## **BREEDING AND GENETICS**

# Fiber Quality of Cultivars and Breeding Lines in the Cotton Winter Nursery and US Environments

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## ABSTRACT

**Researchers in the United States routinely** use the Cotton Winter Nursery (CWN) located in Tecomán, Mexico, for seed increase and generational advancement of breeding materials in cotton (Gossypium spp.). The objective of this study was to characterize the fiber quality of cotton grown in the CWN. High Volume Instrument (HVI) fiber quality parameters from cultivars and breeding lines were examined using correlations and principle component analysis as a means to determine similarities in germplasm response between the CWN and US environments. The US location mean values for elongation, length, micronaire, strength, and uniformity, along with correlation means, appear not to differ substantially from the values obtained for these traits in the CWN. These data indicate that the CWN could serve as an additional environment to select for HVI fiber quality traits.

Vinter nursery facilities traditionally are used for advancing generations, seed increase, and/ or hybridization activities. All major crops have winter nurseries or off-season environments (e.g., maize-Puerto Rico, Hawaii (Mayor and Bernardo, 2009); soybean-Puerto Rico, Chile (Mansur and Orf, 1995); cotton-Mexico (Miller, 1998)) to produce multiple generations of a crop that should enhance genetic gain per year. The Cotton Winter Nursery (CWN) was started in Iguala, Mexico in 1950 and later moved to Tecomán, Colima, Mexico in 1979 (Miller, 1998). The US National Cotton Council, USDA-Agricultural Research Service, and the Mexico Insituto Nacional de Investigationes Agrícolas collaborated on the initial cooperative agreement establishing the CWN.

The CWN has a major role in maintaining accessions in the US Cotton Germplasm Collection. Approximately 10% of the collection (1,000 accessions) is sent to the CWN yearly for seed increases to ensure an adequate seed supply is available to meet the needs of requestors. Researchers also routinely use the CWN for advancing breeding materials one generation and for seed increases of progeny lines (Campbell et al., 2010; Gutierrez et al., 2006). Fiber quality of genotypes grown in the CWN is rarely determined, although such data could be obtained with little difficulty, thus allowing for evaluation and selection. However, the representative quality of fiber samples obtained in the CWN needs to be determined in relation to environments in the US before using data from this off-season environment to make breeding decisions.

The objective of this study was to establish the quality of fiber samples obtained in the CWN. High Volume Instrument (HVI) data from cultivars grown in the CWN and US National Cotton Variety Tests (NCVT) (National Cotton Variety Testing Program, 1994-2007), along with data from breeding lines in experimental yield trials, were examined using correlations and principle component analyses as a means to determine similarities in genotypic response to the CWN and US environments. Determination of similarities among environments should allow for more effective use of the CWN.

### MATERIALS AND METHODS

**Germplasm**—**Cultivars.** Data from two sets of cotton germplasm were used to evaluate fiber properties of cotton grown in the winter nursery in Tecomán, Mexico. The first set of germplasm included five *G. hirsutum* L. cultivars that are a subset of a fiber standards panel of 10 genotypes that has been established to represent a range of commercially available fiber properties. The entire fiber standards panel has been planted with the routine seed increases of the US Cotton Germplasm Collection for a number of years in an effort to monitor fiber quality in the CWN.

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Region	Locations (regions)	Region	Locations (regions)
Arizona	Maricopa, AZ	High Quality	Belle Mina, AL
	Safford, AZ		Bossier City, LA
Blacklands	Dallas, TX		College Station, TX
	Thrall, TX		Florence, SC
Central	Beeville, TX		Keiser, AR
	Bossier City, LA		Lubbock, TX
	College Station, TX		Portageville, MO
	Dallas, TX		Stoneville, MS
	Thrall, TX		Tifton, GA
	Weslaco, TX	Plains	Altus, OK
Delta	Clarkedale, AR		Lamesa, TX
	Keiser, AR		Lubbock, TX
	Portageville, MO		Tipton, OK
	St. Joseph, LA	San Joaquin	Five Points, CA
	Stoneville, MS		Shafter, CA
Eastern	Auburn, AL	Western	Artesia, NM
	Belle Mina, AL		El Paso, TX
	Florence, SC		Pecos, TX
	Starkville, MS		University Park, NM
	Tifton, GA		

Table 1. Regio	ns and locations	within regions in t	he National Cottor	Variety Te	ests where five <b>c</b>	cultivars were g	grown between
1993 and 20	06 <sup>z</sup> .						

<sup>z</sup> National Cotton Variety Testing Program, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007.

The five cultivars analyzed here include 'Acala 1517-99' (Cantrell et al., 2000; PI 612326; PVP 200000181), Acala 'Maxxa' (PI 540885; PVP 9000168), 'All-Tex Atlas' (PVP 9200188), 'Fiber-Max 832' (Constable et al., 2001; PI 603955; PVP 9800258), and 'SureGrow 747' (PVP 9800118). These cultivars have been grown both in Mexico and across nine regions in the US as reported in the NCVT between 1993 and 2006 (Table 1). Appropriate experimental designs and cultural practices were used, although they were not standardized across locations participating in the NCVT. This working data set from the NCVT varied in the combination of regions, locations within regions, and years providing fiber information for each cultivar (Table 2). Cultivars grown in the CWN were hand-planted in September and October as hill-plots (approximately 14 plants per plot), selfpollinated, and hand-harvested in early spring for 3 yr between 2006 and 2008.

 
 Table 2. Distribution of data set used to evaluate cultivars grown in the National Cotton Variety Tests.

Cultivar	Total Data Points	Regions	Locations (regions)	Years
Acala 1517-99	82	8	24	8
Acala Maxxa	157	9	27	9
All-Tex Atlas	106	8	25	9
FiberMax 832	49	3	11	7
SureGrow 747	87	9	27	5

**Germplasm**—**Breeding Lines.** The second set of germplasm included 70 *G. hirsutum* breeding lines developed to simultaneously improve heat tolerance and fiber quality, particularly strength and length. Individual plant selection was practiced in the  $F_2$  and  $F_3$  generations in the high temperature environment of Maricopa, AZ. The  $F_4$  increases were evaluated in the CWN, and the  $F_5$  progeny were evaluated in 2006 at three US locations (Florence, SC; Maricopa, AZ; and Shafter, CA) using a modified augmented design with unreplicated plots. Four commercial checks including 'Phytogen 72' (PVP 200100115), 'FiberMax 958' (PVP 200100208), 'Suregrow 747' (PVP9800118), and 'Deltapine 393' (PVP200400266) were grown at each location.

For both cultivars and breeding lines, samples were collected to measure HVI fiber parameters, including fiber bundle strength, upper-half mean length, length uniformity index, elongation at break, and micronaire. Fiber samples from the five established cultivars grown in Mexico were sent to Starlab, Inc. (Knoxville, TN) for comparison with data previously reported from the same lab in the NCVT. Samples from the breeding lines grown in Mexico in 2005 and the US in 2006 were sent to Cotton Incorporated (Cary, NC) for HVI analysis.

Statistical Analysis. For each set of germplasm, mean values and standard deviations were calculated for the five fiber properties as a measure of overall performance in the CWN versus known US environments. Within the set of breeding lines, further comparisons of fiber trait expression at the CWN versus at known US environments were made by comparing the linear relationships between US environments with linear relationships between these environments and the CWN. Linear relationships cannot be calculated for cultivars because the data set is unbalanced as all cultivars were not grown in all regions, locations, and/or years. Pearson correlations were used to calculate the linear relationships and were determined using the PROC CORR procedure in SAS v. 9.2 (SAS Institute, 2007). The correlation coefficient(r) is a parametric measure of association based upon the actual data values. The formula is:

$$r_{xy} = \frac{\sum_{i} ((x_i - \overline{x})(y_i - \overline{y}))}{\left(\sum_{i} (x_i - \overline{x})^2 \sum (y_i - \overline{y})^2\right)^{1/2}}$$

where  $\overline{x}$  is the mean of entry *x* grown in Mexico, and  $\overline{y}$  is the mean of entry *y* grown in the US. The significance of the correlation is calculated as:

$$t = (n-2)^{1/2} \left(\frac{r^2}{1-r^2}\right)^{1/2}$$

with (n-2) degrees of freedom.

Principal component analyses (PCA) were performed on the correlation matrix of traits across all participating locations for each set of germplasm. The PROC PRINCOMP procedure in SAS v. 9.2 (SAS Institute, 2007) was used to evaluate relationships for a graphical representation of patterns of variation in fiber quality across sets of germplasm and locations.

The analysis of similarities in germplasm response between the CWN and US environments presented here is based on data assembled from several routine testing programs and from a germplasm development project. Therefore this experiment was not specifically designed to test for genotype-byenvironment interactions. In particular, the cultivar data set is biased by the variable number of data points analyzed across environments and years.

#### **RESULTS AND DISCUSSION**

**Cultivars.** Fiber elongation and micronaire means obtained at the Mexico CWN appear to be lower than those obtained from regional tests within the US (Table 3). Conversely, fiber length, strength, and length uniformity means obtained in the CWN appear to be greater than means for these traits obtained in US regional tests. Despite these apparent differences, the average trait values in the US do not appear to differ appreciably from those in the CWN, when one considers the standard deviations attached to the values. Although some biasing occurred in US fiber trait means due to differing numbers of years and cultivars evaluated, these means provided adequate estimates for comparison with CWN fiber data.

In a PCA to discern patterns of variation in fiber quality across environments, the first two principal components (PCs) explained 45.2% and 18.6% of the total variation, respectively (Fig. 1). Coefficients of fiber trait variables in equations used to plot PCs are a good measure of the amount of variability expressed by each trait in the multivariate analysis (Brown, 1991). These coefficients indicate the first PC separated the cultivar data set on the basis of fiber length and length uniformity, whereas variation along the second PC reflected differences due to length uniformity, in addition to micronaire and strength. Fiber data from the CWN in Mexico lie within the distribution of variability for data from the NCVT, but are definitely skewed toward the right side of this distribution (Fig. 1). The failure of data from the CWN to produce a discrete cluster, separate from a NCVT cluster, indicates that CWN fiber data can be considered as a part of the overall distribution of variation for fiber traits.

As mentioned previously, the five cultivars evaluated here are part of a larger fiber standards panel, which also includes the three *G. hirsutum* 

Environment	Region	NZ	Elongation %	Length mm	Micronaire	Strength kN m kg <sup>-1</sup>	Length Uniformity %
Mexico		15	$\textbf{7.2} \pm \textbf{0.6}$	$\textbf{30.2} \pm \textbf{0.7}$	$\textbf{4.1} \pm \textbf{0.3}$	$\textbf{345.5} \pm \textbf{44.6}$	$\textbf{85.2} \pm \textbf{1.2}$
United States	Arizona	11	$\textbf{9.0} \pm \textbf{0.4}$	$\textbf{28.8} \pm \textbf{1.4}$	$\textbf{4.7} \pm \textbf{0.6}$	$\textbf{295.9} \pm \textbf{29.6}$	$82.1 \pm 1.5$
	Blacklands	27	$\textbf{8.7} \pm \textbf{0.8}$	$\textbf{27.8} \pm \textbf{1.6}$	$\textbf{4.3} \pm \textbf{0.5}$	$312.5\pm37.9$	$83.1 \pm 1.1$
	Central	92	$\textbf{8.7} \pm \textbf{0.7}$	$\textbf{28.6} \pm \textbf{1.6}$	$\textbf{4.6} \pm \textbf{0.5}$	$\textbf{318.9} \pm \textbf{31.8}$	$83.7 \pm 1.2$
	Delta	42	$\textbf{8.7} \pm \textbf{0.7}$	$\textbf{29.0} \pm \textbf{1.4}$	$\textbf{4.3} \pm \textbf{0.5}$	$311.4\pm26.1$	$\textbf{84.2} \pm \textbf{1.1}$
	Eastern	56	$\textbf{9.2} \pm \textbf{0.8}$	$\textbf{28.3} \pm \textbf{1.5}$	$\textbf{4.4} \pm \textbf{0.6}$	$317.3\pm32.3$	$83.5 \pm 1.3$
	High Quality	115	$\textbf{8.7} \pm \textbf{0.8}$	$29.2 \pm 1.5$	$\textbf{4.3} \pm \textbf{0.6}$	$315.6\pm30.3$	$\textbf{84.1} \pm \textbf{1.4}$
	Plains	75	$\textbf{9.3} \pm \textbf{0.8}$	$\textbf{27.7} \pm \textbf{1.6}$	$\textbf{4.4} \pm \textbf{0.5}$	$312.2\pm36.5$	$\textbf{82.8} \pm \textbf{1.3}$
	San Joaquin	16	$9.1 \pm 1.6$	$\textbf{29.0} \pm \textbf{0.9}$	$\textbf{4.1} \pm \textbf{0.3}$	$311.2\pm22.2$	$83.2\pm0.8$
	Western	47	$\textbf{8.8} \pm \textbf{0.7}$	$\textbf{29.2} \pm \textbf{1.7}$	$\textbf{4.3} \pm \textbf{0.5}$	$301.2\pm25.2$	$83.5 \pm 1.7$
	U.S. average	481	$\textbf{8.9} \pm \textbf{0.8}$	$\textbf{28.6} \pm \textbf{1.6}$	$4.4\pm0.5$	313.4 ± 31.5	$83.6 \pm 1.4$

Table 3. Summary of means and standard deviations of fiber properties for five cultivars grown in the Mexico CWN and multiple regions in the US National Cotton Variety Tests.

<sup>z</sup> N = number of observations used to calculate mean fiber properties in each environment and region.



Figure 1. Results of the principal component analysis for five cultivars grown in the Mexico CWN and in multiple regions of the US National Cotton Variety Tests. The percent variation explained by each principal component (PC) is given in parenthesis.

entries 'TM1' (Kohel et al., 1970; PI 607172), 'MD51ne' (Meredith, 1993; PI 566941), and 'Tamcot CAMD-E' (Bird, 1979; PI 529633; PVP 7800073) along with two *G. barbadense* L. entries, 'Pima S-6' (PI 608346) and 'Pima 3-79.' It is our intention to grow this panel with two replications in the CWN yearly to serve as a reference standard for continuously monitoring fiber quality. Researchers using the CWN would have access to the HVI fiber data



Figure 2. Results of the principal component analysis for 70 breeding lines grown in the Mexico CWN and three locations in the US. The percent variation explained by each principal component (PC) is given in parenthesis.

from this reference panel. This replicated panel is also included with increases for accessions grown at College Station, TX. The utility of the CWN for fiber evaluation will continue to be measured by comparing the performance of the fiber standards panel in the CWN with its performance in College Station, TX.

**Breeding Lines.** The mean fiber values of 70 breeding lines, averaged across the Maricopa, AZ;

Shafter, CA; and Florence, SC locations and at the CWN in Tecomán, Mexico, are shown in Table 4. The average US location mean values of elongation, length, micronaire, strength, and length uniformity appear not to differ substantially from the values obtained for these traits in the CWN. The mean value for elongation at the CWN was slightly higher than in the US environments, whereas the mean value for micronaire at the CWN was slightly lower. However, all fiber trait means, with their standard deviations, obtained in the CWN overlap with the means and standard deviations obtained across US locations.

Average fiber trait correlations among the Maricopa, AZ; Shafter, CA; and Florence, SC, locations were equivalent in size to the average correlation of these locations with the CWN (Table 4). These correlation values differ from those reported in earlier studies; in particular, fiber strength and length tend to be more highly repeatable in previous reports (Meredith, 1984; Smith, 1992). In this study, the precision and magnitude of fiber trait correlations between locations were undoubtedly influenced by the lack of replication within locations as well as the wide geographic range of locations. Nonetheless, the CWN produced correlations with US locations comparable in size to the correlations among US locations. It would appear that for the purpose of selection, HVI fiber data obtained from the CWN is equivalent in predictive value to data obtained from the Florence, Maricopa, and Shafter locations. This preliminary comparison suggests the CWN cannot only serve the function of generation advance and

seed increase, but could also serve as an additional environment for selection of fiber traits.

Distribution of breeding lines following a multivariate analysis shows data from Mexico overlapping with data from three US environments (Fig. 2). The first two PCs account for no less than 59% of the total variance of all traits. Similar to the multivariate analysis of the cultivar germplasm set, the first PC of the breeding lines is affected primarily by fiber length uniformity, elongation, and length. On the opposite axis, values for micronaire, length, and elongation can be interpreted to explain the spread of data points.

Screening and selection of breeding material may be conducted in the CWN to advance generations since data reported herein indicate average values and correlations for HVI fiber traits fall within the range of standard deviations across US environments. Similar results have been reported supporting the use of off-season nurseries for evaluation and selection for yield, plant height, lodging, protein, oil, and long duration of seed-filling period in soybean (Mansur and Orf, 1995; Rodriguez de Cianzio et al., 1985, 1991) and for kernel weight in spring barley (Rutger and Schaller, 1967).

#### 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 US Department of Agriculture.

Table 4. Summary of means, correlations between locations, and standard deviations of fiber properties averaged across 70 *G. hirsutum* breeding lines grown in the Mexico CWN and three locations (Maricopa, AZ; Florence, SC; and Shafter, CA) in the US.

	Elongation %	Length mm	Micronaire	Strength kN m kg <sup>-1</sup>	Length Uniformity %
CWN breeding line average	6.0 ± 1.1	$\textbf{31.5} \pm \textbf{0.8}$	$\textbf{4.3} \pm \textbf{0.3}$	$\textbf{320.7} \pm \textbf{13.6}$	$\textbf{85.2} \pm \textbf{1.0}$
US locations breeding line average	$\textbf{4.9} \pm \textbf{0.7}$	$31.6 \pm 1.3$	$\textbf{4.7} \pm \textbf{0.5}$	$\textbf{328.1} \pm \textbf{14.1}$	$\textbf{84.7} \pm \textbf{1.2}$
Among U.S. locations correlation average	$\textbf{0.84} \pm \textbf{0.02}$	$\textbf{0.43} \pm \textbf{0.07}$	$\textbf{0.57} \pm \textbf{0.11}$	$\textbf{0.39} \pm \textbf{0.09}$	$\textbf{0.24} \pm \textbf{0.06}$
Between CWN and US locations correlation average	$\textbf{0.85} \pm \textbf{0.01}$	$\textbf{0.42} \pm \textbf{0.10}$	$\textbf{0.66} \pm \textbf{0.06}$	$\textbf{0.29} \pm \textbf{0.11}$	$\textbf{0.38} \pm \textbf{0.09}$

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