was compiled. Leaf shape was found to have consistent effects on boll rot resistance, earliness, flowering rate, chemical spray penetration, lint trash, and yield. Reported effects on various insect resistances, photosynthetic rate, water use efficiency, and fiber quality were not consistent across studies. An ideal cotton cultivar would produce normal leaves up until the point canopy closure is obtained and then it would switch over to an open canopy of okra or super okra. Major leaf shapes of Upland cotton are a multiple allelic series of a single incompletely dominant genetic locus *L-D<sub>1</sub>* on chromosome 15-D<sub>1</sub> (Chr15). Genetic analysis studies have precisely mapped the major effect leaf shape genes in cotton and deciphered the causal nucleotide and gene expression changes leading to leaf shape phenotypic diversity in cotton. Recent advances in understanding the molecular processes underlying leaf shape phenotypic changes could help open new avenues for developing cotton cultivars with ideal leaf shape and could enhance sustainable and profitable cotton production.

Despite the vast majority of cotton cultivars carrying “normal (NL)” or broad leaves, there has been periodic interest in the use of the major leaf shape “mutants” to improve particular aspects of cotton production. These alternate leaf shapes; sub-okra/Sea-Island (subOL), okra (OL), and super-okra (superOL), have been reported to influence a wide range of characteristics from disease and insect resistance to yield and fiber quality. The four leaf shapes are well-established as alleles at a single locus called *L* or *L-D<sub>1</sub>*. All four are easily distinguishable by brief visual observation (Figure 1) and heterozygotes are intermediate between the parental types.



Figure 1: The four major leaf shapes of tetraploid cotton, from left: normal, sub-okra/Sea-Island, okra and super-okra. The leaf shape series is characterized by a decrease in area per leaf caused by an increase in leaf lobing and corresponding decrease in lobe width. At maturity, super-okra has been reduced to a single strip emanating from the petiole. Leaves are from members of the BC<sub>8</sub> isoline series developed in LA 213 background by Kennedy et al. (1986).

Allometric analysis has indicated that the gene underlying this locus acts very early during leaf development and during the initiation of the primordium (Dolan and Poethig, 1991; Hammond, 1941a). After the shape has been determined, it remains constant through the remaining growth and development of the leaf (Dolan and Poethig, 1991). In OL, the underlying factor delays the appearance of lateral lobes, increases growth of the lobe relative to the sinus, and increases lobe elongation compared to lobe widening (Dolan and Poethig, 1991; Hammond, 1941a). This may be done by substantially extending the early growth pattern of the primordia (Dolan and Poethig, 1991). The use of genetic mosaics has also shown that the factor underlying OL is cell specific and cannot move between cells (Dolan and Poethig,

R.J. Andres and V. Kuraparthi, Department of Crop Science, North Carolina State University, 101 Derieux Place, Raleigh, NC 27695; D.T. Bowman, North Carolina Foundation Seed Producers, 8220 Riley Hill Road, Zebulon, NC 27597; and D.C. Jones, Cotton Incorporated, Cary, NC 27513.

\*Corresponding author: [vasu\\_kuraparthi@ncsu.edu](mailto:vasu_kuraparthi@ncsu.edu)

1991). The alleles at the major leaf shape locus in the A genome diploids also acts very early in leaf development, similar to OL in the tetraploid (Hammond, 1941b).

The objective of this study is to provide a comprehensive review on the advantages and disadvantages that can be ascribed to the major leaf shapes of cotton. In addition, a history of the genetic dissection of the leaf shape trait from its first reports to its cloning is compiled.

### Applications of the Major Leaf Shapes in Cotton Production

**Boll Rot Resistance.** OL cultivars reduce losses due to boll rot by 7-11% compared to NL when boll rot conditions are low to moderate (Jones and Andries, 1967; Rao and Weaver, 1976) and 43-45% when environments are more severe (Andries et al., 1969; Karami and Weaver, 1972). SuperOL shows an even greater reduction in boll rot (55%) than NL under severe conditions (Andries et al., 1970). This reduction in boll rot is generally attributed to microclimatic differences. OL and superOL may develop a more open canopy allowing for greater air circulation and light penetration, creating a less favorable environment for microbial growth (Andries et al., 1969; 1970).

**Earliness to Maturity and Flowering Rate.** OL cultivars are earlier to flowering and maturity than NL with effects reported from two to twelve days earlier (Andries et al., 1969; Heitholt and Meredith, 1998; Jones and Andries, 1967; Karami and Weaver, 1972; Rao and Weaver, 1976). SuperOL matures nine to twelve days earlier than NL (Andries et al., 1970). Both OL and superOL also display drastically increased flowering rates with OL flowering 25-50% and superOL 85-100% more than NL (Andries et al., 1969; 1970; Gonias et al., 2011; Heitholt, 1993). This increase in flowering is generally offset by a marked increase in boll abortion (Heitholt, 1993; Heitholt, 1995; Kerby and Buxton, 1976). Flowering rates and boll abscission of subOL are not different than NL (Heitholt, 1993). The increase in flowering rate and decrease in time to maturity may be due to plant physiology (Landivar et al., 1983). Since OL and superOL need less photosynthate to both develop new leaves and maintain existing ones, they can contribute more assimilate to reproductive development and do so earlier (Landivar et al., 1983). However, these cultivars initiate more bolls than they can support, leading to higher rates of abortion (Landivar et al., 1983).

**Insect Resistance.** Studies using near-isogenic lines (NILs) have credited OL with reduced damage from the pink bollworm (PBW) *Pectinophora gossypiella* (Wilson et al., 1979; Wilson and George, 1982). This reduction in PBW damage was at least partially attributed to the earliness of OL (Wilson et al., 1979). However, a later study using seven pairs of leaf shape NILs found a reduction in PBW damage by OL in only three genetic backgrounds (Wilson, 1986). In a follow-up study, Stoneville 7A-Okra, which had twice shown less damage to PBW than its NIL (Wilson et al., 1979; Wilson, 1986), was shown to have decreased boll penetration by PBW larvae (Wilson et al., 1986). The authors hypothesized that OL could result in increased thickness of the carpel (boll) walls, but only in certain genetic backgrounds (Wilson et al., 1986). Wilson et al. (1986) also demonstrated that increased canopy and soil temperatures of Stoneville 7A-Okra had no effect on the growth and development of PBW at multiple life stages. Later, under laboratory conditions, Stoneville 7A-Okra did not have fewer larval entrance holes than Stoneville 7A-Normal but did have fewer larvae per entrance hole in the first 50 minutes after infestation (Wilson et al., 1992). There was no difference in either the number of entrance holes or larvae per hole between Stoneville 213-Okra and Stoneville 213-Normal (Wilson et al., 1992). Furthermore, Stoneville 7A-Okra had thinner carpel walls than Stoneville 7A-Normal while the reverse was true in the Stoneville 213 NILs (Wilson et al., 1992). This indicates that carpel wall thickness has no effect on PBW resistance (Wilson et al., 1992). It appears that there is nothing intrinsic to OL that reduces PBW damage. Anyhow, the more complete control of PBW by *Bt* cotton in the mid-1990s appears to have obviated the need for further research into the PBW resistance inconsistently observed in OL lines.

The silverleaf/sweet potato whitefly (*Bemisia tabaci/argentifolli*) and the banded-winged whitefly (*Trialeurodes abutilonea*) are serious insect pests of cotton. Yield is lost due to direct insect feeding and the transmission of viral diseases while the deposition of sticky honeydew on lint can reduce fiber quality (Centintas and McAuslane, 2009). In numerous germplasm screens, OL has been associated with lower numbers of whitefly adults, nymphs, or eggs (Butler et al., 1988; Butler et al., 1991; Centintas and McAuslane, 2009; Chu et al., 1999; Chu et al., 2000; Chu et al., 2002; Jones et al., 1975; Ozgur and Sekeroglu, 1986; Sippell et al., 1987). One germplasm screen reported that OL did not have a

consistent effect on nymph numbers (Flint and Parks, 1990). In studies using isogenic lines, results were more varying as OL was more resistant in one study by Jones et al. (1975) while in other studies, OL had either mixed effects on whitefly resistance (Butler et al., 1986; Butler and Wilson, 1986) or had no or a negative effect (Butler and Wilson, 1984). The decrease in whitefly numbers was largely attributed to microclimatic differences (Chu et al., 1999; Chu et al., 2002; Ozgur and Sekeroglu, 1986; Sippell et al., 1987). A more open canopy may result in a reduced number of protected sites for feeding and oviposition and therefore create greater exposure to sunlight, high temperature, and low humidity. The majority of these studies do not investigate the impact of these reduced whitefly populations on yield and those that do provide mixed results (Butler et al., 1988; Chu et al., 1999). In conclusion, there may be some ability of OL to reduce whitefly populations but the effect may also depend on other characters such as leaf hairiness (Walker and Natwick, 2006) and the depth of vascular bundles from the leaf surface (Chu et al., 1999).

In a germplasm screen for resistance to the two-spotted spider mite *Tetranychus urticae*, Bailey et al. (1978) noted that OL tended to support lower mite population numbers than NL under insecticide treated fields. This difference was not seen in untreated fields and was therefore attributed to greater pesticide penetration in OL (Bailey et al., 1978). Bailey and Meredith (1983) later showed that an OL isolate had fewer mites than frego, smooth, NL, nectariless, and glandless isolines. Comparing only Siokra (PI 607166) to NL Deltapine 90 (PI 529529), Wilson (1993) demonstrated that mite populations developed slower and reached lower peak levels on Siokra than Deltapine 90. This resulted in smaller yield reductions due to mite infestations in Siokra than Deltapine 90 (Wilson, 1993). The mechanism of resistance of OL to two-spotted spider mite was later attributed to reduced protected area for oviposition and feeding in a study utilizing isolines (Wilson, 1994).

OL has been mentioned as providing some resistance to the boll weevil *Anthonomus grandis* (Pieters and Bird, 1977; Jones, 1982). The mechanisms of this resistance have been attributed to earliness (Pieters and Bird, 1977) and a hotter and drier microclimate (Jones, 1982). However, more detailed studies on the effect of OL on boll weevil were likely abandoned by successful eradication of boll weevil through the Boll Weevil Eradication Program.

**Application of Foliar Chemicals.** OL has been credited with increased spray penetration of insecticides under field conditions (Jones et al., 1987). While the efficacy of this spray penetration was not tested, it was hypothesized that more insecticide delivered deeper in the canopy would reduce insect damage and subsequently the number of applications (Jones et al., 1987). SuperOL likely has the same or greater effect on spray penetration but this has not been documented.

### Leaf Area

In studies that measured the area of individual leaves, OL are roughly 60-70% the size of NL (Andries et al., 1969; Karami and Weaver, 1972; Pettigrew et al., 1993; Pettigrew 2004; Wells and Meredith 1986). SuperOL leaves are 52% the size of NL (Andries et al., 1970) while subOL are not significantly different in size from NL (Wells and Meredith 1986). In terms of leaf area index (LAI), most studies report OL to be statistically less than NL (Heitholt et al., 1992; Kennedy et al., 1986; Wells et al., 1986; Zhu et al., 2008). However, variability exists and in certain instances the difference has not been significant (Kerby et al., 1980; Pegelow et al., 1977; Peng and Krieg 1991). The LAI of subOL is similar to that of NL (Zhu et al., 2008) while superOL is always significantly smaller than NL and usually statistically smaller than OL (Pegelow et al., 1977; Wells et al., 1986; Zhu et al., 2008).

### Photosynthesis and Water Use Efficiency

Based on models and air-flow experiments, it was hypothesized that an OL would have a thinner boundary layer than NL (Baker and Myhre, 1969). This would make it easier for CO<sub>2</sub> to move into an OL, thereby allowing a higher photosynthetic rate but also an increase in water lost through transpiration (Baker and Myhre, 1969). However, single okra leaves failed to fix more CO<sub>2</sub> than NL under experimental conditions and it was hypothesized that the boundary layer does not have a large enough effect on diffusion resistance to have an impact (Baker and Myhre, 1969). Based on modeling studies, it was proposed that on a single leaf basis OL fixes 5% more CO<sub>2</sub> than NL but loses 7.5% more water through transpiration under conditions of low relative humidity (Buxton and Stapleton, 1970). However, there was no difference in transpiration rates under high relative humidity, indicating that OL would have a photosynthetic and water use

efficiency advantage under high humidity conditions (Buxton and Stapleton, 1970). This advantage might be even larger when expanded to a full canopy effect rather than just a single leaf (Buxton and Stapleton, 1970). However, later field studies showed no difference in CO<sub>2</sub> fixation rates between NL and OL canopies while superOL fixed 29% less CO<sub>2</sub> (Pegelow et al., 1977). There was also no difference in transpiration rates or photosynthesis-to-transpiration ratios among the three leaf shapes (Pegelow et al., 1977). Karami et al. (1980) showed that there was no difference in water relations or photosynthetic rates among single leaves of NL, laciniate, and superOL under well-watered greenhouse conditions. However, under drought-stress, superOL had statistically improved water relations and carbon fixation with laciniate an intermediate between the two (Karami et al., 1980). This was attributed to the mutant leaves requiring less water for maintenance and thus being less susceptible to water stress (Karami et al., 1980). Two studies showed that there was no consistent difference in CO<sub>2</sub> canopy fixation rates among leaf shape isolines (Kerby et al., 1980, Peng and Krieg, 1991). On a single leaf basis, one study found higher per unit leaf area fixation in OL compared to NL (Peng and Krieg, 1991) while another found no consistent differences among NL, OL and superOL (Perry et al., 1983). Wells et al. (1986) using leaf shape isolines in a MD 65-11 background found that OL and superOL canopies had reduced canopy photosynthetic rates compared to NL and subOL. This was attributed to insufficient leaf area failing to maximize light interception in OL and superOL (Wells et al., 1986). However, using the same leaf shape isolines but single leaf measurements Pettigrew et al. (1993) found that OL and superOL fixed more CO<sub>2</sub> than NL. Combined with lower stomatal conductance measurements this lead to higher water use efficiency for the mutants compared to NL (Pettigrew et al., 1993). The effects were largely attributed to increases in leaf thickness and chlorophyll concentration of the mutants (Pettigrew et al., 1993). Contradictions to Wells et al. (1986) were attributed to differences in the time of day measurements were taken (Pettigrew et al., 1993) but no mention was made about single leaf vs. canopy measurements. The advantage of OL in carbon fixation and chlorophyll concentration, but not stomatal conductance, was confirmed in a later study using a different genetic background, but again on single leaves (Pettigrew, 2004). The superiority of

OL in photosynthesis was attributed to improved light adapted photosystem II quantum efficiency, electron transport rate, and non-photochemical quenching (Pettigrew, 2004). Gonias et al. (2011) used single leaf measurements on chamber-grown plants to show no difference in leaf photosynthesis, PSII quantum yield, or membrane leakage between NL and OL in the FM 832 background. OL did display higher chlorophyll content while NL had thicker leaves (Gonias et al., 2011). Therefore, the reported effects of leaf shape on photosynthesis and transpiration indicate that individual leaves of the mutants may have certain advantages. However, when examined over an entire canopy these effects are generally negligible.

### Lint Trash

Novick et al. (1991) investigated the effect of leaf shape on lint trash in two genetic backgrounds. SuperOL was found to reduce motes by 15% and small leaf trash by 20% (Novick et al., 1991). The reduction in motes was attributed to higher pollination rates due to increased pollen viability in the less humid and unshaded superOL canopy (Novick et al., 1991). Both OL and superOL were associated with increased cleanability due to a greater percentage of the leaf trash being harder materials such as veins and petioles that are less prone to shattering (Novick et al., 1991). OL can actually increase lint trash due to its propensity to hang on branches following defoliation (Novick et al., 1991). Overall, superOL and subOL showed improved grades compared to NL following cleaning (Novick et al., 1991).

### Yield and Fiber Quality

Studies comparing lint yield in isolines on a per plot basis have reported OL effects ranging from +20% (Karami and Weaver, 1972) to -8% (Wilson and George, 1982) compared to NL. Using single plant harvesting or hybrid cultivars have led to reports of the OL yield penalty being as high as -17% and -38.5% respectively (Wilson, 1986; Zhu et al. 2008). However, the majority of the isogenic studies found that leaf shape had no effect on yield (Gonias et al., 2011; Heitholt, 1993; Heitholt et al., 1996; Jones and Andries, 1967; Meredith, 1983; 1984; 1985; Riar et al., 2013). Increases in yield of OL isolines have been attributed to reductions in boll rot losses (Andries et al., 1969), earliness to escape late-season stress (Andries et al., 1969), improved harvest index (Karami and Weaver, 1972), positive

response to reduced insecticide application (Thomson et al., 1987), improved suitability to increased planting density (Heitholt et al., 1992; Heitholt 1994), and higher water use efficiency (Stiller et al., 2004). However, increased planting density can be offset by difficulties in adaptation to mechanized production (Heitholt et al., 1996) and increased seed and seed treatment costs (Riar et al., 2013). Decreases in yield of OL isolines have been attributed to their construction via backcrossing in elite backgrounds for NL (Meredith and Wells, 1986), insufficient light interception and photosynthesis (Wells et al., 1986), and increased evaporation of soil moisture (Zhu et al., 2008).

In conclusion, the effect of OL on yield is likely heavily influenced by the environmental conditions. Early season stresses are more likely to negatively affect OL lines by delaying the attainment of canopy closure (Heitholt and Meredith, 1998). As long as OL does not reach canopy closure substantially later than NL, it should be in a position to out-yield NL due to its greater relative partitioning of assimilate to reproductive growth throughout the season. On the other hand, late season stresses are more likely to depress yields in NL lines because they produce fewer flowers and mature later (Heitholt and Meredith, 1998).

The effect of superOL isolines on yield is either none (Heitholt, 1993; Thomson, 1971) or slightly negative (-6-8.8%) (Andries et al., 1970; Meredith, 1984). Although Zhu et al. (2008) reported that superOL hybrids yielded only 42% of a NL check hybrid. The inferiority of superOL leaf in terms of yield is likely attributable to insufficient light interception and canopy photosynthesis as well increased weed competition (Thomson, 1971; Zhu et al. 2008). However, these could be overcome by increasing planting density (Thomson, 1971). The effect of subOL isolines on yield ranged from none (Heitholt, 1993; Meredith, et al., 1996) to a slight increase of 3-4.8% (Meredith, 1984; Meredith and Wells; 1987). The yield advantage of subOL might have resulted from a combination of its ability to quickly reach canopy closure along with the benefits of a slightly more open canopy structure later in the season (Meredith, 1984).

While individual studies occasionally report small statistical differences in either boll or fiber quality characters due to leaf shape, these differences are usually not substantial and inconsistent across studies. Therefore, it can be concluded that

leaf shape has no impact on either boll characteristics or fiber quality.

### **Manipulation of upper plant canopy using mechanical topping and pruning**

Changing and reducing the density of the upper leaf canopy through manual or mechanical topping and pruning was hypothesized to improve the cotton productivity. Few attempts were made to estimate the effect of manual and/or mechanical topping and pruning of upper plant canopy in cotton production. It was shown that there was significant increase in squares, bolls and lint yield because of partitioning of nutrients to reproductive organs (Li et al., 2006). In another study, Bennett et al. (1965) showed that topping increased lint percentage, fiber length and micronaire relative to non-topped cotton while boll rot was shown to reduce significantly by altering the plant geometry. Simulation models showed pruning and topping increased the boll retention, possibly due to significant reductions in abscised fruit sites (Yang et al., 2008). Manually topped plots of cotton also showed decreased infestation by bollworms *Helicoverpa armigera* (56%), *Earias* spp. (68%) and *Diparopsis watersi* (71%) (Renou et al., 2011). Although, manual topping was widely followed in China (Dai and Dong, 2014) manipulation of plant canopy through manual topping is not feasible in large-scale cotton production in North America. However, these preliminary studies indicated that changing the canopy architecture offers opportunities to help improve cotton productivity.

### **Inheritance of Leaf Shape in Cotton**

OL has been known to exist in cotton since before the 19<sup>th</sup> century (Mell, 1890). Shoemaker (1909) first showed that OL was controlled by a single gene. "Sea-island" was also shown to be simply inherited a short time later (McClendon, 1912). SuperOL was first reported as a spontaneous mutant in a population of Acala Okra grown in Trinidad and subsequently observed to be simply inherited (Harland, 1932). The allele symbols for NL (*l*), OL (*L<sup>o</sup>*), and superOL (*L<sup>s</sup>*) were given by Hutchinson and Silow (1939). In 1945, Stephens demonstrated that superOL and sea-island (*L<sup>E</sup>*) were alleles at the same locus as NL and OL (Stephens, 1945). Green (1953) reported on a new leaf shape observed in the progeny of a synthetic tetraploid derived from a cross between the A genome diploid *G. arboreum* and the D genome diploid *G. thurberi*. This novel

leaf shape was named subOL ( $L^U$ ) and shown to be a fifth allele at the leaf shape locus (Green, 1953). Citing independent evidence, Green proposed that the subOL allele originated in *G. thurberi*, thus initiating the belief that the locus resides in the D genome. It is likely that the leaf shapes of the parents played some role in this decision as *G. thurberi* possesses a highly lobed leaf similar to OL while most *G. arboreum* leaves are similar to NL or subOL. However, it has not been definitively proven that subOL is a different allele than Sea-Island (Meredith 1983) and recent research indicates they are likely the same allele (Andres et al., 2016).

In 1955, Stephens showed that the leaf shape locus ( $L$ ) was genetically linked to the crinkle leaf ( $cr$ ) and green lint ( $Lg$ ) loci. Stephens then cited Green (1953) as saying that since subOL came from *G. thurberi*, the whole linkage group must reside in the D genome. Unpublished data from Hutchinson saying that green lint came from the D genome diploid *G. armourianum* was also cited to confirm the placement of the linkage group in the D genome (Stephens, 1955). The chromosome carrying the  $L$  locus was named chromosome 15 (Chr15) in cytogenetic work utilizing monosomes (Endrizzi and Brown, 1964). Later telosomic stocks were used to place the  $L$  locus on the short arm of Chr15, opposite  $cr$  and  $Lg$  on the long arm (Endrizzi and Kohel, 1966).

Jiang et al. (2000) used restriction fragment length polymorphism (RFLP) markers to map leaf shape quantitative trait loci (QTL) in an interspecific *G. hirsutum* (OL) x *G. barbadense* (NL)  $F_2$  population. They identified a large, multiple effect QTL on Chr15, presumably the  $L$  locus, mapped between the RFLP markers pAR019 and pAR1001 (Jiang et al. 2000). Song et al. (2005) also mapped leaf shape QTL using an interspecific *G. hirsutum* (NL) x *G. barbadense* (OL)  $BC_1$  population and simple sequence repeat (SSR) markers. A large, multiple effect QTL was not found in this population on Chr15, but a QTL of minor effect on leaf lobe width was mapped to a ~30cM region bounded by SSRs BNL2440 and JESPR152 (Song et al., 2005). The authors commented that the disparity of their findings to Jiang et al. (2000) may be the result of differences in population size, population type, marker type, or parents (Song et al., 2005). However, based on statements made and data presented on the parental lobe lengths, the *G. barbadense* parent may have been subOL rather than OL. Therefore, the lack of detection for the classical  $L$  locus in that study may be better explained by the

greater similarity in shape of subOL to NL as well as the exclusion of subOL homozygotes in the  $BC_1$  mapping population backcrossed to the NL parent. Lacape et al. (2013) used amplified fragment length polymorphism (AFLP) and SSR markers to map leaf shape and other traits in an interspecific *G. hirsutum* (NL) x *G. barbadense* (subOL) recombinant inbred line (RIL) population. The major leaf shape QTL was placed in a 26.9 cM interval bounded by the SSR BNL2440 and the AFLP E43M52-M326.0 on Chr15 (Lacape et al., 2013). Three markers within this region appeared to be highly associated with the major leaf shape QTL, SSRs BNL1693 and MGHE32 and the AFLP 151u. In a first mapping study to treat leaf shape as a single gene rather than a QTL, Andres et al. (2014) placed the leaf shape locus in a 4cM region between the SSRs Gh565 and NAU2343, completely within the flanking markers of previous studies. Andres et al. (2014) used orthologous mapping and the sequenced diploid D genome donor *G. raimondii* to identify two *LATE MERISTEM IDENTITY1*-like genes, *Gorai.002G244000* and *Gorai.002G244200*, as strong candidates for the leaf shape gene in cotton. Zhu et al. (2014) also placed the major leaf shape gene in an 8.8cM region between two single nucleotide polymorphisms (SNPs) on Chr15 of *G. hirsutum* in an intraspecific OL x NL RIL population. These SNP markers span a physical distance of 881kb on Chr02 in the *G. raimondii* genome that fully encompasses the 337kb candidate region identified by Andres et al. (2014). Thus, all five studies have placed the leaf shape gene in relatively the same position leaving little doubt that the major leaf shape gene of cotton resides in this relatively narrow area of the genome. Zhu et al. (2015) showed that the *G. hirsutum* ortholog of *Gorai.002G244000* was significantly up-regulated in OL compared to NL and proposed variations in protein structure might also be responsible for the different leaf shapes. Subsequently, Andres and co-workers have confirmed the over-expression of the *Gorai.002G244000* ortholog in OL and successfully used Virus-Induced Gene Silencing (VIGS) to produce NL leaves in an OL variety (Andres et al., 2016, Andres, 2015). They proposed that over-expression in OL is due a 133bp tandem duplication in the promoter region of *Gorai.002G244000* while NL results from a non-functional, truncated protein due to an eight bp deletion in the third exon. SubOL is an intermediate between NL and OL, lacking both the promoter duplication and the exonic deletion.

SuperOL also possesses the promoter duplication, but what differentiates it from OL was unknown.

An alternate leaf shape locus is also known to exist in the A genome of cotton. In a series of crosses among and between the diploid A genome species *G. arboreum* and *G. herbaceum* Hutchinson (1934) demonstrated that there existed five leaf shapes in Asiatic cotton, all of which are allelomorphic: laciniate ( $L^L$ ), arboreum ( $L$ ), recessive broad ( $I$ ), mutant broad ( $L^B$ ) and mutant intermediate ( $L^I$ ). Only laciniate, which is phenotypically similar to OL, was transferred from *G. arboreum* to *G. hirsutum* by a Dr. C. Rhyne ~1960 (Endrizzi and Stein, 1975; Jones, 1982). The laciniate locus was placed on Chr01 of the A genome in cytogenetic work using monosomes (White and Endrizzi, 1965). Since OL and laciniate alleles have similar effects on leaf shape, they were considered to be genes at “duplicate” loci in the two genomes (White and Endrizzi, 1965). This served as the basis for establishing that Chr01 and Chr15 are homeologous chromosomes in tetraploid cotton (White and Endrizzi, 1965). Recently, it has been shown that the laciniate gene in *G. arboreum* resides in a homoeologous region of the genome as the OL locus and is also likely conditioned by modifications to a *LM11*-like gene (Kaur et al., 2015). There exists no mention of any of the other leaf shape alleles being transferred to *G. hirsutum* from Asiatic cotton. However, since they are considered an allelic series in Asiatic cotton, it is assumed that they could also make up an allelic series at the Chr01 A genome homeolog in *G. hirsutum* (Jones, 1982; Meredith, 1984). Some *G. hirsutum* lines still exist today that purportedly carry the laciniate allele. However, the term laciniate is occasionally used incorrectly and interchangeably with OL and superOL in the literature. Therefore, which of these lines truly carry the laciniate allele is unknown.

### CONCLUSIONS

**The major leaf shape alleles in tetraploid cotton are: normal, sub-okra/Sea-Island, okra, and super-okra.** A summary of leaf shape effects on production characteristics is provided in Figure 2. The OL, most common of the three “mutant” leaf shapes in cotton production, was found to have consistently positive effects on chemical spray penetration, boll rot reduction, earliness to maturity, and flowering rate. However, these advantages were offset by a decrease in boll retention. Yield response was highly variable across studies ranging from 8% to

+20%. The effect on yield appears highly dependent on environment and would likely be neutral across years in a long-term study. The SuperOL provides an even greater advantage than OL for boll rot reduction, earliness to maturity, and flowering rate, in addition to a marked decrease in lint trash. However, excessive boll shed in superOL almost always results in lower yield. The SubOL is comparable to NL for most traits, but may have a slight advantage in boll retention and yield due to more efficient allocation of resources to reproductive growth.

|            | Normal  | Spray penetration   | Boll rot   | Lint trash  | Earliness   | Flowering   | Boll retention  | Yield   |
|------------|---|---|--|---|---|---|---|---|
| Sub-Okra   |  |  |  |  |  |  |  |  |
|            | ?   | None  | None   | None  | None  | +3%   | 0 → +4.8%   |   |
| Okra       |  | +26%  | -44%   | +15%  | -6.5 days   | +38%  | -13%  | -8% → +20%  |
| Super-Okra |  | ?   | -55%   | -20%  | -10.5 days  | +93%  | -21%  | 0 → -9%   |

**Figure 2: Summary of the advantages and disadvantages of the major leaf shapes in cotton production. Only those traits determined to have a consistent impact are listed. Values are unweighted averages or ranges compiled from multiple studies reporting on that particular trait and are set relative to normal leaf shape genotype.**

Major leaf shapes of Upland cotton are a multiple allelic series of a single incompletely dominant genetic locus  $L-D_1$  on chromosome 15-D<sub>1</sub> (Chr15). Genetic analysis studies have precisely mapped the major effect leaf shape genes in cotton and deciphered the causal nucleotide and gene expression changes leading to leaf shape phenotypic diversity in cotton.

**Leaf shape ideotype.** Keeping in view the unique benefits of normal, okra and sub-okra leaf shape genotypes described in this paper, an ideal cotton cultivar would produce normal leaf shape up until the point canopy closure is obtained. This would protect against adverse early season growing conditions while minimizing inputs and seeding rates. Then, once canopy closure was obtained, the cultivar would switch over to an open canopy of okra or even super-okra leaf shape. Subsequent wasteful vegetative growth through shading would be minimized and more photosynthate would be directed towards reproduction. Lower bolls and leaves would remain relatively unshaded leading to lower rates of low position boll shedding. The upper open canopy would render the crop less susceptible to late

season stresses. Therefore, consistently higher yields may be obtained than with either okra leaf shape or normal leaf shape alone. Using virus-induced gene silencing (VIGS) of the *GhLMI1-D1b* gene at the *L-D1* locus, Andres et al. (2016) temporarily induced normal leaf shape formation in an okra leaf shape genotype, and showed proof of concept for creating the leaf shape ideotype in cotton. Recent advances in understanding the molecular genetic processes underlying leaf shape phenotypic changes in cotton could help open new avenues for developing cotton cultivars with ideal leaf shape and could enhance sustainable and profitable cotton production.

### ACKNOWLEDGEMENT

We thank Cotton Incorporated and NC Cotton Producers Association for supporting this research.

### DISCLAIMER

Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by the North Carolina State University and does not imply its approval to the exclusion of other products that may also be suitable.

### REFERENCES

- Andres R.J., D.T. Bowman, B. Kaur, and V. Kuraparthi. 2014. Mapping and genomic targeting of the major leaf shape gene (*L*) in Upland cotton (*Gossypium hirsutum* L.). *Theor. Appl. Genet.* 127: 167-177.
- Andres R.J. 2015. Genetic mapping and molecular cloning of the major leaf shape gene (*L-D1*) in Upland cotton (*Gossypium hirsutum* L.) Ph.D. diss. North Carolina State Univ., Raleigh, NC
- Andres R.J., V. Coneva, M.H. Frank, J.R. Tuttle, S.W. Han, L.F. Samayoa, B. Kaur, L. Zhu, H. Fang, D. Bowman, M. Rojas-Pierce, C.H. Haigler, D.C. Jones, J.B. Holland, D.H. Chitwood, and V. Kuraparthi. 2016. Modifications to a *LMI1*-like gene are responsible for the major leaf shapes of Upland cotton (*Gossypium hirsutum* L.) *Proc. Natl. Acad. Sci. U.S.A.*: In Press.
- Andries J.A., J.E. Jones, L.W. Sloane, and J.G. Marshall. 1969. Effects of okra leaf shape on boll rot, yield, and other important characters of Upland cotton, *Gossypium hirsutum* L. *Crop Sci.* 9: 705-710.
- Andries J.A., J.E. Jones, L.W. Sloane, and J.G. Marshall. 1970. Effects of super okra leaf shape on boll rot, yield, and other characters of Upland cotton, *Gossypium hirsutum* L. *Crop Sci.* 10: 403-407.
- Baker D.N., and D.L. Myhre. 1969. Effects of leaf shape and boundary layer thickness on photosynthesis in cotton (*Gossypium hirsutum*). *Physiol. Plant.* 22: 1043-1049.
- Bailey J.C., R.E. Furr., B.W. Hanny, and W.R. Meredith. 1978. Field populations of two-spotted spider mites on sixteen cotton genotypes at Stoneville, MS 1977. *J. Econ. Entomol.* 71: 911-912.
- Bailey J.C., and W.R. Meredith. 1983. Resistance of cotton, *Gossypium hirsutum* L., to natural field populations of two-spotted spider mites (Acari: Tetranychidae). *Environ. Entomol.* 12: 763-764.
- Bennett O.L., Ashley D.A., Doss B.D., and Scarsbrook C.E. 1965. Influence of topping and side pruning on cotton yield and other characteristics. *Agron. J.* 57: 25-27.
- Butler G.D., and F.D. Wilson. 1984. Activity of adult whiteflies (Homoptera: Aleyrodidae) within plantings of different cotton strains and cultivars as determined by sticky-trap catches. *J. Econ. Entomol.* 77: 1137-1140.
- Butler G.D., T.J. Henneberry, and F.D. Wilson. 1986. *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton: adult activity and cultivar oviposition preference. *J. Econ. Entomol.* 79: 350-354.
- Butler G.D., and F.D. Wilson. 1986. Whitefly adults in okra-leaf and normal-leaf cotton. p. 223-226. *In Cotton: A College of Agriculture Report. 370063, Series P-63, College of Agriculture, Univ. of Arizona, Tucson, AZ.*
- Butler G.D., D. Rimon, and T.J. Henneberry. 1988. *Bemisia tabaci* (Homoptera: Aleyrodidae): populations on different cotton varieties and cotton stickiness in Israel. *Crop Prot.* 7: 43-47.
- Butler G.D., F.D. Wilson, and G. Fishler. 1991. Cotton leaf trichomes and populations of *Empoasca lybica* and *Bemisia tabaci*. *Crop Prot.* 10: 461-464.
- Buxton D.R., and H.N. Stapleton. 1970. Predicted rates of net photosynthesis and transpiration as affected by the microenvironment and size of cotton leaf. p. 31-33 *In Proc. Beltwide Cotton Conf., Proc. Beltwide Cotton Conf., National Cotton Council of America, Memphis, TN.*
- Centintas R., and H. McAuslane. 2009. Effectiveness of parasitoids of *Bemisia tabaci* (Hemiptera: Aleyrodidae) on cotton cultivars differing in leaf morphology. *Fla. Entomol.* 92: 538-547.
- Chu C., A.C. Cohen, E.T. Natwick, G.S. Simmons, and T.J. Henneberry. 1999. *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype B colonization and leaf morphology relationships in Upland cotton cultivars. *Aust. J. Entomol.* 38: 127-131.



- Chu C., T. Freeman, E.T. Natwick, J.S. Buckner, D.R. Nelson, and T.J. Henneberry. 2000. *Bemisia argentifolii* adult, nymph, and egg densities and egg distribution on selected Upland cottons. *J. Entomol. Sci.* 35: 39-47.
- Chu C., E.T. Natwick, and T.J. Henneberry. 2002. *Bemisia tabaci* (Homoptera: Aleyrodidae) biotype B colonization on okra- and normal-leaf Upland cotton strains and cultivars. *J. Econ. Entomol.* 95: 733-738.
- Dolan L., and R.S. Poethig. 1991. Genetic analysis of leaf development in cotton. *Dev. Suppl.* 1: 39-46.
- Dai J., and Dong H. Intensive cotton farming technologies in China: Achievements, challenges and countermeasures. 2014. *Field Crops Research.* 155: 99-110.
- Endrizzi J.E., and M.S. Brown. 1964. Identification of monosomes for six chromosomes in *Gossypium hirsutum*. *Am. J. Bot.* 51 (2): 117-120.
- Endrizzi J.E., and R.J. Kohel. 1966. Use of telosomes in mapping three chromosomes in cotton. *Genetics* 54: 535-550.
- Endrizzi J.E., and R. Stein. 1975. Association of two marker loci with chromosome 1 in cotton. *J. Hered.* 66: 75-78.
- Flint H.M., and N.J. Parks. 1990. Infestation of germplasm lines and cultivars of cotton in Arizona by whitefly nymphs (Homoptera: Aleyrodidae). *J. Entomol. Sci.* 25:223-229.
- Gonias E.D., D.M. Oosterhuis, and A.C. Bibi. 2011. Light interception and radiation use efficiency of okra and normal leaf cotton isolines. *Environ. Exp. Bot.* 72: 217-222.
- Green J.M. 1953. Sub-okra, a new leaf shape in Upland cotton. *J. Hered.* 44 (6): 229-232.
- Hammond D. 1941a. The expression of genes for leaf shape in *Gossypium hirsutum* L. and *Gossypium arboreum* L. I. The expression of gene for leaf shape in *Gossypium hirsutum* L. *Am. J. Bot.* 28: 124-137.
- Hammond D. 1941b. The expression of genes for leaf shape in *Gossypium hirsutum* L. and *Gossypium arboreum* L. II. The expression of gene for leaf shape in *Gossypium arboreum* L. *Am. J. Bot.* 28: 138-150.
- Harland S.C. 1932. The genetics of *Gossypium*. *Bibliographica Genetica* 9: 107-182.
- Heitholt J.J., W.T. Pettigrew, and W.R. Meredith. 1992. Light interception and lint yield of narrow-row cotton. *Crop Sci.* 32: 728-733.
- Heitholt J.J. 1993. Cotton boll retention and its relationship to lint yield. *Crop Sci.* 33: 486-490.
- Heitholt J.J. 1994. Canopy characteristics associated with deficient and excessive cotton plant population densities. *Crop Sci.* 34: 1291-1297.
- Heitholt J.J. 1995. Cotton flowering and boll retention in different planting configurations and leaf shapes. *Agron. J.* 87: 994-998.
- Heitholt J.J., W.R. Meredith, and J.R. Williford. 1996. Comparison of cotton genotypes varying in canopy characteristics in 76-cm vs. 102-cm rows. *Crop Sci.* 36: 955-960.
- Heitholt J.J., and W.R. Meredith. 1998. Yield, flowering, and leaf area index of okra-leaf and normal-leaf cotton isolines. *Crop Sci.* 38: 643-648.
- Hutchinson J.B. 1934. The genetics of cotton. Part X. The inheritance of leaf shape in Asiatic *Gossypiums*. *J. Genet.* 28 (3): 437-513.
- Hutchinson J.B., and R.A. Silow. 1939. Gene symbols for use in cotton genetics. *J. Hered.* 30 (10): 461-464.
- Jiang C., R.J. Wright, S.S. Woo, T.A. DelMonte, and Paterson A.H. 2000. QTL analysis of leaf morphology in tetraploid *Gossypium*. *Theor. Appl. Genet.* 100: 409-418.
- Jones J.E. 1982. The present state of the art and science of cotton breeding for leaf-morphological traits. p. 93-99. *In Proc. Beltwide Cotton Conf., Proc. Beltwide Cotton Conf., National Cotton Council of America, Memphis, TN.*
- Jones J.E., and J.A. Andries. 1967. Okra leaf cotton for boll rot control. *La. Agric.* 10 (4): 4-5, 11.
- Jones J.E., D.F. Clower, M.R. Milam, W.D. Caldwell, and D.R. Melville. 1975. Resistance in Upland cotton to the banded-winged whitefly *Trialeurodes abutilonea* (Haldeman).p. 98-99. *In Proc. Beltwide Cotton Conf., Proc. Beltwide Cotton Conf., National Cotton Council of America, Memphis, TN.*
- Jones J.E., D. James, F.E. Sistler, and S.J. Stringer. 1987. Spray penetration of cotton canopies as affected by leaf and bract isolines. *La. Agric.* 29: 14-15, 17.
- Karami E., D.R. Krieg, and J.E. Quisenberry. 1980. Water relations and carbon-14 assimilation of cotton with different leaf morphology. *Crop Sci.* 20: 421-426.
- Karami E., and J.B. Weaver. 1972. Growth analysis of American Upland cotton, *Gossypium hirsutum* L., with different leaf shapes and colors. *Crop Sci.* 12: 317-320.
- Kaur B., R.J. Andres, and V. Kuraparthy. 2015. Major leaf shape genes in diploid (*Gossypium arboreum*) and polyploid Upland cotton (*G. hirsutum*) map to an orthologous genomic region. *Crop Sci.*: 56: 1095-1105.
- Kennedy C.W., W.C. Smith, and J.E. Jones. 1986. Effects of early season square removal on a three leaf types of cotton. *Crop Sci.* 26: 139-145.

- Kerby T.A., and D.R. Buxton. 1976. Fruiting in cotton as affected by leaf type and population density. p. 66-70. *In*: J.M. Brown (ed). Proc. Beltwide Cotton Conf., National Cotton Council of America, Memphis, TN.
- Kerby T.A., D.R. Buxton, and K. Matsuda. 1980. Carbon source-sink relationships with narrow-row cotton canopies. *Crop Sci.* 20:208-213.
- Lacape J.M., G. Gawrysiak, T.V. Cao, C. Viot, D. Llewellyn, S. Liu, J. Jacobs, D. Becker, P.A. Vianna Barroso, H. De Assuncao, O. Palai, S. Georges, J. Jean, and M. Giband. 2013. Mapping QTLs for traits related to phenology, morphology, and yield components in an inter-specific *Gossypium hirsutum* x *G. barbadense* cotton RIL population. *Field Crops Res.* 144: 256-267.
- Landivar J.A., D.N. Baker, and J.N. Jenkins. 1983. Application of GOSSYM to genetic feasibility studies. Analyses of fruit abscission and yield in okra-leaf cotton. *Crop Sci.* 23: 497-504.
- Li L., Huang Z.W., Chen G.W., Lv Z.Z., and Li Z.J. 2006. Study on the change of hormone and nutrient absorption of cotton after decapitation. *Arid Zone Res.* 23: 604-608.
- McClendon C.A. 1912. Mendelian inheritance in cotton hybrids. *Ga., Agric. Exp. Stn., Bull* 99: 139-228.
- Mell P.H. 1890. A microscopic study of the cotton plant. *Bull.* 13 – Ala. Agric. Exp. Stn. Agric. Mech. Coll., Auburn.
- Meredith W.R. 1983. Effect of environments and genetic backgrounds on evaluation of cotton isolines. *Crop Sci.* 23: 51-54.
- Meredith W.R. 1984. Influence of leaf morphology on lint yield of cotton – enhancement by the sub-okra trait. *Crop Sci.* 24: 855-857.
- Meredith W.R. 1985. Lint yield genotype x environment interaction in Upland cotton as influenced by leaf canopy isolines. *Crop Sci.* 25: 509-512.
- Meredith W.R., and R. Wells. 1986. Normal vs okra leaf yield interactions in cotton. I. Performance of near-isogenic lines from bulk populations. *Crop Sci.* 26: 219-222.
- Meredith W.R., and R. Wells. 1987. Sub okra leaf influence on cotton yield. *Crop Sci.* 27:47-48.
- Meredith W.R., W.T. Pettigrew, and J.J. Heitholt. 1996. Sub-okra, semi-smoothness, and nectariless effect on cotton lint yield. *Crop Sci.* 36: 22-25.
- Novick R.G., J.E. Jones, W.S. Anthony, W. Aguilard, and J.I. Dickson. 1991. Genetic trait effects of nonlint trash of cotton. *Crop Sci.* 31: 1029-1034.
- Ozgur A.F., and E. Sekeroglu. 1986. Population development of *Bemisia tabaci* (Homoptera: Aleurodidae) on various cotton cultivars in Cukurova, Turkey. *Agric., Ecosyst. and Environ.* 17: 83-86.
- Pegelow E.J., D.R. Buxton, R.E. Briggs, H. Muramoto, and W.G. Gensler. 1977. Canopy photosynthesis and transpiration of cotton as affected by leaf type. *Crop Sci.* 17: 1-4.
- Peng S., and D.R. Krieg. 1991. Single leaf and canopy photosynthesis response to plant age in cotton. *Agron. J.* 83: 704-708.
- Perry S.W., D.R. Kreig, and R.B. Hutmacher. 1983. Photosynthetic rate control in cotton. *Plant Physiol.* 73: 662-665.
- Pettigrew W.T. 2004. Physiological consequences of moisture deficit stress in cotton. *Crop Sci.* 44: 1265-1272.
- Pettigrew W.T., J.J. Heitholt, and K.C. Vaughn. 1993. Gas exchange differences and comparative anatomy among cotton leaf-type isolines. *Crop Sci.* 33: 1295-1299.
- Pieters E.P., and L.S. Bird. 1977. Field studies of boll weevil resistant cotton lines possessing the okra leaf-frego bract characters. *Crop Sci.* 17: 431-433.
- Rao M.J., and J.B. Weaver. 1976. Effect of leaf shape on response of cotton to plant population, N rate, and irrigation. *Crop Sci.* 68: 599-601.
- Riar R., R. Wells, K. Edmisten, D. Jordan, and J. Bachelier. 2013. Cotton yield and canopy closure in North Carolina as influenced by row width, plant population, and leaf morphology. *Crop Sci.* 53: 1704-1711.
- Renou A., I. Téréta I., and Togola M. 2011. Manual topping decreases bollworm infestations in cotton cultivation in Mali. *Crop Prot.* 30: 1370-1375.
- Shoemaker D.N. 1909. A study of leaf characters in cotton hybrids. *J. Hered.* 5 (1): 116-119.
- Sippell D.W., O.S. Bindra, and H. Khalifa. 1987. Resistance to whitefly (*Bemisia tabaci*) in cotton (*Gossypium hirsutum*) in the Sudan. *Crop Prot.* 6: 171-178.
- Song X.L., W.Z. Guo, Z.G. Han, and T.Z. Zhang. 2005. Quantitative trait loci mapping of leaf morphological traits and chlorophyll content in cultivated tetraploid cotton. *J. Integr. Plant Biol.* 47 (11): 1382-1390.
- Stephens S.G. 1945. A genetic survey of leaf shape in new world cottons – a problem in critical identification of alleles. *J. Genet.* 46 (2-3): 313-330.
- Stephens S.G. 1955. Linkage in Upland cotton. *Genetics* 40 (6): 903-917.
- Stiller W.N., P.E. Reid, and G.A. Constable. 2004. Maturity and leaf shape as traits influencing cotton cultivar adaptation to dryland conditions. *Agron. J.* 96: 656-664.
- Thomson N.J. 1971. Effects of the superokra leaf gene on cotton growth, yield, and quality. *Aust. J. Agric. Res.* 23: 285-293.

- Thomson N.J., P.E. Reid, and E.R. Williams. 1987. Effects of the okra leaf, nectariless, frego bract, and glabrous conditions on yield and quality of cotton lines. *Euphytica* 36: 545-553.
- Walker G.P., and E.T. Natwick. 2006. Resistance to silverleaf whitefly, *Bemisia argentifolii* (Hem., Aleyrodidae), in *Gossypium thurberi*, a wild cotton species. *J. Appl. Entomol.* 130: 429-436.
- Wells R., W.R. Meredith, and J.R. Williford. 1986. Canopy photosynthesis and its relationship to plant productivity in near-isogenic cotton lines differing in leaf morphology. *Plant Physiol.* 82: 635-640.
- White T.G., and J.E. Endrizzi. 1965. Tests for the association of marker loci with chromosomes in *Gossypium hirsutum* by the use of aneuploids. *Genetics* 51: 605-612.
- Wilson F.D. 1986. Pink bollworm resistance, lint yield, and lint yield components of okra-leaf cotton in different genetic backgrounds. *Crop Sci.* 26: 1164-1167.
- Wilson L.J. 1993. Spider mites (Acari: Tetranychidae) affect yield and fiber quality of cotton. *J. Econ. Entomol.* 86: 566-585.
- Wilson L.J. 1994. Resistance of okra-leaf cotton genotypes to two-spotted spider mites (Acari: Tetranychidae). *J. Econ. Entomol.* 87: 1726-1735.
- Wilson R.L., F.D. Wilson, and B.W. George. 1979. Mutants of *Gossypium hirsutum*: Effect on pink bollworm in Arizona. *J. Econ. Entomol.* 72: 216-219.
- Wilson F.D., and B.W. George. 1982. Effects of okra-leaf, frego-bract, and smooth-leaf mutants on pink bollworm damage and agronomic properties of cotton. *Crop Sci.* 22: 798-801.
- Wilson F.D., B.W. George, K.E. Fry, J.L. Szaro J.L., T.J. Henneberry, and T.E. Clayton. 1986. Pink bollworm (Lepidoptera: Gelechiidae) egg hatch, larval success, and pupal and adult survival of okra- and normal-leaf cotton. *J. Econ. Entomol.* 79: 1671-1675.
- Wilson F.D., J.L. Szaro, and B.A. Hefner. 1992. Behavior of pink bollworm larvae (Lepidoptera: Gelechiidae) on bolls of normal-leaf and okra-leaf cotton isolines under laboratory conditions. *J. Agric. Entomol.* 9 (1): 55-63.
- Yang Y., Ouyang Z., Yang Y., and Liu X. 2008. Simulation of the effect of pruning and topping on cotton growth using COTTON2K model. *Field Crops Res.* 106: 126-137.
- Zhu Q.H., A. Spriggs, J.M. Taylor, D. Llewellyn, and I. Wilson. 2014. Transcriptome and complexity-reduced, DNA-based identification of intraspecies single-nucleotide polymorphisms in the polyploidy *Gossypium hirsutum* L. G3: Genes, Genomes, Genet. 4 (10): 1893-1905.
- Zhu Q.H., J. Zhang, D. Liu, W. Stiller, D. Liu, Z. Zhang, D. Llewellyn, and I. Wilson 2015. Integrated mapping and characterization of the gene underlying the okra leaf trait in *Gossypium hirsutum* L. *J. Exp. Bot.* Online First: doi: 10.1093/jxb/erv494
- Zhu W., K. Liu, and X.D. Wang. 2008. Heterosis in yield, fiber quality, and photosynthesis of okra leaf oriented hybrid cotton (*Gossypium hirsutum* L.). *Euphytica* 164: 283-291.