



# Boll sampling protocols and their impact on measurements of cotton fiber quality



Neha Kothari<sup>a,\*</sup>, Steve Hague<sup>b</sup>, Lori Hinze<sup>c</sup>, Jane Dever<sup>a</sup>

<sup>a</sup> Texas A & M AgriLife Research, 1102 East FM 1294, Lubbock, TX 79403, United States

<sup>b</sup> Texas A & M University, 370 Olsen Blvd, College Station, TX 77843, United States

<sup>c</sup> United States Department of Agriculture, 2881 F & B Road, College Station, TX 77845, United States

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## ABSTRACT

Within plant fiber variability has long contributed to discrepancy and inconsistency in fiber quality and yarn quality. Fiber quality uniformity is a primary plant breeding objective related to cotton commodity economic value. The physiological impact of source and sink relationships render stress on the upper sympodial branches of the cotton plants thereby leading to reduced genetic potential for fiber growth. Objectives of this study were to quantify variability within the top and bottom halves of the plants and evaluate various boll sampling protocols for efficient fiber quality estimation. This research was conducted in College Station, Texas for three years in 2009, 2010 and 2011 at the Texas A & M AgriLife Research farm. Ten fiber samples were collected from each plot varying in boll numbers and regions within the plant and compared against a machine harvested sample. Fiber quality testing was done using High Volume Instrument (HVI) and Advanced Fiber Information System (AFIS). Statistical analyses inclusive of Analysis of Variance (ANOVA) and Best Linear Unbiased Prediction (BLUP) and correlations among fiber properties were performed. It was concluded that the best boll sampling method for accurate fiber quality estimation included a mixed sample of at least 25 bolls picked from all regions of the plant. A statistic to identify the least variable genotype was calculated using the difference between top and bottom bolls. It was concluded that the extent of variability within a plant for fiber length and length uniformity are genotype dependent. Correlation analyses revealed strong relationships among fiber length uniformity, strength, UHML, maturity ratio, and fineness. Strong negative relationships of fiber length measurements with short fiber content and immature fiber content were observed. Correlation analysis within the bottom and top bolls of the plants revealed opposite trends in fiber relationships.

## 1. Introduction

Improving fiber quality is a driving force for breeders to maximize the value of cotton. With increasing demand for textile products and increasing competition from synthetic fibers, cotton breeders are faced with the challenge to improve fiber quality and yield to sustain advances in spinning technologies. Genetic improvement of cotton based on additive gene action for traits of interest has been a go-to for cotton breeders for several decades. It is imperative for US breeders to develop alternate methods and approaches for improving fiber.

Cotton fiber quality traits are environmentally and genetically controlled. Variability in fiber quality is one of the compelling issues faced by breeders for improving cotton. Variability in cotton can be

seen within a single plant, across a field, within a single genotype and even within a single boll (Clouvel et al., 1998; Davidonis et al., 1999; Wilkins and Jernstedt, 1999; Kothari et al., 2015). Fiber quality is also affected by biotic and abiotic stress factors, genetic components and interactions among these factors. Minimized intra-plant variability is one strategy to improve fiber uniformity, leading to better strength and fiber maturity needed to meet commercial yarn specifications. Keeping pace with the requirements set by spinning and weaving technologies, the need to control cotton fiber variability concomitant with maximizing yield is urgent (Davidonis et al., 2004; Bednarz et al., 2006; Krifa, 2012). Growing environment is probably one of the greatest factors affecting variability in fiber quality and yield within a single genotype. Fiber quality variability has been attributed to soil chemistry,

**Abbreviations:** AB, all bolls; AFIS, Advanced Fiber Information System; BB, bottom bolls; BLUP, Best Linear Unbiased Predictor; CSIRO, Commonwealth Scientific and Industrial Research Organization; FBRI, Fiber and Biopolymer Research Institute; HVI, High Volume Instrument; IFC, immature fiber content; SFC (number), short fiber content (by number); SFC(weight), short fiber content (by weight); TB, top bolls; UHML, upper half mean length

\* Corresponding author.

E-mail address: [neha.kothari@ag.tamu.edu](mailto:neha.kothari@ag.tamu.edu) (N. Kothari).

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fertility, (Johnson et al., 2002), moisture content (Elms et al., 2001) and organic matter (Pettigrew et al., 1996; Johnson et al., 1999). Cotton boll development is affected by temperature changes in the growing environment. This affects the fiber quality within the bolls. (Gipson and Joham, 1968; Gipson and Joham, 1969). Weeds and pests contribute to declining fiber quality and lead to quality inconsistency (O'Berry et al., 2009; Reeves et al., 2010). In order to improve and optimize the textile processing of cotton fibers, it is important to have an accurate measurement of cotton fiber traits (Moore, 1996).

Understanding intra-plant fiber variability is important to determine if genetic improvement to control/minimize this trait is possible. In a previous study investigating intra-plant fiber variability using the Advanced Fiber Information System (AFIS) to measure fiber traits, it was determined that the extent of variability within a single plant for fiber length (number) was genotype dependent (Kothari et al., 2015). Fiber quality from various fruiting positions within the plants was determined across multiple environments and phenotyped for quality estimation. The difference in percent variability of fiber length (number) was calculated and analysis showed a significant genotypic effect. Results suggested that intra-plant variability was possibly a genotypic effect even though it was always assumed to be singularly environmental. Intra-plant variability renders a specific 'top crop' concern in fiber quality. 'Top crop' refers to the fibers that are harvested from the upper sympodial positions of the cotton plants that typically have poor overall fiber quality. This means the upper sympodial branches of the plants exhibit lower fiber quality (especially in terms of fiber length and maturity) thereby reducing the overall economic value of the harvested crop (Bednarz and Nichols, 2005; Kothari et al., 2007).

The current study builds on our previous work and was designed specifically to measure the effects of genotype on intra-plant variability. The primary objective of the current study was to evaluate various boll sampling protocols to estimate within-plant variability of fiber quality using both HVI and AFIS testing. Two genotypes, Fibermax 832 (Constable et al., 2001) and Deltapine 491 (PI 618609) were planted for three years to understand variability across environments, and within plants, genotypes and boll sample sizes. The degree of variability was estimated to partition genotype and environmental effects. Detailed correlation analyses were performed to understand the behavior of fiber properties at various regions within the plants.

## 2. Materials and methods

### 2.1. Genotypes

Two genotypes were selected for this study based on fiber quality and yield estimates. FiberMax 832 (FM 832) and Deltapine 491 (DPL 491) are commercial upland cultivars with excellent fiber quality. FM 832 is an okra leaf type plant which is known for high fiber quality and acceptable yields (Constable et al., 2001). FM 832 was developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia and marketed by Bayer CropScience in the US. DPL 491 is a commercial cultivar with normal leaves, high yields and excellent fiber quality. It was released in 2002 and described to have large boll size, adaptable widely to growing environment and long staple fibers (Keim et al., 2002).

### 2.2. Experimental design

The two cultivars were grown in three environments in 2009, 2010 and 2011 at the Texas A & M AgriLife Research farm at College Station, Texas. Both entries were grown in four-row plots (12 m × 1.0 m) with four biological replications in each year. The soil type was Westwood silt loam, a fine-silty, mixed thermic Fluventic Ustochrept, intergraded with Ships clay which is a fine, mixed, thermic Udic Chromustert. The experiment was arranged in a randomized complete block design.

### 2.3. Harvest and fiber testing

Ten methods were evaluated in the seed cotton sampling protocol. Sampling techniques included a grab sample machine-picked from an entire row within the plot (method #1). Three random handfuls of seed cotton were taken from a harvest sack from each plot and combined. This sample was considered as representative of reference fiber quality of the plot and was designated as 'all bolls (AB)'. The other sampling techniques included three sets of 15, 25 and 50 hand-picked boll samples. The first set of 15, 25 and 50 boll samples were picked only from the bottom half of the plant (method #2, 3, and 4), the second set of 15, 25, 50 boll samples were picked only from the top half of the plant (method #5, 6, and 7) and the last set of 15, 25 and 50 boll samples were picked from all parts of the plant(s) to obtain a uniform mix (method #8, 9, and 10). The first set was designated as 'bottom bolls (BB)', the second set designated as 'top bolls (TB)' and the last set designated as 'mixed (MIX)'. Bolls for all hand-picked samples were selected randomly within the designated zone(s) of the plants (i.e. no preference was given for boll positions within a branch). Within the four-row plots for each entry, row 1 was used for the 15 boll samples, row 2 was used for the 25 boll samples, row 3 was used for the 50 boll samples and row 4 was machine picked. Seed cotton samples were ginned on a ten-saw laboratory scale gin, and the fiber was tested with HVI and AFIS systems.

Fiber testing was done at the Fiber and Biopolymer Institute (FBRI) at Texas Tech University, Lubbock, Texas. Upper half mean length (UHML), micronaire, length uniformity, strength and elongation were measured using HVI. Neps, length by weight and number (length (w), length (n)), short fiber content by weight and number (SFC (w), SFC (n)), immature fiber content (IFC), maturity ratio, fineness and standard fineness were measured using AFIS. Conditioning and testing were carried out under constant climate controlled conditions. The standard temperature for textile testing is  $20 \pm 2^\circ\text{C}$  and  $65 \pm 2\%$  relative humidity. Prior to testing, samples were arranged in single layers and allowed to equilibrate for 48 h under standard atmospheric conditions. In order to minimize experimental error, the same technician ran all the samples. Eight check samples are run twice every morning on the HVI for calibrating the machine. HVI testing included a single, non-replicated measure for micronaire and two replications for fiber length and strength. One non-replicated sample was used for AFIS testing. For AFIS sample preparation, a 500 mg tuft of fibers was drawn into a 25 cm length sliver and 10,000 fibers were measured from that sample. Fiber samples were blended before AFIS testing was performed.

### 2.4. Statistical analysis

The trial was a split-plot design where the factorial arrangement of treatment (genotypes) was the main plot and the ten sampling techniques (sample) were the split-plot factor for each year. Replications (rep), rep x factorial treatments (genotypes), and rep x samples were considered random effects. Years, years x factorial treatments, and years x sample were also considered random effects. Factorial effects, genotypes and sampling techniques were considered as fixed effects. For mean separation, the Waller-Duncan method was used. For the purpose of mean separation testing, replications and years were considered random effects. Fiber data for the samples were analyzed and ANOVA with means, BLUPs, standard deviation and mean separations (Waller-Duncan method) calculated using the PROC MIXED procedure in SAS 9.4 (SAS Institute, 2012). Correlation analysis and least square means (LSM) calculations were done using JMP genomics 6.0 using the REML method (JMP, 1989). The fibers samples were independent from each other because each set of boll samples (15, 25 and 50) were picked from different rows of the plot.

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