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MOLECULAR BIOLOGY AND PHYSIOLOGY

Comparison of Growth, Yield, and Fiber Quality of the Obsolete SA30 Yellow Leaf with Four Sets of Modern Yellow and Green Leaf Near Isogenic Cotton (*Gossypium hirsutum* L.) Lines

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ABSTRACT

The Virescent Yellow leaf cotton line Stoneville Accession 30 (SA30, PI 528447) was crossed with four modern parental lines (DP5690, DES119, SG747 and MD51ne) to develop four sets of near isogenic lines (NILs) segregating for green and yellow leaves. Comparisons of these lines were made in the field in a two-year replicated study between the obsolete SA30 line and four modern NIL sets. Yield measurements, including hand (bolls/plant) and machine harvested (kg/plot) samples, of the four modern NIL sets compared to SA30 resulted in a twofold difference except for DP5690 yellow leaf (192%) and MD51ne yellow (167%) in the kg/plot ratios. Other yield measurements (seed cotton weight and 100 boll seed weight) reflected the "Mebane" cotton background of the SA30 line with larger bolls, whereas lint yields reflected the higher lint percentages of the modern NILs. Growth parameters including plant height and number of nodes were measured at predetermined intervals and height-to-node ratios were determined with the green leaf lines growing faster than the yellow leaf lines. The yellow leaf NILs and the SA30 line grew at the same rate. Cotton fiber quality was measured with both AFIS and HVI and both similarities and differences are reported in the paper. Even though the NILs used in this study were created to evaluate various yield measurements along with plant height and height-to-node ratios, this study also demonstrated that these lines can be used to search for the genes involved in increased partitioning to the reproductive structures.

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Cotton is a crop that is valued for its fiber. In 2012, the cotton crop in the U.S. was valued at $5.52 billion (USDA National Agricultural Statistics Service, 2014) to $5.97 billion (National Cotton Council, 2014a) making the U.S. cotton industry a vital part of the U.S. economy. U.S. cotton production amounts to approximately 14% of the world production (National Cotton Council, 2014b). To preserve the competitiveness of the U.S. in the world cotton markets, yield and fiber quality improvement of cotton fiber is a research priority in the U.S.

Improvement in cotton yields in the latter part of the 20th century has been attributed to increased dry-matter partitioning into reproductive growth (Pettigrew and Gerik, 2007; Wells 2016). This has been accomplished through selection of high-yielding plants from various populations; however, this type of selection method will become ineffective as the amount of photosynthesizing leaf area becomes the major limiting factor (Pettigrew and Gerik, 2007). Therefore, other avenues for improving cotton fiber yields and quality need to be identified and evaluated.

One avenue for improvement could be to identify components of cotton photosynthesis that could be manipulated to improve cotton yields (Cornish et al., 1991). The problem with this approach is the complexity of the photosynthesis apparatus, which uses sunlight, water, CO_2, and various nutrients to manufacture the basic building blocks for plant growth and, therefore, is essential in increasing yields and fiber quality of cotton (Turley and Pettigrew, 2011). The use of genetics to target and improve photosynthesis in cotton has not been emphasized by breeders/geneticists. Questions still remain as to which traits should be targeted to select for improved photosynthesis. These traits have to be measured easily in a rapid, nondestructive manner if possible. Pettigrew and Turley (1998) evaluated the variation in photosynthesis components of six

cotton genotypes that differed in carbon exchange ratios. They determined that the narrow range of variation of photosynthetic components made it difficult to choose superior breeding lines.

Using a variant allele from an obsolete cotton (*Gossypium hirsutum* L.) line and comparing the expression of this same allele in modern lines will facilitate the identification of physiological changes associated within the modern selections that result in increased yields. The expression of this detrimental virescent allele was reported to double the Chl_a/Chl_b ratios of the SA30 line over two wild-type cotton lines due to a reduction of Chl_b most likely from a reduction in the light harvesting complex (Turley and Pettigrew, 2011). This scenario reflects on the difficulty of not only identifying the virescent allele but identifying the mechanism that increases reproductive development. Evaluation of virescent cotton plants provides a system to identify possible factors that influence the photosynthetic performance of leaves (Habash et al., 1994; McCourt and Somerville, 1987; Miles, 1980). Reducing leaf chlorophyll diminishes the leaf's ability to capture light efficiently, and in some cases, can create an imbalance in the excitation rates between PS_I and PS_{II} (Baker and Ort, 1992). Equal excitation rates between the two photosystems are required to achieve the maximum quantum efficiency of CO_2 assimilation. A reduction of CO_2 assimilation in these yellow lines theoretically would reduce yields. Currently, there are 22 recessive alleles reported to express the virescent leaf phenotype (Zhang et al., 1997). Zhang categorized the virescent phenotypes into two subcategories: deep yellow and yellow. Plants of the deep yellow phenotype were dwarfed in size and retained the virescent phenotype for most of the growing season. They characteristically had a $Chl_{a/b}$ ratio approximately twice that of the yellow category. The yellow category characteristically had $Chl_{a/b}$ ratios similar to wild-type cotton (Zhang et al., 1997).

In 2011, Turley and Pettigrew reported on three virescent upland cotton lines including SA30 (PI 528447), SA174 (PI 528567), and SA31 (PI 528448). This study measured the photosynthesis strength of these obsolete cotton lines and compared various growth parameters with two modern wild-type lines. The virescent lines SA30 and SA174 were of the deep yellow phenotype reported by (Zhang et al., 1997) and during this study also were found to be allelic (Turley and Pettigrew, 2011). The virescent plant SA31 also expressed a virescent and red leaf phenotype that gave its leaves a bronze color. SA30 was initially named Virescent Yellow and discovered in 1925 in a field of "Mebane" cotton (Killough and Horlacher, 1933). SA30 was reported to grow slowly and retain its yellow phenotype through most of the summer.

The development of near isogenic lines (NIL) lines was initiated in 1995 with the goal of comparing the obsolete virescent SA30 line, that is, Virescent Yellow as originally designated by Killough and Horlacher (1933) in newly developed NILs carrying the same virescent leaf allele (v_1v_1) in modern cotton backgrounds: DP5690 (DP5690), Sure-Grow 747 (SG747), Delta Experiment Station 119 (DES119), and Mississippi Delta 51ne (MD51ne). This allowed for the direct comparison of the allele in an obsolete line with the same allele expressed in four modern lines. These lines were planted in a replicated study over a two-year period allowing for a direct comparison of plant growth, lint yields, and fiber quality.

MATERIALS AND METHODS

Near-Isogenic Line Development . A backcross breeding method was used to develop the NILs for virescent and wild-type (green) leaf sets from four modern upland cotton varieties. The four modern upland cotton genotypes selected in 1995 as the wild-type parents were cultivars DP5690 (Monsanto Company, St. Louis, MO, USA; PVP 9100116), SG747 (Monsanto Company, St. Louis, MO, USA; PVP 9800118), DES119 (Mississippi Agricultural and Forestry Experiment Station, Mississippi State, MS, USA; PI 606809; PVP 8500176) and MD51ne (USDA-ARS, Stoneville, MS, USA; PI 566941). These four parental lines were developed into pure inbred lines by self-pollination accompanied with single seed descent (SSD) through nine generations using both the greenhouse and field at the USDA-ARS in Stoneville, MS. The virescent parent was SA30 (PI 528447). SA30 was obtained from the Mississippi Obsolete Variety Sub-Collection, USDA-ARS, College Station, TX, also available online at GRINglobal https://npgsweb.ars-grin.gov/gringlobal/search.aspx (Percival, 1987; Yu et al., 2014; https://npgsweb.ars-grin.gov/gringlobal/search.aspx). Pollen from SA30 was used to fertilize emasculated flowers from each of

the four SSD inbred genotypes described above. The F_1 seed from each cross were grown and self-fertilized in the greenhouse. The F_2 seed were then planted in the field the following spring and the segregating yellow plants were used in the back crossing events with the F_9 SSD parents.

After 6 years of crossing/back crossing, followed by self-pollinating in the greenhouse, BC_5F_2 NILs expressing the two leaf colors, i.e., green and yellow, were selected. Each NIL set was theoretically 98.44% identical to their recurrent parent DP5690, SG747, DES119, or MD51ne. Seed was increased from these individual plants by self-pollination in the greenhouse or in the field. Greenhouses were kept free of pollinating insects through the use of screens and insecticides. Cross pollination of field-grown flowers was prevented by placing 10.2 x 15.2 cm organza bags (Gifts International, Ontario, CA) over the flower buds prior to opening to exclude pollinators, because cotton pollen is not windborne. Seed preparation for planting was by standard practices of saw ginning and acid delinting to remove seed fuzz.

Field Study. This study was conducted at Stoneville, MS in 2014 and repeated in 2015. Field plots consisted of 4 rows spaced 1.02 m apart. Each plot was 9.14 m long with a 3.04 m alley between plots. Field plots were established in a Bosket very fine sandy loam soil (fine-loamy, mixed, active, thermic Mollic Hapludalfs) (Soil Survey Staff, 2014) in a field that had only been planted to upland cotton during the preceding season. Plots were mechanically planted on May 5, 2014 and April 30, 2015. The fungicide pentachloronitrobenzene (Terraclor Super X 18.8 G, Chemtura USA Corporation, Middlebury, CT) was applied in furrow at 11.2 kg/ha to manage seedling diseases. The insecticide spinetoram (Radiant SC, Dow AgroSciences, Indianapolis, IN) was applied to manage thrips twice in 2014 (110 ml/ha on May 20, 146 ml/ha on May 27) and twice in 2015 (110 ml/ha on May 14, 110 ml/ha on May 30). Plots were over-seeded and after the plants reached the first true leaf stage, seedlings were thinned to 6.5 plants m^{-2}. Standard agronomic practices for cotton production in the Mississippi Delta region were used to manage the crop (http://msucares.com/crops/cotton/index.html). Plots were furrow irrigated as needed each year to minimize moisture stress.

Three plant growth parameters were measured on a biweekly schedule and expressed as days after planting (DAP): plant height (cm), number of mainstem nodes, and height to mainstem node ratio (cm/node). During 2014, measurements were taken on June 4 (30 DAP), June 18 (44 DAP), July 2 (58 DAP), July 16 (72 DAP), July 30 (86 DAP), and August 13 (100 DAP). These same measurements were taken in 2015 on June 6 (41 DAP), June 24 (55 DAP), July10 (71 DAP), July 24 (85 DAP), and August 5 (97 DAP). It was randomly chosen that the fourth plant from the end of the middle two rows of each plot (4 plants) would be used for measurements. Height was determined by measuring from the base of the mainstem (swelled corky region) to the apex of the plant. Nodes were counted on the mainstem beginning with the cotyledonary node (=1) and continuing to the apical meristem. Height to mainstem node ratio was calculated by dividing the height of the individual plant by the number of mainstem nodes. All graphs were created with GraphPad 7 (GraphPad Software, Inc. LaJolla, CA).

Preceding the hand harvest of boll samples to determine the number of bolls per plant from each line, the plots were treated with defoliant (thidiazuron and diuron; Ginstar EC, Bayer CropScience, Research Triangle Park, NC) and boll opener (ethephon; Boll Buster, Loveland Products, Inc., Greeley, CO). Samples were collected by harvesting all open bolls on sequential plants in one of the middle plot rows beginning with the fifth plant from the end of the plot and working towards the center of the plot until a minimum of 100 bolls had been collected. Once 100 bolls were obtained and any additional bolls on the last plant were harvested the total weight of seed cotton was determined. The number of plants were counted and recorded along with the number of bolls and the weight of boll samples was calculated for each plot. These weights (seed cotton, seed weights and lint weights) were then adjusted and reported on a 100-boll basis. Plot weights were obtained by harvesting the two center rows of the plots with a cotton picker and weighing these harvests. The seed cotton weights from the hand-picked samples were added to get the final plot weight. The boll samples were collected October 6, 2014 and October 2, 2015. Plots were mechanically harvested on October 9, 2014 and October 8, 2015.

Advanced Fiber Information System (AFIS) Measurements. Fiber samples from each subplot were analyzed on an Uster AFIS PRO (Uster

Technologies, Inc., Knoxville, TN) at Cotton Incorporated (Cary, NC). This instrument tests three slivers (0.5 g each) to generate a mean value for each of the properties analyzed. Parameters measured were: mean length (mm) of fiber calculated based on fiber weight, $L_{(w)}$ or fiber number, $L_{(n)}$; upper quartile length (mm) calculated based on fiber weight, $UQL_{(w)}$; length (mm) of the longest 5% of fibers calculated based on fiber number, $5\%_{(n)}$; percentage of short fibers (fibers less than 12.7 mm long) calculated based on fiber weight, $SFC\%_{(w)}$ or fiber number, $SFC\%_{(n)}$; fiber fineness in mTex; number of neps per g; number of seed coat neps per g; percentage of immature fibers (those with less than 0.25 maturity), IFC%; and maturity ratio, MR. Maturity ratio is calculated by dividing the amount of fibers with a 0.50 or greater circularity ratio by the amount of fibers with a 0.25 or less circularity ratio (Calhoun et al., 1997; Williams and Yankey, 1996).

High Volume Instrument (HVI) Measurements. Fiber samples from each subplot were sent to the Cotton Fiber Testing Laboratory at the LSU AgCenter (Baton Rouge, LA) for testing. Fiber samples were evaluated using an Uster 900 SA HVI (Uster Technologies, Inc., Knoxville, TN). This instrument tests each sample four times to generate a mean value for each of the properties analyzed except for micronaire, which is determined only twice. Parameters measured were: upper half fiber length (mm), which is the mean length of the longer half of the fibers in the sample, UHL; fiber length uniformity index, which is the ratio between the mean length and the upper half mean length in percent; short fiber index, which is the percentage of short fibers (fibers less than 12.7 mm long), SFI; strength required to break a fiber bundle (g/tex); elongation, or distance that the fiber bundle extends before it breaks during strength determination (%); maturity, which is the maturity ratio as determined based on measurements made by this instrument; and micronaire, which is a measurement of fiber fineness based on resistance to airflow (Stetina et al. 2014).

Statistical Design. This study utilized a randomized complete block design with 3 replications. The main plot treatment was genotype, which consisted of the four sets of NILs (described in detail above) and the virescent parent SA30. Data from both years were combined, and all statistical analyses were performed using SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). The mixed models procedure (PROC MIXED) with the Kenward-Roger denominator degrees of freedom option was used for analysis of variance (ANOVA) for all data. Years, replications, and their interactions were modeled as random effects. Differences of least squares means identified differences between means at $p \leq 0.05$.

RESULTS

The virescent and wild type phenotypes of the modern and the obsolete line SA30 are shown in the field (Fig. 1). Figure 1 illustrates the organization of the randomized, replicated four-row plots of yellow and green leaf plants. It is noticeable in Fig. 1 that the yellow lines are shorter than the green lines. This observation is supported in Fig. 2 in that SA30 and the yellow NIL lines exhibited slower growth than their green leaf NIL counterparts but by the end of the growing season they would often reach similar heights, especially in 2015. The yellow NIL lines in the four cotton backgrounds had the tendency to grow slightly faster than SA30. There were only a few instances where the yellow leaf NILs were statistically greater in height than SA30. These occurred in 2014 in the DES119 line at 72 and 86 days after planting (DAP). The green leaf NILs grew faster than the yellow leaf lines between 44 and 86 DAP for 2014 and between 41 and 85 DAP in 2015. These can be noted where the standard deviation bars appear to be absent which occurs often in 2015.

Figure 1. Picture of field plot design in 2014 showing randomized four-row plots of green and yellow near isogenic lines in modern backgrounds along with the obsolete parent SA30.

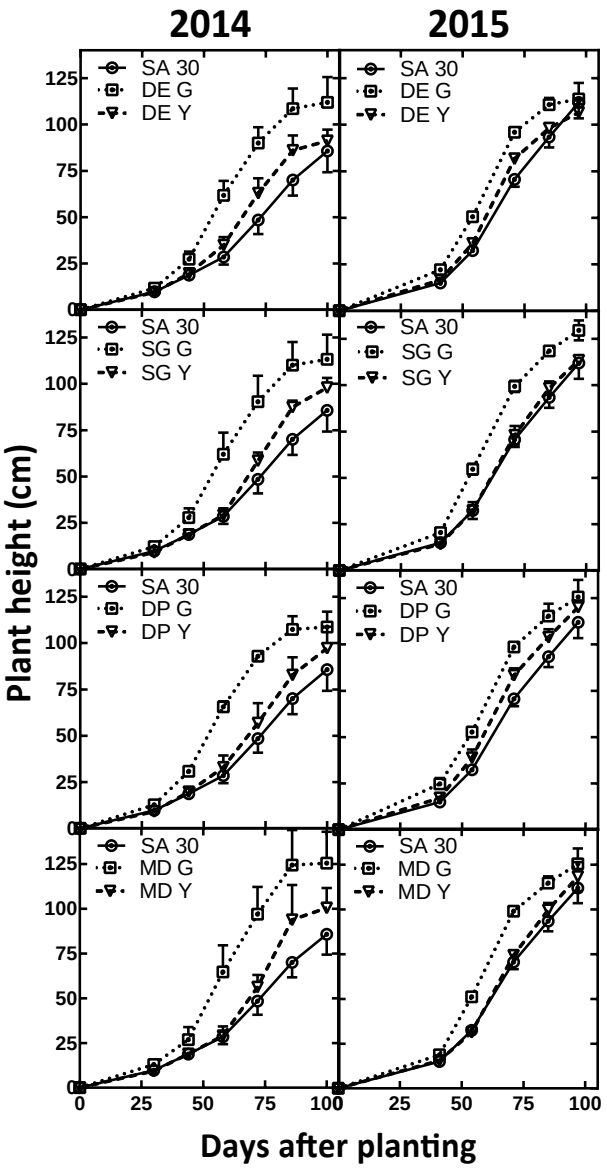

Figure 2. Plant height comparisons at different intervals of growth for segregates of green (G) and yellow (Y) leaf near isogenic lines in modern cotton backgrounds DES119 (DE), SG747 (SG), DP5690 (DP), and MD51ne (MD) and the obsolete parent SA30. Standard deviation bars when smaller than the symbol are not drawn.

Plant nodes were also counted but no differences were identified between the genotypes at any measurement interval (data not shown). However, height/node ratios presented in Fig. 3 show some differences between the green and yellow leaf plants. For DES119 differences occurred at 58 and 72 DAP (2014) and 55, 71 and 85 DAP (2015). For SG747 differences occurred at 44 DAP (2014) and 55, 71 and 85 DAP (2015). For DP5690 differences occurred at 55, 72 and 86 DAP (2015) and for MD51ne differences occurred at 55 and 71 DAP (2015). No differences between the modern and obsolete yellow lines were observed as was expected. In similar nodal events in 2014 the first flower was found 56 DAP and each plot had a flower open by 62 DAP. In 2015 the first flower was found 57 DAP and each plot had their first flowers by 59 DAP.

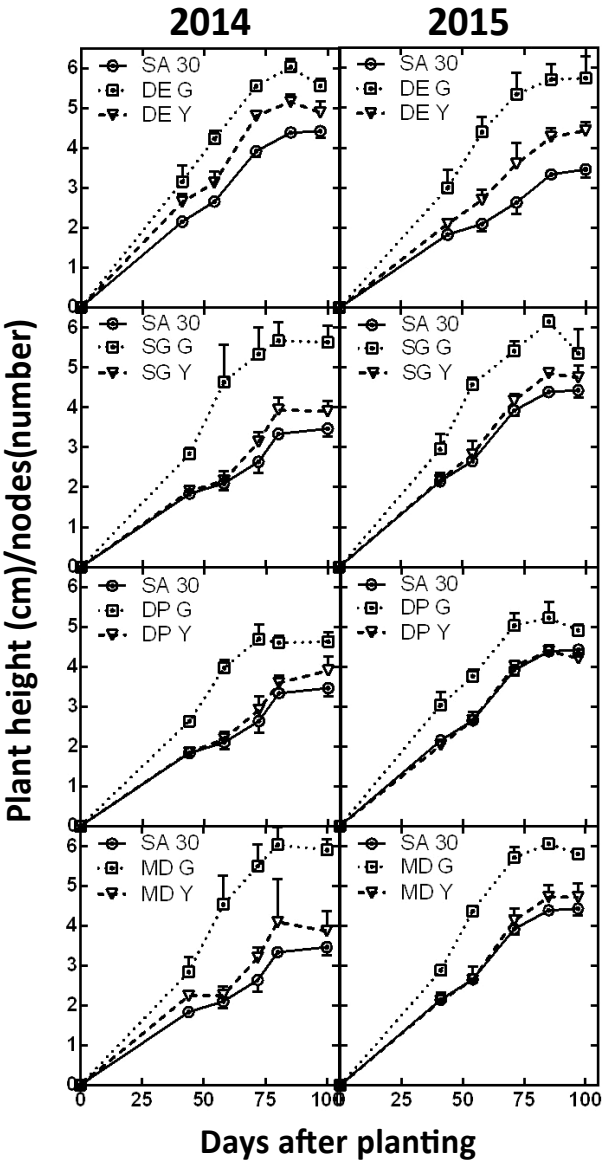

Figure 3. Height/node ratios at different intervals of growth of green (G) and yellow (Y) leaf near isogenic lines in modern cotton backgrounds DES119 (DE), SG747 (SG), DP5690 (DP), and MD51ne (MD) and in the obsolete parent (SA30). Standard deviation bars when smaller than the symbol are not drawn.

Cotton yield results are reported in Table 1. For bolls/plant the SA30 yield was statistically smaller than the yields of the NILs regardless of leaf color. These differences were all greater than a two-fold increase. The NIL sets reported as (% increase) of the SA30 boll/plant and reported in the parenthesis by leaf color (green, yellow leaf) were DES119 (247%, 226%), SG747 (242%, 232%), DP5690 (213%, 251%) and MD51ne (228%, 201%). Seed cotton weight per 100 bolls and 100-boll seed weights for the SA30 harvest were larger than the modern NIL lines. Only DES119 yellow leaf was equivalent for the seed cotton weight. Unlike the seed cotton, the 100-boll lint weight of SA30 was equivalent to both the DP5690 and MD51ne NIL sets; all of these lines had lower 100-boll lint weights than the DES119 and SG747 NIL sets. The 100-boll lint weights mirrored the increased lint percentages in Table 1. Plot weights had the same trends as bolls per plant except DP5690 yellow leaf and MD51ne yellow leaf had less than 200% increases.

Fiber length measurements from AFIS and HVI measurements are summarized in Table 2. The mean length based on weight ($L_{(w)}$) measurements for SA30 were similar to all NILs except DP5690 and MD51ne green and yellow lint measurements. Similarly the mean length based on fiber number ($L_{(n)}$) was equivalent to that of SA30 in all NILs except DP5690 yellow leaf. The upper quartile length ($UQL_{(w)}$), $5\%_{(n)}$ and the UHL measurements for SA30 were similar to the SG747 green and yellow leaves but were lower than the measurements from the yellow and green leaves of DES119, DP5690 and MD51ne. Other AFIS fiber measurements such as short fiber content $SFC_{(n)}$, (F=2.65, p=0.0948) and $SFC_{(w)}$, (F=3.13, p=0.0634) were all similar.

Variation in fineness, strength, and elongation are reported in Table 3. The fineness of SA30 was similar to both the DES119 green and yellow leaf and MD51ne yellow leaf line. Fiber strength was similar between SA30 and all the NILs except two; DES119 green leaf had weaker fibers than SA30 and MD51ne yellow leaf had stronger fibers. SA30 also had the highest elongation extension of all the lines. These NIL lines in most cases were also different. HVI fiber measurements the fiber length uniformity (F = 1.87, p= 0.0898), short fiber index (SFI, F=1.97, p=0.1794), micronaire (F=3.12, p=0.0642) and maturity (F=0.48, p=0.8435) did not vary among the genotypes tested.

Table 1. Boll counts, seed, lint and plot weights from 100 hand harvested samples collected from sequential cotton (*Gossypium hirsutum* L.) plants for four sets of green and yellow leaf near isogenic lines and their obsolete Virescent Yellow parent SA30 in a field study in Stoneville, MS.

Genotype, leaf color	Bolls/plant	Seed Cotton Wt (g)	100-boll Seed Wt (g)	100-boll Lint Wt (g)	Plot Wt (kg)	Lint %
SA30, Yellow	**5.87 b**	**517.24 a**	**363.83 a**	**153.40 b**	**3.03 d**	**29.6 e**
DES119, Green	**14.50 a**	**440.47 bcd**	**258.51 cd**	**181.96 a**	**6.36 ab**	**41.3 b**
DES119, Yellow	**13.27 a**	**470.46 bc**	**278.30 bc**	**192.16 a**	**6.23 ab**	**40.9 b**
SG747, Green	**14.21 a**	**463.02 bc**	**266.89 cd**	**196.13 a**	**6.98 a**	**42.3 a**
SG747, Yellow	**13.52 a**	**446.56 bcd**	**263.20 cd**	**183.36 a**	**6.30 ab**	**41.0 b**
DP5690, Green	**12.52 a**	**463.86 bc**	**303.83 b**	**160.02 b**	**7.03 a**	**34.4 d**
DP5690, Yellow	**14.73 a**	**464.09 bc**	**302.05 b**	**162.04 b**	**5.81 bc**	**34.8 d**
MD51ne, Green	**13.37 a**	**399.29 d**	**243.34 d**	**155.95 b**	**6.17 abc**	**39.0 c**
MD51ne, Yellow	**11.82 a**	**422.72 cd**	**260.41 cd**	**162.31 b**	**5.07 c**	**38.3 c**
F	**7.98**	**5.19**	**16.78**	**8.06**	**11.86**	**206.86**
P>F	**<0.0040**	**0.016**	**0.0003**	**0.004**	**0.0011**	**<0.0001**

Analysis based on combined data from 54 observations over two years.

Means followed by the same letter are not significantly different at P≤0.05 based on differences of least square means.

Table 2. Length of fibers measured using Advanced Fiber Information System (AFIS) and High Volume Instrument (HVI) automated testing equipment from 9 hand-harvested cotton (*Gossypium hirsutum* L.) lines for four paired yellow and green leaf near isogenic lines and their obsolete parent SA 30 in a field study in Stoneville, MS.

Genotype, Leaf Color	$L_{(w)}$[a]	$L_{(n)}$[b]	$UQL_{(w)}$[c]	$5\%_{(n)}$[d]	UHL[e]
SA 30, Yellow	26.54 de	23.24 bc	30.61 d	34.72 d	28.83 d
DES119, Green	26.87 cde	22.40 c	32.13 bc	36.19 bc	31.62 a
DES119, Yellow	27.38 cd	23.11 bc	33.51 b	36.39 bc	30.48 b
SG747, Green	26.24 e	22.17 c	31.06 d	34.87 d	28.70 d
SG747, Yellow	26.24 e	22.02 c	31.37 cd	35.13 cd	28.32 d
DP5690, Green	28.45 ab	24.00 ab	33.86 a	38.61 a	29.92 bc
DP5690, Yellow	28.78 a	24.69 a	33.73 a	38.35 a	29.67 c
MD51, Green	27.86 abc	24.00 ab	32.64 b	36.88 b	29.59 c
MD51, Yellow	27.74 bc	23.95 ab	32.21 bc	36.40 bc	29.51 c
F	7.43	3.71	12.77	12.32	21.27
P>F	<0.0001	0.0025	0.0008	0.0009	<0.0001

[a] $L_{(w)}$ = mean length (mm) of fiber calculated based on fiber weight (AFIS).

[b] $L_{(n)}$ = mean length (mm) of fiber calculated based on fiber number (AFIS).

[c] $UQL_{(w)}$ = upper quartile length (mm) calculated based on fiber weight (AFIS).

[d] $5\%_{(n)}$ = length (mm) of the longest 5% of fibers calculated based on fiber number (AFIS).

[e] UHL = mean length (mm) of the longer half of the fibers in the sample (HVI).

Analysis based on combined data from 54 observations over two years.

Within each column means followed by the same letter are not significantly different at $P \leq 0.05$ based on differences of least squares means.

Table 3. Fiber properties determined using Advanced Fiber Information System (AFIS) and High Volume Instrument (HVI) automated testing equipment from 9 hand-harvested cotton (*Gossypium hirsutum* L.) lines for four paired yellow and green leaf near isogenic lines, their obsolete parent SA30 in a field study in Stoneville, MS.

Genotype, Leaf color	Fineness[a]	Strength[b]	Elongation[c]
SA 30, Yellow	200.50 a	30.12 bc	8.78 a
DES119, Green	187.83 ab	27.18 d	7.57 b
DES119, Yellow	192.33 ab	27.72 bc	7.58 b
SG747, Green	185.00 bc	29.21 bcd	6.98 c
SG747, Yellow	181.33 bc	28.60 bcd	7.15 bc
DP5690, Green	170.00 d	30.17 ab	5.50 d
DP5690, Yellow	165.33 cd	30.95 ab	5.93 d
MD51ne, Green	183.17 bc	30.60 ab	6.73 c
MD51ne, Yellow	186.17 ab	32.60 a	6.87 c
F	5.10	5.10	26.71
P>F	0.0162	0.0166	<0.0001

[a] Fineness = fiber fineness in mTex (AFIS).

[b] Strength = strength required to break a fiber bundle (g/Tex) (HVI).

[c] Elongation = distance that the fiber bundle extends before it breaks during strength determination (%) (HVI).

Analysis based on combined data from 54 observations over two years.

Within each column and treatment, means followed by the same letter are not significantly different at $P \leq 0.05$ based on differences of least squares means.

DISCUSSION

This study was designed to evaluate differences between the obsolete SA30 and the BC_5F_2 NIL progeny of yellow and green leaf segregates in DES119, SG747, DP5690 and MD51ne backgrounds. One problem with making these lines near isogenic is the loss of heterogeneity during the single seed descent process which could change some of the original qualities of these lines. These lines were selected in consultation with Dr. William Meredith in 1995. The DES119 was a parent of SG747 and therefore comparisons of the different yield and fiber quality measurements would act as an internal control. The line DP5690 was popular in 1995, and MD51ne was bred for improved fiber quality (Meredith, 1993). SA30 is a Mebane cotton variety and has been reported to have large bolls some with five locules (Killough and Horlacher, 1933).

These modern yellow leaf NILs carry the same virescent allele as SA30 and looked uniformly similar. SA30 was also the first non-lethal virescent leaf cotton line reported in the literature with the virecent allele later designated as the v_1v_1 allele. Twenty-one additional alleles have been identified for virescent phenotypes in cotton from alleles v_2 to v_{22} (Zhang et al., 1997). Future work with these NILs will attempt to further characterize the v_1v_1 gene.

Plant height and nodes were measured in the field in both 2014 and 2015. Plant height-to-node ratios were calculated from these measurements. Turley and Pettigrew (2011) made similar comparisons between SA30 and two other virescent lines - SA174 and SA31 (bronze leaf) along with two wildtype lines. Kerby and Keeley (1987) reported that plant height was more sensitive to environmental stress than the number of mainstem nodes. They also found that removal of leaves and/or cotyledons on young plants could significantly reduce number of mainstem nodes. This removal of leaves and or cotyledons would reduce the total photosynthesis. This is theoretically comparable to what is occurring in reduced photosynthesis in the virescent (Turley and Pettigrew, 2011) leaf cotton lines used in this work. However, neither in the Turley and Pettigrew (2011) paper nor in the research presented in this paper did we ever see a reduction in the number of nodes. Kerby et al. (1997) reported that plant height and height-to-node ratios are indicators of the strength of vegetative growth of cotton before anthesis. In this paper, both plant height and height-to-node ratios were affected.

Virescent lines are therefore at a disadvantage unless a genetic adaption can compensate for lack of pigment accumulation. Habash et al. (1994) postulated that the decrease in light absorbance in a virescent line of cowpea was compensated for by a positive change in canopy architecture. No noticeable change of canopy architecture was observed in this work, however, a greening of leaves in late August/September was observed in these NIL yellow lines along with the SA30 in the field. The greening of the SA30 leaves late in the growing season was originally reported by Killough and Horlacher (1933). They also reported the same occurrence when growing SA30 in a greenhouse.

The main objective for developing these NIL sets in modern lines was to compare the growth rates, yields and fiber quality with the obsolete parent SA30. One objective in evaluating these virescent lines was to show how single nuclear genes can affect growth in cotton, and determine if these plants have developed ways to compensate for the lower chlorophyll accumulation by modifying specific growth factors, that is, plant height, number of mainstem nodes, and height/mainstem node ratio. Habash et al. (1994) postulated that the decrease in light absorbance in a virescent line of cowpea was compensated for by a positive change in canopy architecture. Moving the virescent allele from SA30 to DES119, SG747, DP5690 and MD51ne did not change the canopy. However, it did put the v_1v_1 allele in modern lines that have been bred for increased yields. Improving the partitioning of resources to the reproductive structures would be a better explanation for what has occurred in these NIL lines especially when evaluating the boll yields and the plot weight measurements. There was essentially a 2 fold increase in yield in the yellow NILs derived from the SA30.

Further molecular/biochemical characterization could give insights into cellular mechanisms for increased reproductive structure yields. Comparisons of transcriptomes of the SA30 and the NILs reported in this paper could provide a means to identify the gene(s) that are responsible for these yield increases. The use of NILs would facilitate identification of possible candidates genes, however, the question becomes where do you look for these important genes? Similarities of the growth rates of NIL yellow lines and the SA30 line suggest that the virescent gene functions the same in all these lines.

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DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

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