PROOF OF CONCEPT: NOVEL GENE BASED BREEDING VS FIELD BASED BREEDING IN IMPROVING FIBER QUALITY TRAITS IN COTTON

A Dissertation

by

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ABSTRACT

Field Based Breeding (FBB) has been the conventional approach of plant breeders in making individual plant selections in Cotton (Gossypium. sp). Gene Based Breeding (GBB) provides a unique approach for breeders in assessing the fiber quality of a cotton plant before harvest. GBB uses Single Nucleotide Polymorphisms (SNPs) as the genetic markers in making selections for two traits: Upper Half Mean Length (UHML) and Fiber Bundle Strength (FBS). These SNPs are located within the fiber quality genes as proposed by Dr. Hongbin Zhang. Two distinct populations were analyzed for a proof of concept study where selections were based on both methods mentioned above. An F_2 population of 13P-54 ELSU // 11K-13 ELSU / NMSI 1331, an interspecific cross (Pima introgression), and an F_3 population of 11K-13 ELSU / 06WE-621 ESU, an intraspecific cross (Upland x Upland) were used. Two hundred and fifty individual plants were visually selected in each population for the FBB. Two hundred fifty plants were randomly selected for the GBB and then screened using 11 SNP based genes for UHML and FBS. The top and the bottom 10% for UHML and FBS among these selections in each population were carried forward in a randomized complete block design with two replications for two years. A regression analysis was conducted to estimate the association between the GBB and FBB predictions and actual HVI measurements of advanced progeny. With only 11 SNPs used as markers in the GBB protocol, R^2 values for UHML were as good as those in the FBB protocol relative to the longest 10% of the progeny. Neither GBB or FBB was effective in identifying foundational individual

plants that were predictive of advanced progeny FBS. Based on R^2 values, GBB was more effective in predicting UHML in the interspecific population as compared with the intraspecific upland population.

DEDICATION

I would like to dedicate this dissertation to my grandparents, Dr. Dilbagh Singh Bhangu, and Mrs. Gurminder Kaur Bhangu. I would like to thank them for their love and guidance in my life.

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NOMENCLATURE

AFLPs-	Amplified Fragment Length Polymorphisms
CIL-	Cotton Improvement Lab
ELON-	Elongation
ELSU –	Extra Long Staple Upland
ESU-	Extra Strength Upland
FBB-	Field-Based Breeding
FBS-	Fiber Bundle Strength
GAS-	Gene Allele Selection
GBB-	Gene-Based Breeding
GEPS-	Gene Expression Profile-based Selection
GFL-	Gossypium Fiber Length
GSTR-	Gossypium Strength
GxE	Genotype by Environment
HVI –	High Volume Instrument
IPS-	Individual Plant Selection
MAS -	Marker Assisted Selection
MIC –	Micronaire
MS –	Mean Squares
PCR-	Polymerized Chain Reaction
QTL-	Quantitative Trait Loci

RAPDs- Random-Amplified Polymorphic DNAs

RCBD- Randomized Complete Block Design

RILs- Recombinant Inbred Lines

RFLPs- Restriction Fragment Length Polymorphisms

SNP- Single Nucleotide Polymorphism

SSRs- Simple Sequence Repeats

TAMU- Texas A&M University

USDA- United States Department of Agriculture

U.S. – United States of America

UHML – Upper Half Mean Length

UI – Uniformity Index

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CHAPTER I

INTRODUCTION

Cotton (*Gossypium*. sp.) is an essential global fiber crop with production in 80 countries. The United States produced 19,238,000 bales of cotton fiber in 2018 (USDA, 2019) and accounted for a 16.92% share of global production. Despite being the third-largest producer of cotton behind India and China, the United States accounted for 38.94% of the global export market in 2017 for raw cotton fibers (USDA, 2019). The export market of the United States relies heavily on superior fiber quality cotton for processing and, ultimately, high-quality finished products. Advances in spinning technologies, such as air-jet spinning, which can produce yarn at a faster rate (Eldessouki et al., 2015), requires longer fibers with higher strength. Moreover, increased competition from synthetic fibers with competitive spinning properties has accentuated the need to enhance cotton fiber properties through breeding.

New cotton cultivars must be produced with improved fiber quality to compete with synthetic fibers. The goal of a cotton breeder is to improve desired traits in cotton plants to produce a cultivar that is either superior in yield, fiber quality, or increased stress resistance. However, desired selections are made in the early generations of a cotton cultivar program when that particular cultivar's population is still segregating for the traits of interest. Depending upon the breeder's choice, individual plant selections (IPS) usually are either made in the F_2 or F_3 generation. IPS in cotton breeding programs

are also dependent upon a trait or a combination of traits, e.g., fiber length, fiber strength, plant architecture, boll size, boll count, etc. Selection criteria are determined based on the end goal of a particular project such as the development of a superior fiber quality cultivar for better spinning quality. However, these often produce less lint percentage compared to other cultivars that exhibit lower fiber quality. Cotton breeders are consistently pushing their efforts to break some of these antagonistic relationships between fiber quality traits and yield.

The Cotton Improvement Lab (CIL) at Texas A&M AgriLife Research has developed germplasm with a focus on improved fiber quality. Superior fiber quality has a direct relationship with better fabric production. The demand for stronger and longer fibers by the textile industry, as well as the competition from synthetic fibers, has prompted cotton breeders to produce germplasm consisting of higher strength and length while maintaining an acceptable yield.

Cotton breeders at the CIL have adopted a novel approach to speed up the selection process based on superior fiber quality standards. An expedited and quantitative process is desirable, given the limitations presented by the subjective nature of visually making individual plant selections in a large population.

DNA markers may hold the answers to predicting superior fiber quality traits while the plants are in the vegetative stage of growth, thus allowing for selection for

other traits such as yield only among plants that will produce superior fiber. Single Nucleotide Polymorphism (SNP) markers are the simplest form of variations where a single base pair change in the DNA of a crop can lead to phenotypic diversity (Majeed et al., 2019). Cotton breeders hope to identify genetic diversity through SNPs to further the improvement of selections based on fiber quality.

Field-based breeding (FBB) has been the conventional approach of plant breeders in making individual plant selections in cotton (*Gossypium* sp). DNA marker technologies such as SNP markers are proving to be more useful in predicting the effect of a Quantitative Trait Loci (QTL) associated with fiber quality traits, while providing better coverage of a crop's genome when compared with RFLPs, AFLPs, and SSRs. Gene-based breeding (GBB) provides a unique approach for breeders in assessing the fiber quality of a cotton plant before harvest. GBB uses SNPs as genetic markers when making individual plant selections. The SNPs used in this project are located within the fiber quality genes, as proposed by Dr. Hongbin Zhang at Texas A&M.

Earlier technologies detected and utilized SNPs only as markers flanking a sequence of DNA, i.e., QTL, or any other genomic sequence of interest in any desired crop. The uniqueness of GBB lies in its fiber quality genes identified with a SNP marker located within the sequence. The reliability of SNPs closely associated with a trait locus can often be invalidated when the marker-trait association fails to express its effect in a multiple environment study with statistical significance (Nadeem et al., 2018). GBB

platform offers an alternative approach to overcome this challenge by introducing SNP markers that are part of the fiber quality genes rather than flanking a QTL, thereby at least assuring that the SNP is associated with the fiber quality traits. The mutation rates of SNPs and the apparent genotype by environment (GxE) interaction would undoubtedly affect the expression of fiber quality genes associated with desired traits in the GBB approach as it did in conventional Marker-Assisted Selection (MAS). GBB can at least allow cotton breeders to assess segregating populations for superior fiber quality because of the reliability of these fiber quality genes, rather than focusing on QTLs associated with a flanking marker which may become less reliable due to factors such as plant growth stages, multiple locations, and cross over events that change the coupling/repulsion relationship (Nadeem et al., 2018).

The overarching goal of this research was to compare the effectiveness of IPS in an F_2 and an F_3 populations of CIL germplasm based on two selection methodologies. A proof of concept study will allow breeders to compare the accuracies of predictions made by the use of limited SNP markers for GBB with the conventional FBB approach. This study should provide useful comparisons in early generation estimations of the fiber length and strength of individual plants in these populations. Early and accurate estimations of fiber quality traits in the GBB platform can facilitate the screening of a larger number of plants in segregating populations. The success of GBB in selecting an individual plant with superior fiber quality will depend upon how closely it can estimate its actual phenotype.

Improvements in fiber properties are feasible only if new genetic combinations can be derived. The Texas A&M AgriLife CIL seeks to exploit such genetic variation. An improved portfolio of cotton fiber quality cultivars allows the textile industries to utilize superior fiber quality to maintain cotton as a viable commodity for Texas and U.S. producers.

CHAPTER II

LITERATURE REVIEW

Gossypium spp.

Cotton (*Gossypium spp*.) is the most widely used fiber crop relative to the global textile industry. There are four species of cultivated cotton, two A-genome diploid (2n=2x=26) and two AD-genome tetraploid (2n=4x=52) species; however, one of the two AD genomes, *G. hirsutum* (L.), dominates contemporary production. While yield is the essential trait from the breeding and production points of view, improvements of the cotton fiber quality is significant (Feng et al., 2015). Cotton breeders are continually trying to break the antagonistic relationship between yield and fiber quality traits in cotton (Groves et al., 2016). A renewed interest in breeding for superior fiber quality cotton allows breeders to explore the existing genetic variation in the development of superior fiber properties in cultivars. Fiber quality data are determined by High Volume Instrument (HVI) testing, which includes Upper Half Mean Fiber Length (UHML), Fiber Bundle Strength (FBS), Micronaire (MIC), Elongation (ELON) and Uniformity Index (UI) (Smith and Coyle, 1997). Classical breeding techniques and methodologies have resulted in significant fiber quality improvement (Culp, 1992; Cooper, 1992; Gannaway and Dever, 1992; Elzik and Thaxton, 1992; Smith, 1992; Smith et al., 2017). The conventional breeding techniques, however, are slow and time-consuming, thus pushing cotton breeders to look for new ways to speed up the selection process of superior fiber quality cultivars.

Fiber Quality

HVI measurements have been a reliable source of fiber quality measurements since the 1960s (Kelly et al., 2013). UHML, MIC, FBS, UI, and ELON of fibers before rupture (ELON) can be determined on a commercial bale (218 kg) or on a single plant yielding 20 g of lint (Kelly et al., 2013). HVI provides a further classification of cotton fibers by including color grades for white, lightly spotted, spotted, tinged, and yellow stained cotton (Cotton Inc, 2020). It is the preferred method of measurement of fiber quality parameters due to its speed and efficiency (Kelly et al., 2012). It uses a fibrosampler in which a beard of fibers is created with fibers parallel (Hertel, 1940) and then optically scanned for length characteristics (Ramey, 1999). HVI measurements are the basis for determining the value of cotton grown in the United States.

UHML is the standard fiber length measurement in the U.S. cotton industry (Smith et al., 2009). The UHML is defined as the mean length of the longer half of the fibers by weight (Woo, 1967). HVI uses a fibrosampler in which a beard of fibers is created with fibers parallel (Hertel, 1940) and is then optically scanned for measurements (Ramey, 1999). The fibrogram method is the basis for determining the UHML (Ramey, 1999; Cui et al., 2009) and can be programmed to provide staple length in 1/32nd inch increments. A common goal of breeders is to increase the UHML for a better yarn and, ultimately, a better fabric. HVI UHML is measured in inches and can be used when making individual plant selections since a single plant produces sufficient lint

quantity for HVI measurement (Kelly et al., 2012). UHML is widely accepted as the standard in determining cotton length (Smith et al., 2009), though recent studies have shown increased variability in measuring fiber lengths less than 0.5 in. under the HVI measuring system (Cui et al., 2007; Cai et al., 2010). Upland cotton is classified by four categories based on UHML: short (≤1.06 in.), medium (1.06–1.14 in.), medium-long (1.18–1.26 in.), long (1.26-1.37 in.), and ELSU (>1.375 in.). Pima UHML is divided into long (1.33-1.375 in.) and extra-long (>1.375 in.) (Braden and Smith, 2004; Smith, 2009).

FBS is measured by HVI, where a random tapered specimen (fiber samples) is clamped on each end along its length and then tested with applied force (in grams) needed to break the bundle (Sasser et al., 1991). The fiber bundle strength refers to a standard weight bundle composed of fibers of unequal lengths clamped between the jaws (Ciu et al., 1999). The beard is clamped on both sides; thus, the strength measurement is made from the average of the two sides. FBS is measured as tenacity, i.e., g/tex, and is calculated from the force to break divided by the bundle mass and multiplied by the width of the clamp assembly (Ramey, 1999). The breaking load is the mass in grams, whereas tex refers to the linear density in g/km (Munro, 1987; Taylor, 1994). Fiber strength is categorized into five categories: weak (23 g/tex and below), intermediate (24-25 g/tex), average (26-28 g/tex), strong (29-30 g/tex), and very strong (31 g/tex and above), (Ramey, 1999). The strength of cotton fibers directly affects yarn quality and production speed (Liu et al., 2016).

Markers and SNPs

DNA markers are heritable entities where the position of a DNA sequence is associated with a trait of interest (Staub et al., 1996). Markers have been used to exploit genetic diversity and germplasm organization, e.g., *Arachis* (Lanham et al., 1992) and *Brassica* (dos Santos et al., 1994; Thormann et al., 1994), *Vaccinium* (Novy et al., 1994). Restriction Fragment Length Polymorphisms (RFLPs) were the first DNA markers to be used in a marker-based selection strategy in tomatoes (Young, 1999). Newer marker technologies replaced RFLPs with Amplified Fragment Length Polymorphism (AFLPs), Random-Amplified Polymorphic DNAs (RAPDs), and Simple Sequence Repeats (SSRs) (Young, 1999). While qualitative traits are less complicated and follow a more straightforward mode of inheritance in plants, DNA markers can be more useful in understanding the genetic complexity and inheritance of quantitative traits (Peleman and Voort, 2003). Marker-assisted selection and breeding emphasize selecting superior lines based on the genotype rather than the phenotype.

SNPs refers to a change in a nucleotide base sequence between two DNA samples. SNPs are more abundant and more stable than SSRs and represent the most common type of genetic polymorphism (Edwards et al., 2007). SNP markers are advantageous because of the high level of polymorphism and allelic variation they can reveal in a genome. Moreover, the low mutation rate of SNPs makes them an apt choice of genetic markers in understanding complex traits (Eathington et al., 2007). SNPs are

easy to develop and portable, thus making it markers of choice. SNPs represent a uniform coverage of the genome and can provide high-resolution markers in the mapping of traits (Mammadov et al., 2012). SNPs can quickly and efficiently screen a segregating population, therefore assisting a breeder with the selection even before the trait of interest is phenotypically expressed.

Several studies have been conducted to associate variation in phenotypic traits such as UHML and FBS to their genotypic markers such as SNPs (Abdurakhmonov et al., 2008; Kantartzi and Stewart, 2008; Zeng et al., 2009; Cai et al., 2014; Mei et al., 2013; Zhang et al., 2013; Su et al., 2016; Huang et al., 2017; Sun et al., 2017; Liu et al., 2018). The SNPs identified by Ma et al. (2018) established distribution of fiber quality SNPs within the A and D subgenomes with the A subgenome possessing 2.3 times more than the D subgenome in 419 accessions of upland cultivars. More recently, SNP chips like CottonSNP80K arrays (Cai et al., 2017) and CottonSNP63K array (Hulse-Kemp et al., 2014) have been developed to use SNP markers to explore genetic diversity and marker-trait association when making selections. Along with the QTLs, candidate genes also have been identified, which are associated with fiber qualities such as UHML and FBS (Liu et al., 2018; Diouf et al., 2018). A genomic analysis conducted by Hinze et al. (2017) further established a strong relationship of SNPs with fiber quality genes, mostly located in the A subgenome.

GBB Platform

The abundance of fiber quality SNPs is yet to be exploited by cotton breeders in selecting superior fiber quality cultivars. The breeders in this research study will utilize 11 of the fiber quality gene-based SNPs developed by the Zhang lab at Texas A&M University to make individual plant selections in two specific breeding populations. The GBB is proposed to design and select superior cultivars based on not only alleles (gene allele selection, GAS) but also expression profiles of the genes, marked by SNPs controlling the targeted trait(s) (gene expression profile-based selection, GEPS) (pers comm., Zhang)

A recombinant inbred line population of a cross between TAM-94L and NM 1331 was used to identify SNP based genes for UHML and FBS (pers comm., Zhang). Two hundred of the $F_{2:8}$ RILs, along with parents, were genotyped using RAD seq technology. The DNA was sequenced, and the expressions of fiber gene QTLs for UHML and FBS were profiled in 10-dpa fibers of the 200 RILs and parents. The Rad-seq data were integrated with the Upland cotton genome physical map (pers comm., Zhang) and then used with a novel high throughput gene and QTL cloning system (undisclosed) developed by the Zhang lab at Texas A&M to develop SNP-based fiber quality genes.

Under the GBB platform, 474 UHML genes were identified and cloned with SNPs as their genetic marker located inside the genes. Only 11.4% of these genes were

positively linked with increasing the UHML while 88.6% decreased the UHML when actively expressed in 10-day post-anthesis (dpa) fibers. These fiber length genes were labeled as Gossypium Fiber Length (GFL) genes, with each gene affecting the UHML ± 2.6% to 7.9%. The GBB identified and validated 756 genes known as Gossypium Strength (GSTR) genes controlling FBS. The GBB platform identified 12.2% of the GSTR genes that were linked in increasing FBS whereas 87.8% decreased FBS when actively expressed in 10-dpa fibers. Twenty-one of the GSTRs were identical to GFL genes and expressed the same additive effect to the fiber quality of an individual plant. The effect of GSTR genes ranged from ± 3.7% to 14.2%. The GBB platform recognizes the additive and dominance effects of these UHML and FBS genes, but more importantly, identified the interaction network among these UHML and FBS genes. The interaction network includes the action, action directions, and the interaction of these UHML and FBS genes, which ultimately affects the development of fiber length and strength in a cotton plant. The UHML and FBS genes were cloned using the gExpress technology (U.S. Patent Serial No.:62/298,606). GBB further classified these gene-based SNPs into A and D subgenomes based on their chromosome location.

In an unpublished study, the GBB system identified 740 SNPs from 226 of the 474 GFL genes that increased or decreased UHML by 2.1% to 22.6%. Based on these GFL based SNPs and their expression profiles, nine statistical predictive models were developed and used to estimate the UHML of a single plant selection with the predictability of $r>0.80$ ($P<0.00$).

While larger breeding nurseries are certainly advantageous in capturing genetic variation, the limitations of resources such as fewer workers measuring fiber quality in the field and other resources are sometimes difficult to overcome. Seed chipping technologies have provided an early selection advantage to the breeding community. However, public breeding programs like the cotton breeding program at TAMU are at a disadvantage in applying such advanced technology due to limited finances. GBB can provide an additional advantage to the cotton breeders at TAMU in making early season selections before harvest on two criteria, UHML and FBS. GBB plant selections can utilize the actual location of the SNP within the gene, thereby eliminating false positives or a loose linkage between the marker and its trait.

CHAPTER III

MATERIALS AND METHODS

Germplasm

TAM 11K-13 ELSU (PI 684656) is an extra-long staple upland germplasm line developed by Texas A&M Agrilife Research and released in 2017. This ELSU line was a result of the cross of 03 B-182-39 and 03 A-106-8. TAM 11K-13 ELSU exhibited a longer UHML than all other comparison genotypes at 1.33 in., which is slightly below the ELSU standard of 1.375 in., when performance tested across Weslaco, Corpus Christi, and College Station in 2014 and 2015 (Smith et al., 2018).

TAM 06WE-621 (PI 671964) is an extra-strength upland (ESU) germplasm line released in 2014 by Texas A&M Agrilife Research. One of the parents was the result of a cross between DP491 and TAM 96WD-18 (Thaxton et al., 2005; PI 635879), and the other parent was derived from the cross between TAM 91C-95Ls (Smith, 2001; PI 614952) and Deltapine Acala 90 (PVP 8100143) (Smith et al., 2014). TAM 06WE-621 has been shown to exhibit FBS values as high as 38.5 g/tex (Smith et al., 2014).

NMSI 1331 (*Gossypium barbadense* L.) was developed under a new class of cotton called ‘New Mexico Sea Island” at New Mexico State University (Smith and Cothren, 1999) and commercially released in 1996. It was derived from a single plant selection from the heterogeneous Montseratt Sea Island (MSI) population at New

Mexico State University (Roberts et al., 1997). The sea island genotypes are often used in efforts to introgress superior Pima type fiber quality alleles in improving fiber quality of Upland genotypes while maintaining desirable agronomic traits (Smith et al., 1999).

Strain 13P-54 ELSU was an unreleased ELSU line that was developed by the CIL at Texas A&M Agrilife Research. This ELSU strain exhibited UHML as long as 1.46 inches and FBS as high as 33 g/tex. The line resulted from the cross of TAM 04 WB-33s (Smith et al., 2011) and breeding strain A-106-30 ELSU, which was a full sib of TAM A 106-16 ESU released in 2009 (Smith et al., 2009).

TAM B182-33 (PI 654362), an extra-long staple upland (ELSU) germplasm line developed by Texas A&M Agrilife Research and released in 2008 (Smith, 2009). Cotton Inc. (2020) identified ELSU as germplasm that exhibits an UHML equal to or exceeding 1.26 in. However, internal to the CIL, ELSU is defined as an UHML equal to or exceeding 1.375, which is the minimum UHML for Pima grade 1. TAM B182-33 was derived from the cross of TAM 94L-25 (Smith et al., 2009) and PSC 161 (May et al., 1995) and has been shown to exhibit an UHML value of 1.39 in. (Smith et al., 2009).

Breeding Populations

Two breeding populations were established in 2016 at the Texas A&M AgriLife Research Farm near College Station. Texas. An interspecific F_2 population of 13P-54 ELSU // TAM 11K-13 ELSU / NMSI 1331 was established for individual plant

selections for the FBB and GBB platforms; this population carried a *G. barbadense* genetic background which made it ideal in GBB selections because of its expected high polymorphism. This interspecific population was expected to provide more allelic variation that can be explored through gene-based SNPs when making selections and thus giving more power to the breeders to choose even within a small sample size population. An intraspecific F_3 population was derived from TAM 11K-13 ELSU /TAM 06WE-621 ESU. This F_3 population was established to make GBB and FBB selections within an upland / upland population with no known *G. barbadense* introgression since the majority of upland cotton breeders are interested in upland by upland breeding populations. Ninety percent of the world's production is accounted for by upland cotton (Zhang et al., 2008).

The selection protocols for GBB (Zhang, pers comm) and FBB platforms (subpopulations) were followed for the breeding nurseries in both populations in the summer of 2016. The interspecific population (13P-54 ELSU // TAM 11K-13 ELSU / NMSI 1331) nursery was established with 2160 individually spaced F_2 plants, and the intraspecific population (TAM 11K-13 ELSU /TAM 06WE-621 ESU) nursery contained 3000 spaced F_3 plants. The soil type was a Belk clay series, a Fine, Mixed, Thermic, Entic Hapluderts. Cultural practices were consistent with cotton production in central Texas, including furrow irrigation. The planting date was April 20th at the Texas A&M AgriLife Research Farm near College Station, Texas. Two hundred fifty individual plants were randomly selected from each population for GBB, and 250 plants were

visually selected for FBB for only long UHML and not FBS since FBS cannot be visually identified. The selections for FBB and GBB platforms created four subpopulations: GBB UHML GBB FBS, FBB UHML and FBB FBS,

Selection Protocol

The FBB selection protocol consisted of individual plant selections based on visual observations within the F_2 and F_3 nurseries for interspecific and intraspecific populations, respectively, in 2016. Breeders usually select for UHML by parallelizing the fibers from individual seeds and visually selecting plants with the longest fibers or sufficiently long fibers. For the research reported herein, visual determination of the length of the fibers of individual plants was determined by observing the length from three bolls, one each from the top, middle, and bottom fruiting zones of an individual plant. Two hundred fifty individual plants with the longest fibers were selected from 2160 individual plants in the interspecific population and 250 were selected from 3000 individual plants in the intraspecific population.

The GBB selection protocol consisted of screening 250 random plants from both populations with 11 gene-based SNPs that were associated with superior UHML and FBS. These SNP markers were developed using a 200 Recombinant Inbred Line (RIL) population from the cross of TAM 94L-25 x NMSI 1331. The RILs were used to construct RAD-SEQ libraries and then sequenced using Illumina Hi-Seq 2000 from which a total of 3703 SNPs were validated for its association with genes for UHML and

FBS. A total of 474 genes were identified and cloned for UHML and 736 genes identified for FBS (pers.comm, Zhang). Two hundred fifty plants were selected for the 11-SNP-GBB platform in determining fiber quality predictions from 2160 individual plants in the interspecific population and 250 were selected from 3000 individual plants in the intraspecific population.

Genotyping Protocol

DNA was collected from young leaf tissues at approximately the first flower growth stage in each population from 250 individual random plant selections for the GBB platform. DNA was isolated, extracted, and labeled in the summer of 2016. A standard Polymerized Chain Reaction (PCR) protocol was followed, as determined by the Zhang lab (Zhang et al., 2014). The PCR samples from both the populations were then genotyped by using the standard gel-electrophoresis protocol developed by the Zhang lab (Zhang et al., 2013). Each sample was compared with a DNA ladder and its original SNP sample for each gene; this comparison was used to determine the SNP orientation i.e., A, T, G, C, or heterozygous.

Establishing the top and bottom 10 percent sub-subpopulations

Two hundred and fifty plants were identified within the intraspecific and within the interspecific populations by visually selecting for long fibers (FBB). At maturity, the seedcotton of each plant was harvested, ginned on a laboratory gin, and HVI fiber quality determined by the Fiber and Biopolymer Research Institute (FBRI) at Texas

Tech University. Subsequently, the top (longest UHML or strongest FBS) and bottom (shortest UHML or lowest FBS) 25% were planted to progeny rows in 2017 for seed increase. Thus approximately 63 $F_{3:4}$ intraspecific progeny were established. The decision was made to proceed with 10% selection criteria rather than 25% criteria and thus the top and bottom 10% based on the original 2016 IP data were selected for the completion of the project. Thus the sub-subpopulations were composed of approximately 29 $F_{4:5}$ FBB progeny established as the top 10% for UHML subpopulation, 28 for the bottom 10% UHML FBB, 29 for the top 10% FBS, and 28 for the bottom 10% FBS sub-subpopulation. Sub-Subpopulations for the GBB platform were developed in a similar fashion, utilizing the 11 SNP evaluation of 250 IP in each of the intraspecific and interspecific populations in 2016. Succinctly outlined, the following populations, subpopulations and sub-subpopulations were developed:

Intraspecific population: TAM 11K-13 ELSU / TAM 06WE-621 ESU

Subpopulation: 250 FBB

Sub-subpopulation: 10 % top and bottom UHML

Sub-subpopulation: 10 % top and bottom FBS

Subpopulation: 250 GBB

Sub-subpopulation: 10 % top and bottom UHML

Sub-subpopulation: 10 % top and bottom FBS

Interspecific population: 13P-54 ELSU // TAM 11K-13 ELSU / NMSI 1331

Subpopulation: 250 FBB

Sub-subpopulation: 10 % top and bottom UHML

Sub-subpopulation: 10 % top and bottom FBS

Subpopulation: 250 GBB

Sub-subpopulation: 10 % top and bottom UHML

Sub-subpopulation: 10 % top and bottom FBS.

The FBB selections and 11-SNP-GBB selections were considered progeny rows in 2017 with each row being an F_2 derived F_3 progeny ($F_{2:3}$) among the interspecific selections and F_3 derived F_4 ($F_{3:4}$) progeny among the intraspecific populations. It was important to note that few selections overlapped for UHML and FBS selections in FBB.

Checks

The two checks were released by the Cotton Improvement Lab at Texas A&M. TAM B182-33 (PI 654362) ELSU exhibited a UHML exceeding 1.37 inches and resulted from a cross between TAM94L-25 and PSC 161. TAM 06WE-621 (PI 671964) is an ESU exhibiting an FBS of approximately 38 g/tex, about 25 % greater than the best current commercial cotton cultivars. TAM 06WE-621 is a product of DP 491/TAM96WD-18//TAM91C-95Ls/DP Acala 90.

Experimental Design and Performance Trials

The single plants selected in 2016 were planted to progeny rows in 2017 for seed increase. The top 10% and bottom 10% selections based on the FBB and GBB platforms were performance tested in 2018 and 2019. The FBB and GBB selections from the

intraspecific population comprised one randomized complete block (RCB) performance trial, while those from the interspecific population comprised the second performance trial. The selections from the intraspecific and interspecific populations were in the $F_{3:5}$ and $F_{2:4}$ generations, respectively, in the RCBD trials.. The first RCBD trial consisted of 136 entries derived from the GBB and FBB selections in the intraspecific population along with two checks. The second RCBD trial included 127 entries from the interspecific GBB and FBB selections along with two checks.

The planting date for both trials was 5 May 2018. These two trials were repeated using 2017 seeds, i.e., the same generations, in 2019 at the same location. The two RCBD trials of 2019 were planted in a different field with the planting date of 20 May 2019. The soil type and the location were the same as the selection trial in 2016. Thirty boll samples were collected from each entry in each trial, ginned by the same persons on the same lab gin for HVI measurements. The lint samples were evaluated at the FBRI for the determination of HVI UHML and FBS

Statistical Analysis

Regression analyses was utilized to regress the UHML and FBS of advanced progeny to their foundational IPS. The analyses provided an estimate of the correlation between progeny performance and their founding IPS fiber properties. Regression analyses were applied to the top and bottom 10% sub-subpopulations as described above

to determine if associations change with selecting within or among elite fiber quality populations. The R^2 values of the correlations were determined using the latest version of JMP 14.0 software. The UHML and FBS distribution in the breeding population were graphed using JMP 14.0 software.

The linear model for this experiment was: $R_{ijr} = m + G_i + Y_j + R(Y_j) + GY_{ij} + e_{ijr}$ for UHML and FBS. The model calculates the observed response R_{ijr} of the genotype i in the year j with the rep effects where m=grand mean, G=genotype, Y=year, R=replications, and e=pooled error.

An analysis of variance was conducted for UHML and FBS. Sums of Squares were reported using JMP Pro 14.0 for reps (R), genotypes (G), year (Y), and genotype x year (GxY). Mean values were calculated for all 127 genotypes and 117 genotypes for the intraspecific and interspecific populations, respectively. LSD values were calculated for the mean values as $LSD = t_{.05} * (2*ems/r)^{-½}$ and used for separating the mean values for all entries. A means separation table was constructed for all the entries in each trial for UHML and FBS to compare the performance with the checks that included TAM B 182-33 for UHML and TAM 06WE-621 for FBS. Entries included in the performance means table were named as DBV (Daman Bhangu Visual) and DBG (Daman Bhangu Genotype) following a label that identified whether it was from the top 10% or bottom 10% sub-subpopulations. DBV was identified as a visual selection determined under the FBB model, and DBG was identified as a 11-SNP-GBB selection determined under the GBB model.

The Expected Mean Squares were calculated for entries for each population under a fixed model effect.

	df	MS	F Test
Year (Y)	Y-1	M1	M1/M5
Reps (R)	(R-1)Y	M2	
Genotypes (G)	G-1	M3	M3/M5
Genotype x Year (GxY)	(G-1)(Y-1)	M4	M4/M5
Error	(R-1)(G-1)Y	M5	

Objectives

Two distinct populations were analyzed for a proof of concept study where selections were based on both the FBB and GBB systems. The F_2 population of 13P-54 ELSU // TAM 11K-13 ELSU / NMSI 1331, an interspecific (Pima introgression) population, and the F_3 population of TAM 11K-13 ELSU / TAM 06WE-621 ESU, an intraspecific (Upland x Upland) were used. Within each of these two populations, two subpopulations were derived by visually selecting 250 individual plants in each for FBB and 250 for GBB using the 11 gene-based SNP markers. Within each of the four subpopulations, two sub-subpopulations were derived by selecting the top and bottom 10 % for UHML and FBS.

Objectives were:

1. Determine if breeders can predict progeny performance form genetic or phenotypic predictive data when selecting within a narrow range of UHML or FBS elite quality;

a. Determine the correlation between the IP HVI UHML and FBS measurements and the UHML and FBS of the resulting F_4 or F_5 progeny within the top and bottom 10% when utilizing the FBB protocol.

b. Determine the correlation between the 11 gene-based SNP predicted fiber IP UHML and FBS and the UHML and FBS of the resulting F_4 or F_5 progeny within the top and bottom 10% when utilizing the GBB protocol.

c. Determine success in selecting improved progenies based on IP data through analyses of variance

2. Determine if GBB, which was based on an interspecific population, can be used in an intraspecific population for UHML and FBS.

CHAPTER IV

RESULTS & DISCUSSION

Pre-Selection Distribution of UHML and FBS Within Populations

The UHML values ranged from 1.11 to 1.51 inches, with half of the values concentrated within the range of 1.36 inches to 1.43 inches in the intraspecific population (Figure 1). The FBS values in the intraspecific population ranged from 28.10 g/tex to 40.90 g/tex, with half of the values concentrated within 34.5 g/tex to 37 g/tex

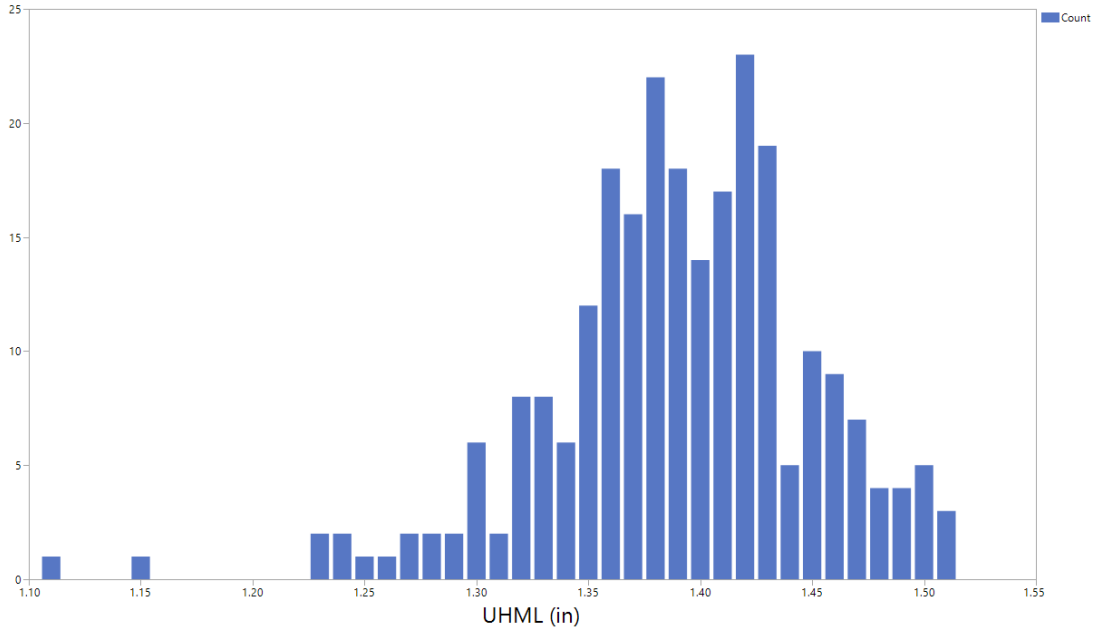

Figure 1. Distribution of UHML (inches) values of individual F_3 plants selected for the GBB genotype screening in the intraspecific population in 2016.

(Figure 2). The range of UHML and FBS was expected to be centered around an average mean of 1.38 inches since this intraspecific population consists of one ELSU parent (TAM 11 K-13). This distribution of UHML and FBS values among the 250 random individual plant selections suggested a well-dispersed range of phenotypic

variation that was used for selections for fiber quality traits. However, the population was developed by crossing upland genotypes.

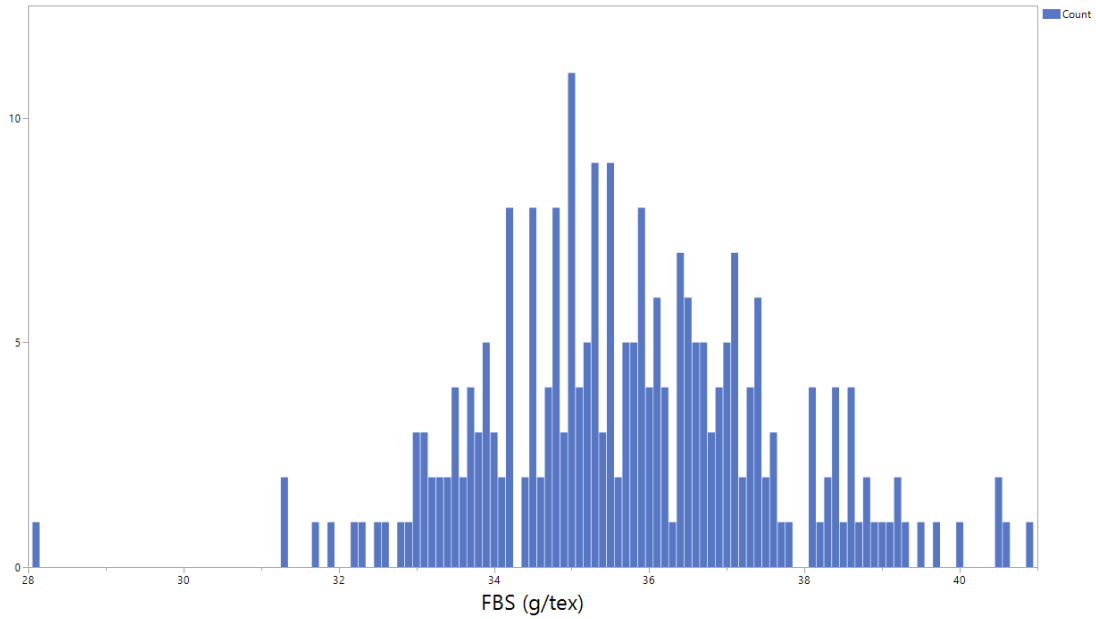

Figure 2. Distribution of FBS (g/tex) values of individual F_3 plants selected for the GBB genotype screening in the intraspecific population in 2016.

The UHML values in the interspecific population ranged from 1.15 to 1.63 inches, with half of the values concentrated within 1.34 inches to 1.48 inches (Figure 3). The FBS values in the intraspecific population ranged from 26.70 g/tex to 39.40 g/tex. Somewhat unexpectedly, the FBS values are slightly narrower in their distribution in the interspecific population, with half of the values concentrated within 32.13 g/tex to 34.90 g/tex (Figure 4).

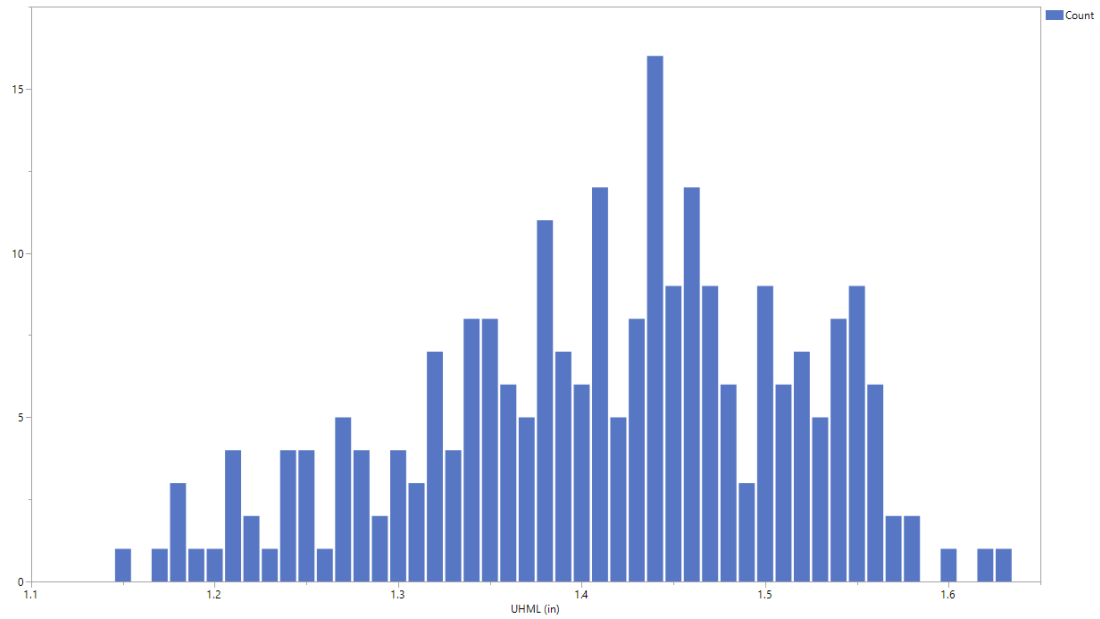

Figure 3. Distribution of UHML (inches) values of individual F_2 plants selected for the GBB genotype screening in the intraspecific population in 2016.

The distribution of UHML values of the interspecific population is larger than the intraspecific population. This expectation of UHML values confirmed that the interspecific population contained plants with greater variation in UHML apparently due to the introgression of *G. barbadense* genetic background found in the NMSI 1331, and, thereby theoretically providing more power in choosing individual plants in GBB method.

The range of UHML and FBS values suggest that both intraspecific and interspecific breeding populations contained sufficient phenotypic variation to be used for the research reported herein.

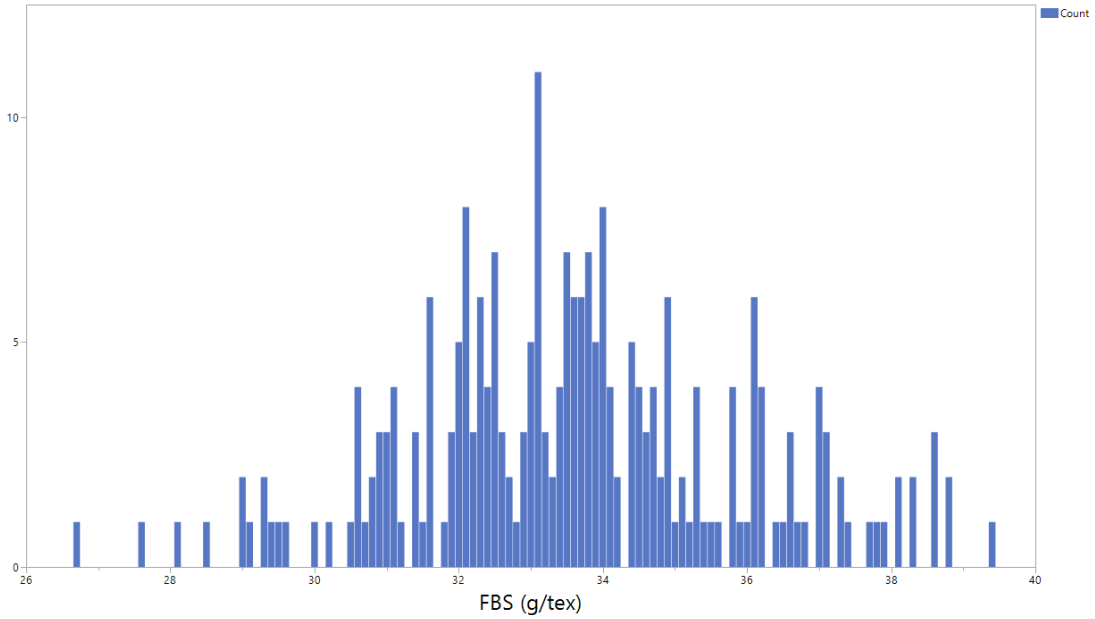

Figure 4. Distribution of FBS (g/tex) values of individual F_2 plant selections of the GBB method in the interspecific population in 2016.

Correlations – GBB – UHML Intraspecific Population

Regression analyses failed to identify a relationship between the 11 SNP predicted HVI values and actual HVI measurements in the intraspecific population in either the top or bottom 10% subpopulations. Figure 5 shows the regression lines and the R^2 values from regressing the 11-SNP predicted UHML on the actual HVI determined UHML within the top and bottom 10% of the 11-SNP predicted $F_{3:5}$ progeny. The 11 SNPs failed to predict $F_{3:5}$ progeny UHML from the foundational IP within the top 10% subpopulation in either year; R^2 for 2018 was 0.089 and 0.004 for 2019. The scatterplot of the UHML values, ranging from 1.07 to 1.36 inches for the top

10% category, is indicative of the lack of relationship between the IP SNP predictions

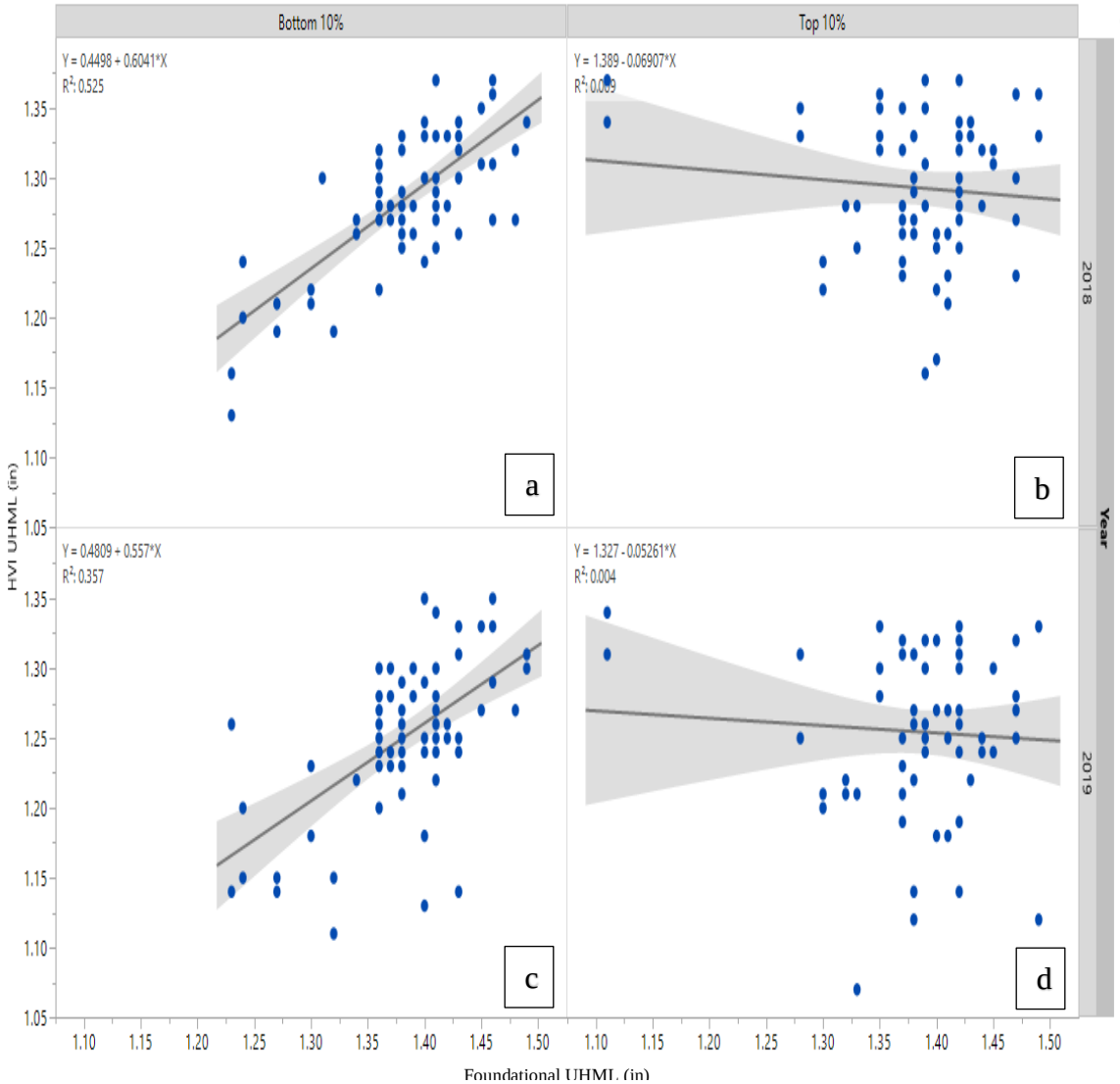

Figure 5. Regression of the resulting $F_{4:5}$ progeny HVI values for UHML on their F_3 individual foundational plant 11-SNP predicted UHML within the intraspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

and the actual HVI measurements for the resulting progeny for 2018 and 2019 within either subpopulation.

The eleven gene-based SNPs applied to the F_3 IPs were insufficient to predict the UHML values of the $F_{3:5}$ progeny within the top 10% sub-subpopulation although a few of the predictions were within the top 10% for superior UHML. This research suggests that the current GBB model using only 11 gene-based SNPs will not predict superior UHML in the resulting progeny or strains within an intraspecific population that is segregating for superior UHML. The GBB prediction accuracy might or should be improved with the inclusion of more gene-based SNPs. Still, given the results for the top 10% interspecific sub-subpopulation discussed below, that may not be an accurate conclusion.

The R^2 values for the bottom 10% sub-subpopulation for UHML of the $F_{3:5}$ progeny as predicted by the F_3 SNP data were 0.53 and 0.36 for 2018 and 2019, respectively (Figure 5). The predicted UHML based on the 11 GBB SNPs was much better for the lowest 10% subpopulation than for the top 10%; however, a closer look at the data suggest caution. The distribution of the $F_{3:5}$ progeny UHML values, ranging from 1.11 to 1.36 for the bottom 10% category, indicated no relationship between the SNP predictions and its actual HVI values for 2018 and 2019. The higher correlation values within the bottom 10% sub-subpopulation for UHML predictions was misleading since only 6 % of the total selections in the bottom 10% exhibited UHML below an ELSU UHML i.e., less than 1.375 inches. The 11-SNP-GBB predictions among the bottom 10% contained more top-performing HVI UHML entries than the predictions in the top 10% category for both years. Despite a higher correlation value than the top

10%, our research suggested a lack of relationship in the prediction accuracy in the GBB method using only 11 gene-based SNPs for estimating the lower UHML within this intraspecific population.

Correlations – GBB – FBS Intraspecific Population

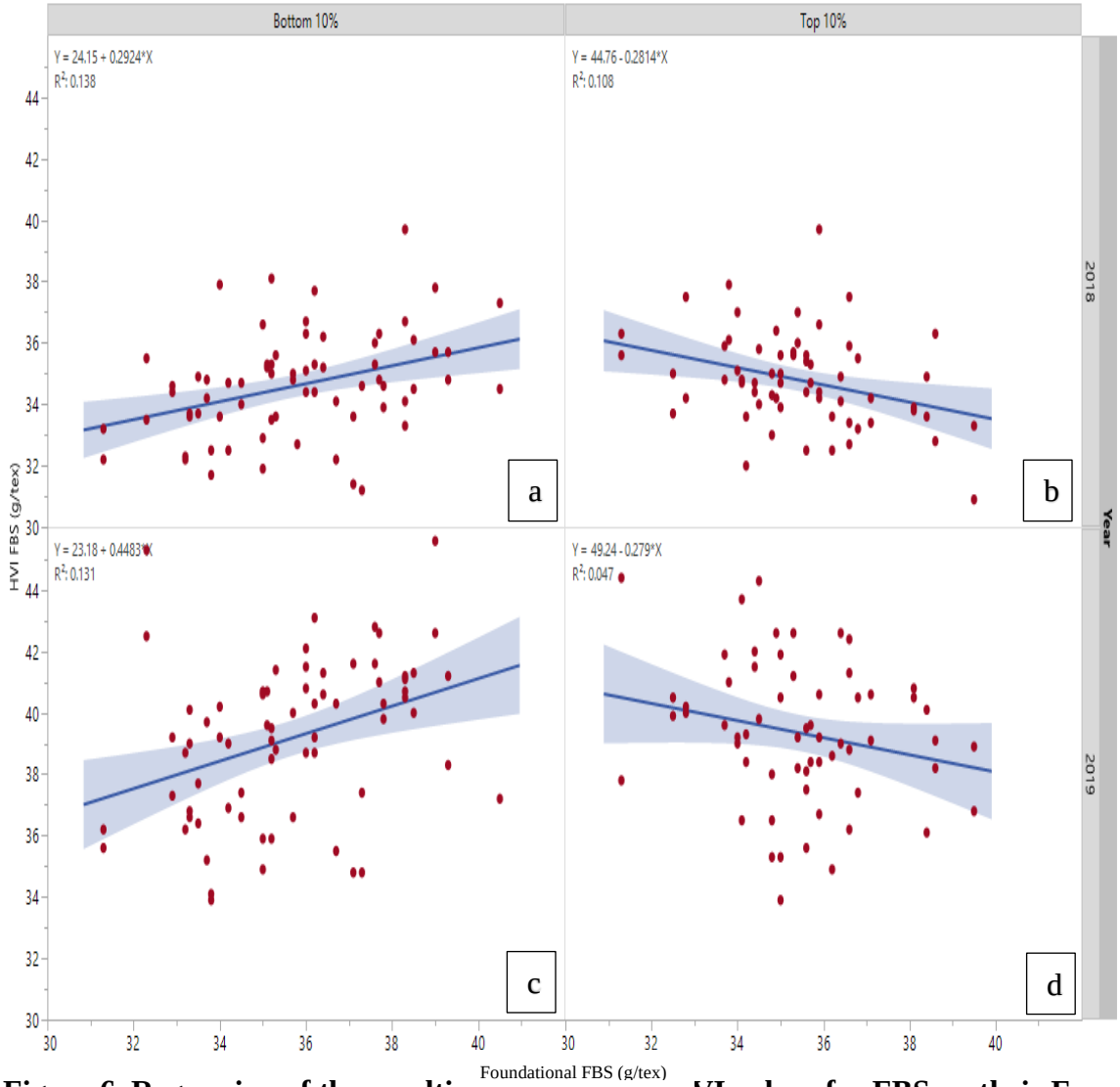

Figure 6. Regression of the resulting $F_{4:5}$ progeny HVI values for FBS on their F_3 individual foundational plant 11-SNP predicted FBS within the intraspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

The 11-SNP-GBB selections failed to predict the top 10% FBS values for randomly selected individual plant selections in this intraspecific population. The scatterplot graph (Figure 6) showed R^2 values of 0.11 and 0.05 for the top 10% plants for FBS in 2018 and 2019, respectively. The FBS predictions were scattered within a range of 31 g/tex to 40 g/tex in 2018 and 34 g/tex to 44 g/tex in 2019. This range of FBS should have provided sufficient variation for prediction purposes but the low R^2 values suggested a lack of robustness in predicting the top 10% of individual plants for the superior FBS. The success of the GBB prediction methods should have accounted for a clearer separation between the lower and higher FBS values. Since this intraspecific breeding population may contain less phenotypic variation for superior FBS in comparison to the interspecific population, the clustering of FBS values around the mean value may present a disadvantage for breeders in statistically identifying superior FBS performing lines from lower-performing lines. This research indicated that there was no correlation between the 11-SNP-GBB predicted FBS values and actual HVI measurements in the top 10% category for both years.

The R^2 value between the 11-SNP-GBB predicted FBS values and HVI measurements in the bottom 10% category for both years was 0.14 and 0.13 in 2018 and 2019, respectively. The distribution of the FBS values in the bottom 10% category ranged from 31 g/tex to 45 g/tex, across both years, again a range that one would expect to provide ample opportunity for selection. The 11 SNP-GBB method failed to predict individual plants with lower FBS quality was supported by the wide range of FBS values

exhibited by the selections in the bottom 10% category (Figure 6). Almost half of the FBS values in the bottom 10% category exhibited a fiber strength above or equal to the in-house standard of 38 g/tex for the project's ESU genotype for both years i.e. TAM 06WE-621. There was no relationship between the 11-SNP- GBB predicted FBS and its HVI FBS values in separating the weak performing lines in comparison to high performing FBS lines. This research suggested no correlation between 11-SNP-GBB predictions with actual measurements for predicting the superior and inferior FBS lines in 2018 and 2019.

The 11-SNP-GBB model failed to predict breeding lines in the intraspecific population for either the best or the worst performance in UHML and FBS. The lack of correlation could have been affected by environmental factors. Davidonis et al. (2004) point out that the effect of high moisture content during harvest can result in a decreased fiber maturity and fiber maturity directly impacts FBS. The presence of rainfall during the harvest of 2018 may have impacted the fiber strength of the 11-SNP-GBB selections, thereby affecting the correlation with the HVI measurements. The GBB predictions in UHML category for the top and bottom 10% could have been affected by high moisture content due to rainfall as demonstrated by Bradow and Davidonis (2010) where environmental fluctuations can severely affect the cotton fiber quality from seedling to harvest.

Data from Bhangu et al. (2017) demonstrate that the parental lines of this intraspecific population, TAM 11 K-13 and TAM 06 WE-621, have the genetic potential to produce superior fiber quality, UHML and FBS, under irrigated and dryland conditions in central and south Texas. This intraspecific cross from the two elite parents suggested that a lower rate of variation for fiber quality traits may have aided these 11 gene-based SNPs to capture genetic variation in UHML and FBS successfully. However, the results of this 11- SNP-GBB predictions in this intraspecific population suggested a lack of robustness of these 11 SNPs that proved to be inconclusive and statistically weak in differentiating progeny in the top 10% and progeny in the bottom 10% of individual plant selections for UHML and FBS.

Correlations – GBB – UHML - Interspecific Population

The R^2 results from the regression analyses of the top and bottom 10 % sub-subpopulations for UHML within the interspecific population were much more encouraging than in the intraspecific population (Figure 7). The R^2 among the predictions in the top 10% was 0.49 for 2018 and 0.34 in 2019 which was an improvement over the 0.089 and 0.004 for the intraspecific population (Figure 5). Such R^2, while greatly improved, remain below what would be necessary for breeders to abandon actual phenotypic data. The R^2 was improved compared with the 11-SNP GBB within the interspecific population as expected given that the SNPs were developed from an interspecific RIL population and the current interspecific population contains the same *G. barbadense* parent. However, the scatterplot for the regression of predicted on

actual UHML values showed actual UHML values ranging from 1.15 to 1.42 inches for the top 10% category. In contrast, the 11 SNPs predicted values ranging from about 1.15 to 1.60. Like the intraspecific populations, the top 10% values should have concentrated above or equal to the average UHML of ELSU lines considering one of the parents in this population was NMSI1331, a superior fiber *G. barbadense* biotype sea island genotype. The R^2 value dropped in 2019 by 15 points, which suggested changing environmental conditions and years as a possible source of variation in UHML values.

It was worth noting that two of the selections in the 11-SNP-GBB method within

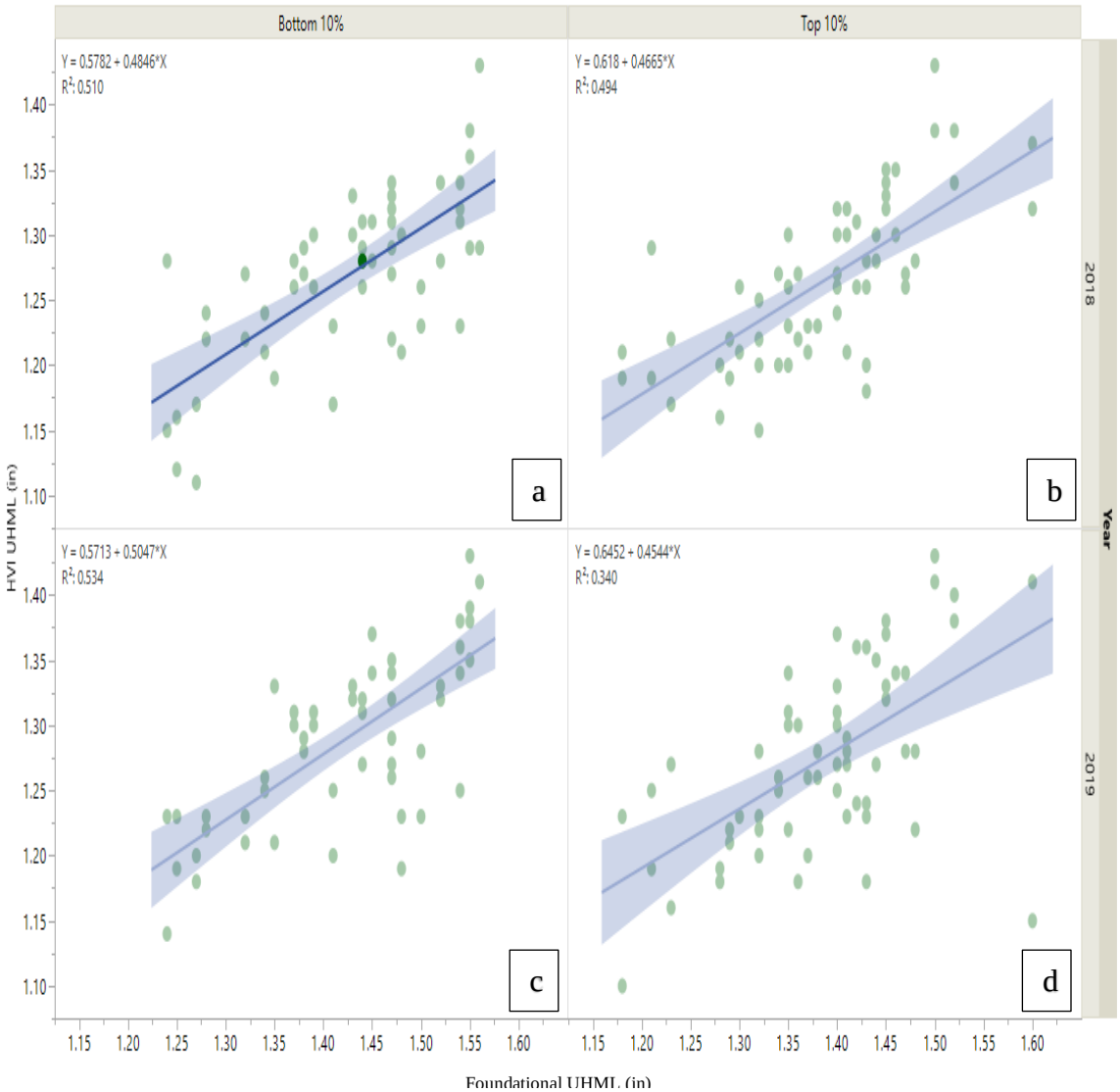

Figure 7. Regression of the resulting $F_{3:4}$ progeny HVI values for UHML on their F_2 individual plant 11-SNP predicted UHML within the interspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

the interspecific population did produce fibers with exceptional UHML of 1.42 and 1.37 inches; however, the 11-SNP-GBB model failed to achieve its overall objective of predicting the best performers within the top% of individual plants with high UHML in

both years just as in the intraspecific. Most of the 11-SNP-GBB predictions for UHML in the top 10% included lines exhibiting an UHML below 1.37 inches, whereas the distribution results of Figure 5 indicated several plant selections with high UHML values even though the R^2 values within this sub-subpopulation for the GBB was essentially zero. Higher correlation numbers are indicative of better prediction accuracy in 2018 than 2019 in estimating the UHML within the interspecific population.

The R^2 value for the11-SNP-GBB predictions in the bottom 10% category of the interspecific population was 0.51 and 0.53 for 2018 and 2019, respectively (Figure 7). The prediction accuracy was not only numerically better but more consistent relative to the top 10% category for UHML. The range of HVI UHML values, as shown in the scatterplot, ranged from about 1.13 to 1.45 in. while the 11 SNPs predicted a range of about 1.13 to about 1.55. A wider distribution of the UHML values was expected of the interspecific population since the introgression of *G. barbadense* allows for more polymorphism in the breeding population than the intraspecific population.

The purpose in establishing these sub-subpopulations was to determine if the associations would be better in less extreme quality or UHML quality more closely resembling standard quality upland. The bottom 10% portion of the sub-subpopulation for UHML in both the intra and interspecific populations did not reflect standard upland fiber quality but rather contained individuals with exceptional UHML and not sufficiently different from the upper 10 % group. Thus, the similar R^2 values are not surprising in this interspecific population given the range of values and the fact that one

parent, NMSI 1331, was the same in this research as in the RIL population from which the SNPs were derived. The bottom 10% category contained three selections that exceeded an UHML of 1.37 in. despite containing selections with low UHML in comparison to ELSU lines. The 11-SNP-GBB prediction method predicted several ELSU individuals but their actual HVI phenotypes were below expectations even in this interspecific population.

Better prediction accuracy of the GBB method will probably require more gene-based SNPs for screening the interspecific population for UHML as was the conclusion in the intraspecific population also. The interspecific population was expected to be more polymorphic. It, therefore, contained higher segregation variation for UHML than the intraspecific population and that was partially true and may have been responsible for the better R^2 values. As mentioned earlier, the GBB model was developed using a RIL population with NMSI 1331 being utilized as one of the parents; this expectation was realized with the GBB prediction model as it was able to capture more allelic variation, but not all of it due to the limited number of gene-based SNPs being utilized.

Correlations – GBB – FBS - Interspecific Population

The R^2 values between the 11-SNP-GBB predicted FBS and their HVI measurements for the top 10% was 0.12 and 0.09 in 2018 and 2019, respectively (Figure 8). The scatterplot (Figure 8) shows a range 28.0 g/tex to 38.0 g/tex in 2018 for

both the SNP predicted and actual HVI phenotype; and about 32.0 g/tex to about 45.0

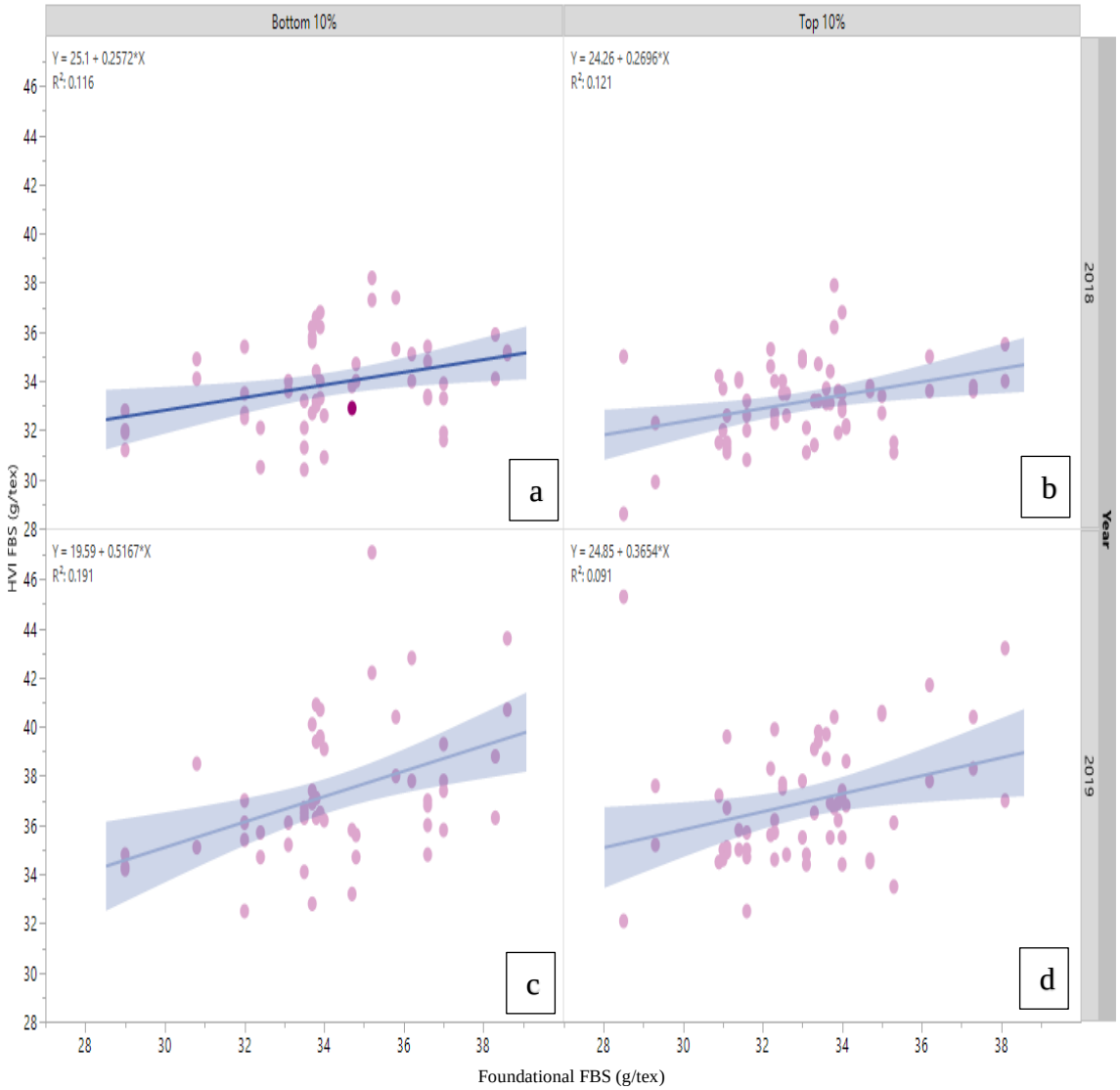

Figure 8. Regression of the resulting $F_{3:4}$ progeny HVI values for FBS on their F_2 individual plant 11-SNP predicted FBS within the interspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

g/tex in 2019, The 11-SNP GBB model predicted only one selection above the ESU standard of 38.0 g/tex in either year. The rest of the 11-SNP-GBB predictions of superior FBS values fell short in actually exhibiting superior FBS measurements in 2018 and 2019. The presence of a broad range of FBS values in the top 10% category suggested

no clear distinction of superior FBS selections between the top 10 and bottom 10% groups. With a low R^2 for both years in the top 10% category, the prediction accuracy failed to estimate the superior FBS selections by the 11-SNP-GBB method.

The R^2 values between 11-SNP-GBB predicted FBS with their HVI measurements for the bottom 10% were 0.11 and 0.19 in 2018 and 2019, respectively (Figure 8). The low R^2 in the bottom 10% sub-subpopulation shows a failure to establish the prediction accuracy that breeders could depend upon between the 11-SNP-GBB predictions and their actual measurements. Again, the populations, subpopulations, and sub-subpopulations developed in this research failed to establish sub-populations that adequately separated standard FBS in the bottom 10% category to compare with elite FBS selections in the top 10 % category. This failure was evident in 2018 and 2019 in the distribution of the FBS values where some of the FBS selections exhibited FBS exceeding 38.0 g/tex. The scatter plot graph of FBS values for the bottom 10% suggested the 11-SNP-GBB method was not predictive of superior and low performing lines for FBS.

The interspecific population in this study did not verify that a highly polymorphic breeding population as defined herein would be better for SNP-GBB for FBS, although as noted above it was promising for UHML. Moreover, the high moisture content may have impacted fiber strength and the overall fiber quality of the random individual plant selections. Like the intraspecific population, our study indicated a lack

of robustness of these 11 gene-based SNPs in predicting the FBS value in comparison to the after-harvest measurement determined by HVI. A higher number of gene-based SNPs probably are required in screening the interspecific as well as intraspecific population.

Correlations – FBB – UHML - Intraspecific Population

Individual F_3 plants were visually selected using standard "cotton breeder" protocol as described above with 250 plants from the intraspecific population so identified. These plants were harvested individually and standard HVI fiber data were obtained from the FBRI in Lubbock. Following a seed increase generation in 2017, the top and bottom 10% for UHML were then planted in a two rep, RCBD experiment in 2018 and 2019. Boll samples were taken from each row and HVI data again obtained as described above. Regression analysis was employed to determine the correlation or association of the individual F_3 plant UHML and the resulting $F_{3:5}$ progeny row UHML within the top and bottom 10% subpopulations based on the F_3 IP data.

The R^2 values for the FBB UHML F_3 selections regressed against their $F_{4:5}$ progeny HVI UHML within the top 10 % subpopulation, based on F_3 data, in the intraspecific population were 0.50 and 0.67 in 2018 and 2019, respectively (Figure 9). Since the individual plants for the top 10% in the FBB method were selected after knowing their actual HVI UHML values, it was expected that these $F_{4:5}$ progeny lines would correlate well with their F_3 foundation plant UHML regardless of year tested. Given the quantitative nature of UHML in upland cotton (Islam et al., 2014), the failure

to find a higher correlation of $F_{4:5}$ progeny with visually selected F_3 plants for UHML

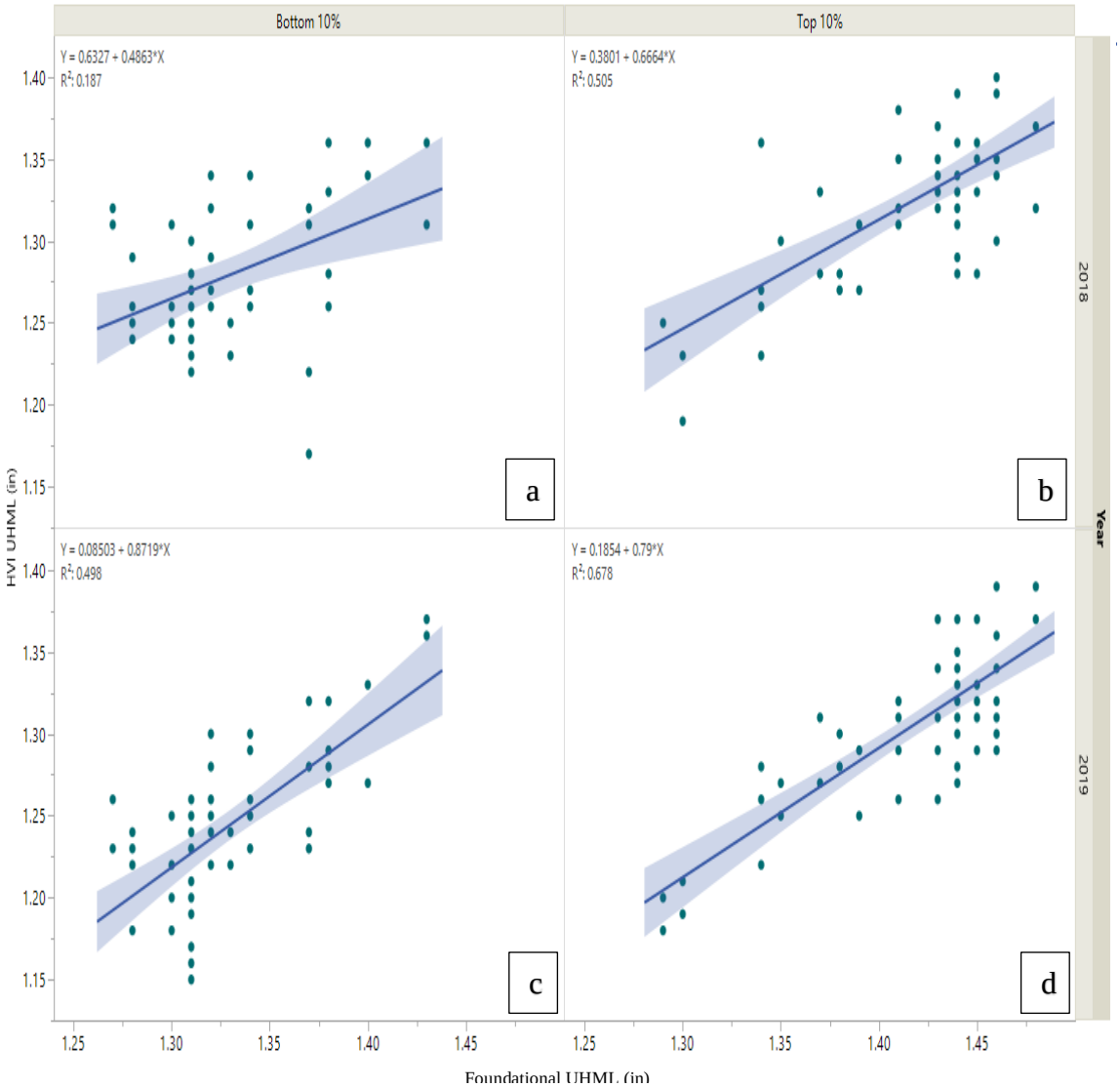

Figure 9. Regression of the resulting $F_{4:5}$ progeny HVI values for UHML on their visually selected F_3 individual foundational plant HVI UHML in the intraspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

was not surprising since the F_3 generation contains considerable heterozygosity. However, experience dictates that early selection for UHML results in progeny with excellent UHML and that experience was verified in these data with a large percentage

of the top 10% subpopulation having UHML above 1.25 and several with UHML values exceeding 1.3 inches given that only one parent exhibited the ELSU trait. Recall that the reported UHML of TAM 11K-13 ELSU was 1.33 and only 1.20 for TAM 06WE-621 (Smith et al., 2014) ESU. However, in 2018 only three $F_{4:5}$ progeny exceeded an UHML of 1.37 inches and in 2019 only five were above 1.37 inches. Thus, R^2 values of 0.5 and 0.67 are indicative of the high heritability of UHML and the efficiency of making field selections for UHML. These R^2 values verify that visual field selection techniques exceed those of the 11 GBB SNPs that showed associations of only 0.009 and 0.004 within the top 10% subpopulation for UHML (Figure 5). The prediction accuracy numerically improved in 2019, possibly due to the lack of moisture content experienced when compared with high moisture encountered during harvest in 2018.

Since this population was derived by visually selecting only for longer fibers, the discrepancies in the top and bottom 10% sub-subpopulations were not large (Figure 9). The actual HVI UHML ranges were similar for the top and bottom 10% categories but the association of F_3 individual plant's UHML with their $F_{3:5}$ progeny UHML was not numerically as good as within the highest 10% sub-subpopulatios, with R^2 values of 0.18 in 2018 and 0.49 in 2019. However, the R^2 for the lowest 10 % subgroup in 2019 was the same as the R^2 for the highest 10% in 2018. These results also support the above conclusion that visual selection for UHML in early generations are effective in producing progeny with longer UHML.

Correlations – FBB – FBS Intraspecific Population

The 250 FBB intraspecific F_3 plants were considered random selections relative to FBS since they were field selected based on visual fiber length. However, UHML and FBS are correlated, so the randomness is not absolute (Hugie et al., 2016). The R^2 values between FBB HVI FBS values for the 2016 individual F_3 plants and their resulting $F_{3:5}$ progeny HVI FBS measurements for the top 10% sub-subpopulation were

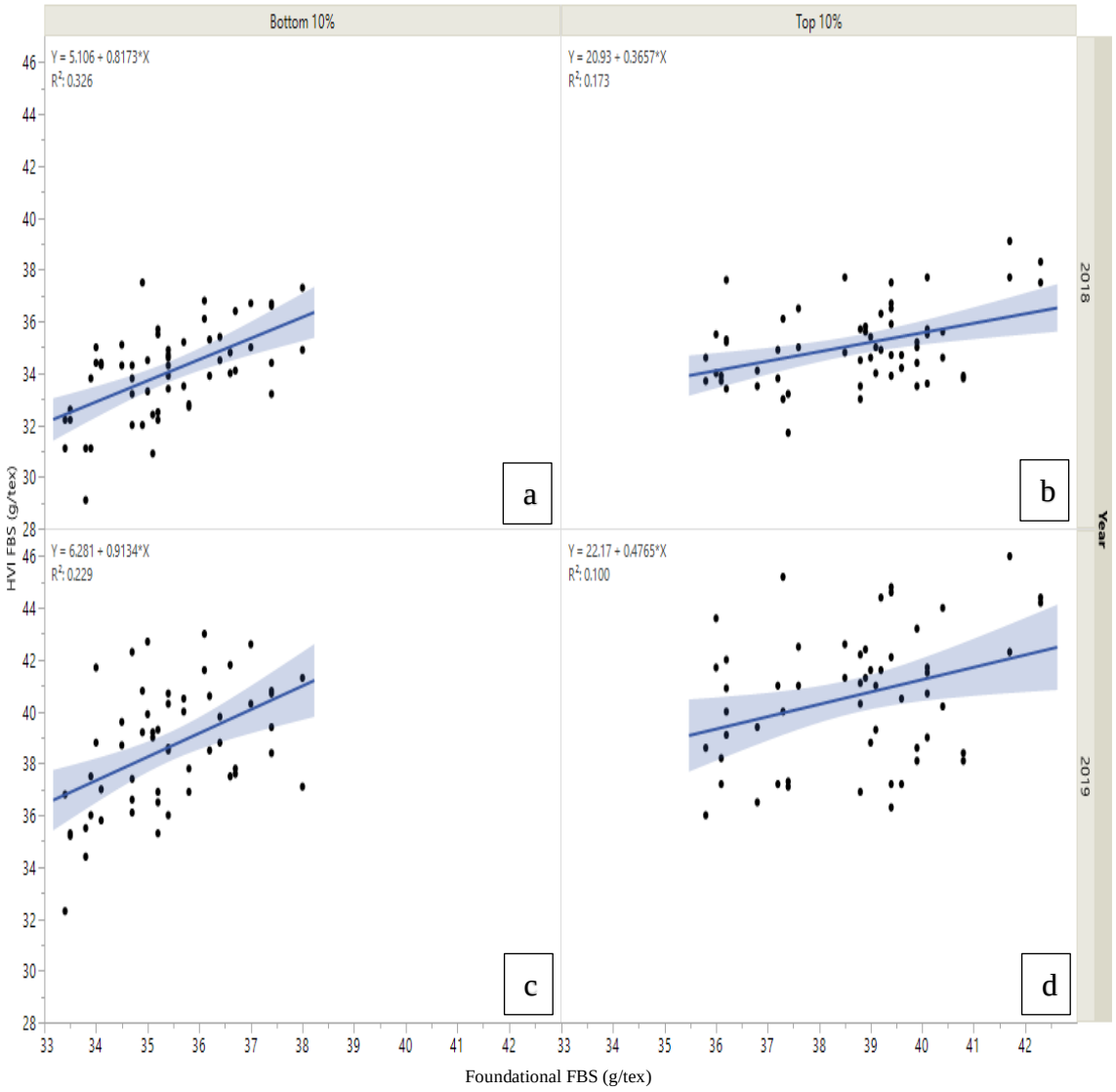

Figure 10. Regression of the resulting $F_{4:5}$ progeny HVI values for FBS on their visually selected F_3 individual foundational plant HVI FBS in the intraspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

0.17 and 0.10 in 2018 and 2019, respectively (Figure 10). While the R^2 values were not as good as with UHML for associations within the top 10% sub-subpopulation under the FBB method, the regression line did suggest that superior FBS lines were indeed selected in 2018 and 2019. The top 10% sub-subpopulation progeny in 2018 had FBS

values below 38.0 g/tex except for two lines, whereas in 2019, most of these same $F_{3:5}$ progeny exhibited FBS equal to or above 38.0 g/tex. This demonstrates the impact of environment on this HVI fiber trait. As mentioned above, the high moisture content during the 2018 harvest cannot be ruled out as a possible source of decrease in FBS values of 2018 in comparison to the FBS value of the original selection. This moisture issue may have also led to an increase in trash content in the samples which may impact FBS.

The R^2 values between the F_3 IP FBS and their resulting $F_{3:5}$ progeny FBS within the bottom 10% subpopulation were 0.32 and 0.22 in 2018 and 2019, respectively (Figure 10), again suggesting that IP selections in early generations will provide a level of probability that the resulting progeny will exhibit improved FBS. However, within the bottom 10% sub-subpopulation, as with the top 10% sub-subpopulation, FBB IP FBS was not a great predictor of their $F_{3:5}$ progeny FBS. On the positive side, within each of the sub-subpopulations, there were individual $F_{3:5}$ progeny that exhibited excellent FBS and the two sub-subpopulations were clearly distinguishable.

Correlations – FBB –UHML - Interspecific Population

Regression analyses of the $F_{2:4}$ progeny on their foundational F_2 single plant HVI UHML in the interspecific populations produced R^2 values as expected (Figure 11) and similar to the R^2 values found in the intraspecific population (Figure 9) except for the bottom 10% in 2018. This expectation holds true for FBB selection platform because of better separation of UHML with data points clustered more towards the lower and higher

end of fiber UHML spectrum since the selections are made after their original HVI

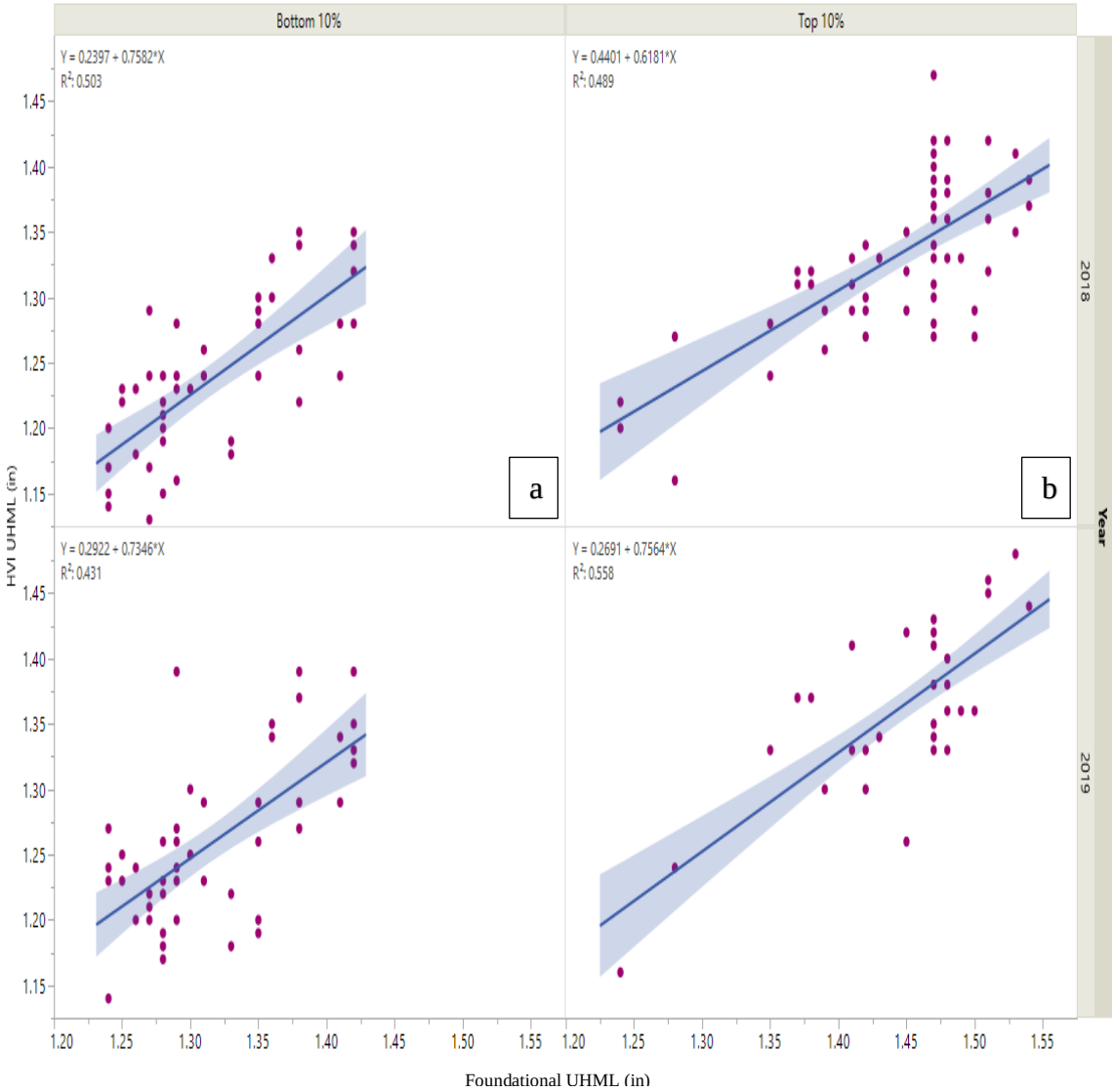

Figure 11. Regression of the resulting $F_{3:4}$ progeny HVI values for UHML on their visually selected F_2 individual foundational plant HVI UHML and in the interspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

measurements. The R^2 values were 0.49 and 0.56 for the top 10% category in 2018 and 2019, respectively (Figure 11). The finding that the R^2 values found in the interspecific population's advanced progeny, whether predicted by the HVI or the 11 SNP GBB

protocol, suggests again that the GBB protocol may be as good as the actual HVI IP data. Most upland cotton breeders probably are not going to accept R^2s of only 0.34 to 0.53 (Figure 7) on which to base IP selections but those are not drastically different from the HVI IP predicted correlations of 0.43 to 0.56 (Figure 11). The scatterplot graph for the FBB $F_{2:4}$ progeny regressed on their F_2 IP HVI UHML, despite a few outliers, contained superior UHML selections clustered towards the higher end of the UHML measurements for both years. The prediction accuracy produced consistent R^2 values suggesting that the interspecific population exhibited more stable UHML values over the two years in comparison to the intraspecific population. The FBB was successful in determining two exceptionally superior fiber length lines with a UHML exceeding 1.46 inches in 2018 and 2019. This scatterplot graph suggests that the FBB method performed numerically better relative to prediction accuracy based on the R^2 indicated associations, as well as clustering of the fiber quality lines with superior UHML from the low UHML exhibiting lines in this interspecific population.

The R^2 values for the bottom 10% sub-subpopulation indicating an association between the F_2 foundational plants and their $F_{2:4}$ progeny for UHML were 0.50 and 0.43 for 2018 and 2019, respectively (Figure 11). The clustering of the UHML values was towards the lower end of the UHML range suggesting that FBB successfully identified or breeder-selected shorter UHML plants in the interspecific population and was essentially identical to the correlation values reported herein for the GBB based on the 11 SNPs (Figure 7). The prediction accuracy was numerically better in 2018 and slightly

less in 2019, where some of the FBS values did exceed the threshold of 1.37 inches, which is considered the accepted starting point for a superior UHML line. This research suggested that FBB works equally well in either intraspecific or interspecific populations (Figures 9 and 11).

Correlations – FBB – FBS Interspecific Population

The R^2 values from regressing the $F_{2:4}$ progeny FBS values on their foundational F_2 IPS for the top 10% were 0.32 and 0.30 in 2018 and 2019, respectively (Figure 12).

There was a lack of clustering towards higher FBS values for the top 10% in 2018, which suggested that superior FBS selections were unable to match the predicted FBS

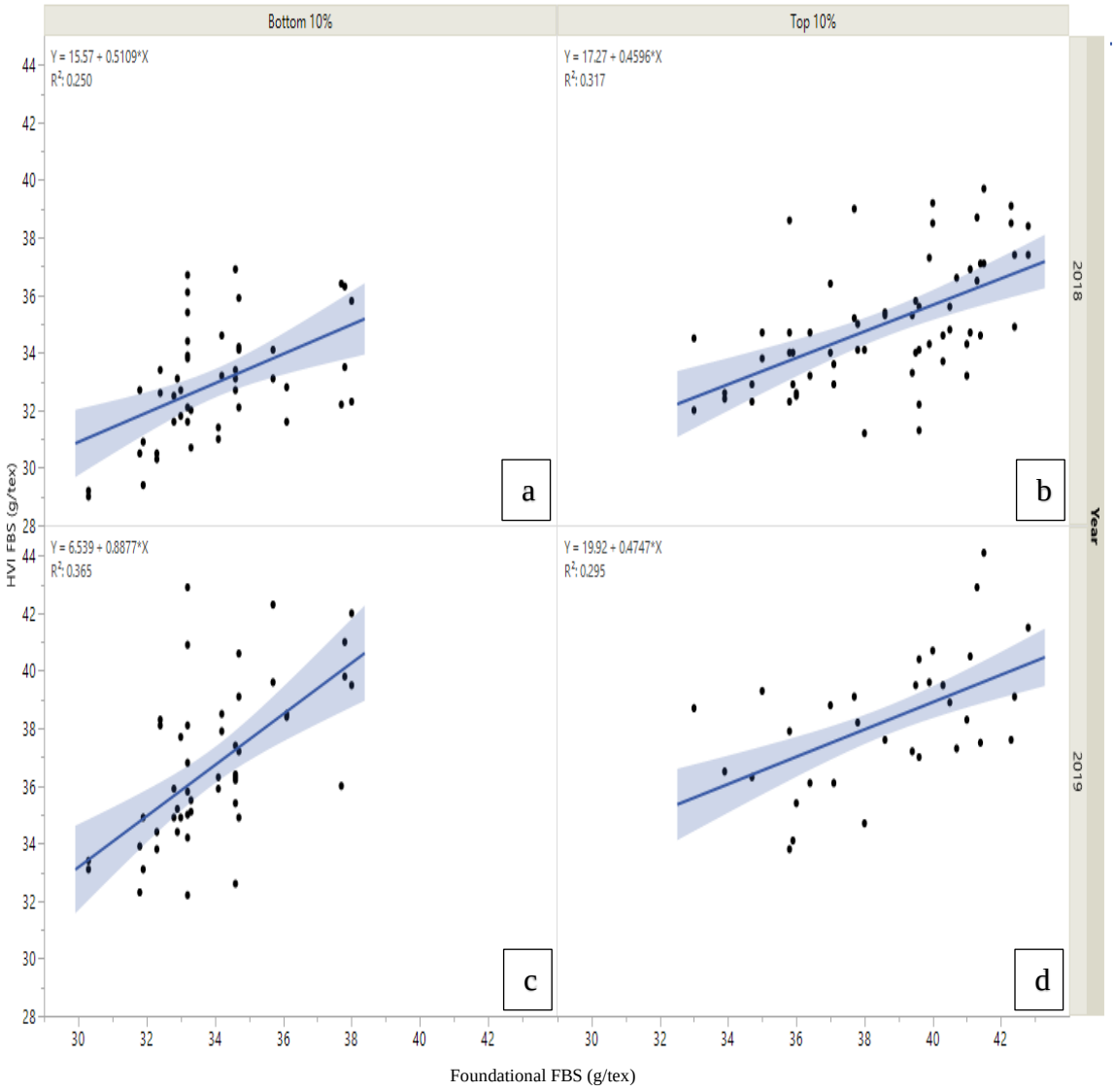

Figure 12. Regression of the resulting $F_{3:4}$ progeny HVI values for FBS on their visually selected F_2 individual foundational plant HVI FBS and in the interspecific population. Panel a=regression for the bottom 10% of the population for 2018; panel b=the top 10% for 2018; panel c=bottom 10% for 2019; panel d=top 10% for 2019.

based on their foundational IPS plants in 2016. A numerical improvement was noted in the clustering of FBS values with more lines performing above 38.0 g/tex in 2019. The

regression plot of the FBB method in the interspecific population's top 10% category did reveal two exceptional FBS lines, one exhibiting a FBS of 42.0 g/tex in 2018 and another exhibiting 44.0 g/tex in 2019. In comparison with the R^2 values reported in Figure 10 for the intraspecific population, those from the interspecific population (Figure 12) are generally higher. The FBB protocol should not have been affected by the common NMSI 1331 parent present in the GBB SNP RILs. Thus, the explanation for the slight improvement in association is not obvious. Again, high moisture during the 2018 harvest could also be a source of a decrease in FBS values of 2018 in comparison to the FBS value of the original IP selections.

The R^2 values between FBB determined FBS with their HVI measurements for the bottom 10% category within the interspecific population were 0.25 and 0.37 in 2018 and 2019, respectively (Figure 12). The R^2 values were lower in 2018 than in 2019 despite a better clustering of the FBS values under 38.0 g/tex. The R^2 value was higher in 2019, but the scatterplot diagram showed 50% of the FBS values that were above or equal to 38.0 g/tex suggesting again that the research protocol did not create a low FBS sub-subpopulation for FBS in the interspecific population. Again, the purpose of comparing top and bottom 10% categories was to compare the regression curves or R^2s obtained with exceptional fiber quality with more conventional quality. Unfortunately, for that purpose, the top and bottom categories were not sufficiently different.

Review of Regression Analyses

R^2 values suggest that GBB produced results similar to those obtained through conventional breeder's phenotypic selection protocols in all UHML sub-subpopulations except the top 10 % of the intraspecific population (Table 1). In the bottom 10 % intraspecific sub-subpopulation and both sub-subpopulations in the interspecific population, the GBB was essentially equal to the FBB protocol. However, for FBS, the FBB protocol appears to provide breeders with a slightly better association between foundational individual plants and the FBS of their resulting progeny than the GBB protocol, again based on the 11 SNPs used in this study.

Additional genic SNPs from the 226 (personal communication) identified by the Zhang lab should provide much improved results. The models provided by the Zhang lab in an unpublished study (pers.comm Zhang) indicates that using all of 226 SNPs that have been correlated with additive alleles for an increased UHML among individual plants can provide a higher correlation between the predicted UHML and FBS with its actual measurements. Such an effort requires more funding and a perhaps a larger sample size to fully study the consistency of the predictability of UHML and FBS in GBB selection platform.

Table 1. Comparison of R square values from regressing progeny HVI data on foundational individual plant data within 2018 and 2019 for UHML and FBS within GBB and FBB protocols.

Selection protocol and HVI trait	Intraspecific Population				Interspecific Population			
	Top 10 %		Bottom 10%		Top 10 %		Bottom 10%	
	2018	2019	2018	2019	2018	2019	2018	2019
GBB UHML	0.09	0	0.53	0.36	0.49	0.34	0.51	0.53
FBB UHML	0.51	0.67	0.19	0.5	0.49	0.56	0.50	0.43
GBB FBS	0.11	0.05	0.14	0.13	0.12	0.09	0.12	0.19
FBB FBS	0.17	0.1	0.33	0.23	0.32	0.3	0.25	0.37

UHML and FBS distributions within subpopulations of Intra and Interspecific populations

Intraspecific Population

A second way to analyze and interpret the research reported herein is to conduct an ANOVA on the UHML and FBS of the advanced progeny of all 2016 IPS that were identified within the top or bottom 10% within either the intraspecific or interspecific populations plus appropriate checks, those being TAM B182-33 ELS for UHML and TAM 06WE-621 ESU for FBS. The subpopulations of the intraspecific population consisted of 125 $F_{3:5}$ progeny and 116 for the $F_{2:4}$ interspecific progeny. As noted above, this number is fewer than the simple sum of 200 (top 25 and bottom 25 each for UHML and FBS in each of the GBB and FBB platforms, because some selections were the same for both UHML and FBS and selections were extended to include any 2016 IPS that equaled the lowest UHML or FBS value selected.

Table 2. Analysis of variance for UHML† and FBS properties of GBB and FBB platform selections within an intraspecific population and two checks grown at College Station in 2018 and 2019 in a randomized complete block design.

Source		**UHML**	**FBS**
	df	MS	MS
Year (Y)	1	0**.1237****	**2823.7****
Reps (R)	2	0.0046	1.4859
Genotypes (G)	126	**0.0083****	**11.219****
Genotype x Year (GxY)	126	**0.0013****	2.6269
Error	252	0.00105	2.4533
Total	255		

**Significant at p should be < or equal 0.05
† UHML=upper half mean length; FBS=fiber bundle strength; GBB=gene based breeding; FBB=field based breeding.

Fiber UHML and FBS were affected by Genotype and Year in the intraspecific populations (Table 2). Genotypes did not respond the same across both years for UHML as indicated by a significant GxY component. Genotypes were expected to be variable for UHML and FBS since the experimental design dictated selection of the top and bottom 10% for either UHML or FBS. The significant Year effects were not unexpected since yearly environments can be considerably different at College Station, TX and the literature supports an environmental component when evaluating these traits across years or locations (Bhangu et al., 2017). In this particular case the high precipitation rate during harvest in 2018 and lack of precipitation in 2019 would suggest a significant Year effect. For FBS, the G*Y interaction was not significant indicating that genotypes were consistent in response across years.

Interspecific Population

Genotypes and Year were highly significant sources of variation for both UHML and FBS in the interspecific population (Table 3). A significant Genotype*year interaction was detected for FBS only. Further investigation of this interaction revealed that the top 10% sub-subpopulation of FBB was significantly different (P=.05) and outperformed than all of the other sub populations.

Table 3. Analysis of variance for UHML† and FBS properties of GBB and FBB platform selections within an interspecific population and two checks grown at College Station in 2018 and 2019 in a randomized complete block design.

Source		**UHML**	**FBS**
	df	MS	MS
Year (Y)	1	**0.0508****	**1456.3****
Reps (R)	2	0.0041	3.9743
Genotypes (G)	116	**0.0178****	**14.789****
Genotype x Year (GxL)	116	0.0013	**3.5249****
Error	228	0.0012	2.2515
Total	235		

**Significant at p should be < or equal 0.05
† UHML=upper half mean length; FBS=fiber bundle strength; GBB=gene based breeding; FBB=field based breeding.

The effect of genotypes result was expected, especially within the interspecific population given the likelihood of a broader range of potential UHML and FBS values because of the *G. barbadense* parent. As noted above, the year effect was significant possibly due to the high precipitation during 2018 harvest and the lack of precipitation in 2019.

The genotype by year effect was a significant source of variation for UHML in the intraspecific trial and FBS in the interspecific trial. For the ANOVA results of the

intraspecific trials, it was interesting to note the mean square (MS) value of genotype by year interaction of .0013 relative to the value of .0083 of the genotypic effect. While both of these sources were significant, but the genotype by year effect appears to minimal when compared with genotype and can possibly be ignored when analyzing the combined performance for both years for each mean value of each genotype. Similarly, for the FBS trait in the interspecific trial, the MS value of genotype is significantly higher than the MS value of with an absolute difference of 12.264. The MS values of these two significant interactions, i.e. UHML in intraspecific and FBS in intraspecific, suggested that while there is an inconsistent response of some genotypes relative to other genotypes across years, however there are only few genotypes responsible for these inconsistencies in the mean response for UHML and FBS.

Top and Bottom Sub-Subpopulation Means Analyses

The regression indicated that there is variation from year to year that affect consistency of selection. The R^2 results were indicative of showing a consistency of response among the type of subpopulations in both populations; however, the means comparison of the bottom and top 10% sub-subpopulations in Table 4 was able to determine the effectiveness of the selection methodologies in UHML and FBS.

The top 10% sub-subpopulation in the FBB protocol exhibited an overall average UHML of 1.31 inches and was significantly higher (P=.05) compared with the top 10% in GBB. Although, the bottom 10% sub-subpopulation from GBB and FBB selections

performed below the top 10% FBB sub-population but did exhibit an UHML no differently than the top 10% sub-subpopulation among GBB $F_{3:5}$ progeny. The FBS data from Table 4. presented similar results like UHML where the top 10% sub-subpopulations performed better with a higher FBS value compared to all other subpopulations including top 10% GBB selections. While the top 10% GBB selections were statistically different with a numerical decline of only .83 g/tex from the top 10% FBB but it was not different from the bottom 10% sub-subpopulations of GBB and FBB selections. The efforts in this study were also concentrated on the prediction accuracy of the GBB platform in selecting the top 10% for UHML and FBS. The means separation among sub-subpopulations suggested that GBB protocol was not able to differentiate individual plants in the original F_2 nursery for superior UHML and FBS. The FBB protocol remained robust in its approach towards selecting individual plants in the $F_{3:5}$ progeny for superior and low performing UHML and FBS.

Further data from Table 4 indicated that the top 10% sub-subpopulation among FBB $F_{2:4}$ progeny performed better (P=.05) with an average UHML of 1.35 inches as compared to the next low-ranking performer i.e. bottom 10% sub-subpopulation with an UHML of 1.28 inches. As the correlation data suggested as well in Table 7, the GBB failed to separate the bottom and top 10% sub-subpopulations as they performed no differently with a numerical difference of .01 inches. This average mean UHML data of the GBB sub-subpopulations suggested a lack of robustness of the 11-SNP-GBB genes used in the screening for superior UHML among individual plants in F_2 progeny of the

interspecific population. The bottom 10% sub-subpopulation for FBB selections did perform the lowest among the selection groups with an UHML of 1.25 inches. The FBS values from Table 4 further jeopardized the prediction accuracy and the overall effectiveness of GBB platform with both the top and bottom 10% sub-subpopulations performing with no difference (P=.05). The bottom 10% sub-subpopulation for FBB was the lowest performing group with 34.80 g/tex FBS, however was no different (P=.05) than both the GBB sub-subpopulations.

Table 4. Average UHML and FBS of top and bottom 10% sub-subpopulations of GBB and FBB platform selections within the intraspecific and interspecific population grown at College Station in 2018 and 2019.

Intraspecific ($F_{3:5}$)			Interspecific ($F_{2:4}$)		
Sub-populations		UHML (inches)	Sub-populations		UHML (inches)
Top 10%,FBB	A	1.31	FBB,Top 10%	A	1.35
Top 10%,GBB	B	1.27	GBB,Bottom 10%	B	1.28
Bottom 10%,GBB	B	1.27	GBB,Top 10%	B	1.27
Bottom 10%,FBB	B	1.26	FBB,Bottom 10%	C	1.25

Intraspecific ($F_{3:5}$)			Interspecific ($F_{2:4}$)		
Sub-populations		FBS (g/tex)	Sub-populations		FBS (g/tex)
Top 10%,FBB	A	37.84	FBB,Top 10%	A	36.65
Top 10%,GBB	B	37.01	GBB,Bottom 10%	B	35.57
Bottom 10%,GBB	B	36.87	GBB,Top 10%	B	35.04
Bottom 10%,FBB	B	36.34	FBB,Bottom 10%	B	34.80

Subpopulations not connected by same letter are significantly different.

The means separation of sub-subpopulations suggests that FBB platform provided enough statistical power in separating the superior and lower performing lines among the interspecific and intraspecific populations. The data for the GBB sub-subpopulations suggests that more than 11-SNP based genes are certainly required to

separate the bottom and top 10% progenies in both populations for UHML and FBS. The GBB platform failed in all of the subpopulations in separating the high and low selection groups based on their similar average UHML and FBS data from the means separation analyses in Table 4.

Intraspecific Population- Means Separation Analyses- 2018 and 2019

Intraspecific – UHML

UHML values for the intraspecific population in Table 5 and 6 support the conclusions stated above from the regression and correlation discussion. Four of the 29 $F_{3:5}$ progeny in Table 5 that were predicted to be in top 10% for UHML based on the UHML of the founding F_3 IPS in 2016 did perform better (P=.05) than the TAM B182-33.

Table 5. UHML† of GBB and FBB platform selections within the intraspecific population plus TAM B-182-33 ELS grown at College Station in 2018.

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBV- 16	Top 10%	1.40
DBV- 69	Top 10%	1.40
DBV- 152	Top 10%	1.39
DBG-218	Bottom 10%	1.37
DBV- 09	Top 10%	1.37
DBG-03	Top 10%	1.36
DBV- 67	Top 10%	1.36
DBV- 40	Bottom 10%	1.36
DBG-227	Top 10%	1.36
DBG-87	Top 10%	1.36
DBV- 78	Top 10%	1.35
DBV- 105	Top 10%	1.35
DBG-95	Top 10%	1.35
DBV- 26	Bottom 10%	1.35
DBV- 35	Top 10%	1.35
DBV- 49	Top 10%	1.35
DBG-66	Top 10%	1.35
DBG-45	Top 10%	1.34
DBG-39	Top 10%	1.34
DBV- 64	Top 10%	1.34
DBG-190	Bottom 10%	1.34
DBV- 08	Top 10%	1.34
DBV- 110	Top 10%	1.34
DBG-216	Bottom 10%	1.34
DBV- 96	Bottom 10%	1.34
DBG-07	Top 10%	1.34
DBG-50	Top 10%	1.34
DBG-204	Bottom 10%	1.34
DBV- 13	Bottom 10%	1.33
DBV- 45	Top 10%	1.33
DBV- 118	Top 10%	1.33
DBG-248	Bottom 10%	1.33
DBV- 70	Bottom 10%	1.33
DBG-205	Bottom 10%	1.33

DBG-79	Top 10%	1.33
DBV- 43	Top 10%	1.33
DBG-88	Top 10%	1.33
DBG-166	Bottom 10%	1.33
DBV- 86	Top 10%	1.33
DBV- 117	Top 10%	1.32
DBG-45	Top 10%	1.32
DBV- 116	Top 10%	1.32
DBG-29	Bottom 10%	1.32
DBG-215	Bottom 10%	1.32
DBV- 33	Top 10%	1.32
DBG-49	Top 10%	1.32
DBG-02	Top 10%	1.32
DBV- 01	Bottom 10%	1.32
DBV- 79	Bottom 10%	1.32
DBV- 112	Top 10%	1.31
DBG-194	Bottom 10%	1.31
TAM B 182-33	UHML Check	1.31
DBV- 22	Top 10%	1.31
DBV- 07	Bottom 10%	1.31
DBG-78	Top 10%	1.31
DBG-201	Bottom 10%	1.31
DBG-118	Bottom 10%	1.31
DBG-117	Bottom 10%	1.31
DBG-214	Bottom 10%	1.31
DBV- 142	Top 10%	1.31
DBV- 94	Top 10%	1.31
DBG-242	Bottom 10%	1.30
DBG-64	Bottom 10%	1.30
DBG-76	Top 10%	1.30
DBV- 14	Bottom 10%	1.30
DBV- 03	Top 10%	1.30
DBG-182	Bottom 10%	1.30
DBG-61	Bottom 10%	1.30
DBG-196	Bottom 10%	1.30
DBG-89	Top 10%	1.30
DBV- 155	Bottom 10%	1.29

DBG-168	Bottom 10%	1.29
DBG-17	Top 10%	1.29
DBG-202	Bottom 10%	1.29
DBV- 143	Top 10%	1.29
DBG-23	Bottom 10%	1.29
DBV- 137	Bottom 10%	1.28
DBG-212	Bottom 10%	1.28
DBV- 104	Bottom 10%	1.28
DBG-42	Top 10%	1.28
DBG-40	Top 10%	1.28
DBV- 158	Bottom 10%	1.28
DBV- 148	Bottom 10%	1.28
DBV- 29	Top 10%	1.28
DBG-69	Top 10%	1.28
DBG-236	Bottom 10%	1.28
DBG-80	Top 10%	1.28
DBG-81	Bottom 10%	1.28
DBV- 57	Bottom 10%	1.28
DBG-203	Bottom 10%	1.28
DBG-60	Bottom 10%	1.27
DBG-241	Bottom 10%	1.27
DBV- 65	Bottom 10%	1.27
DBV- 145	Bottom 10%	1.27
DBG-36	Top 10%	1.27
DBV- 21	Bottom 10%	1.27
DBG-74	Top 10%	1.27
DBG-116	Bottom 10%	1.27
DBG-47	Top 10%	1.26
DBG-53	Bottom 10%	1.26
DBV- 156	Bottom 10%	1.26
DBG-46	Top 10%	1.26
DBG-73	Top 10%	1.26
DBV- 101	Top 10%	1.25
DBV- 30	Bottom 10%	1.25
DBV- 149	Top 10%	1.25
DBG-38	Top 10%	1.25
DBV- 62	Bottom 10%	1.25

DBV- 151	Bottom 10%	1.25
DBV- 139	Bottom 10%	1.25
DBG-170	Bottom 10%	1.25
DBG-93	Top 10%	1.25
DBV- 50	Bottom 10%	1.24
DBV- 54	Bottom 10%	1.24
DBV- 80	Bottom 10%	1.24
DBG-09	Top 10%	1.24
DBV- 133	Bottom 10%	1.23
DBG-43	Top 10%	1.23
DBG-56	Top 10%	1.22
DBG-211	Bottom 10%	1.22
DBG-183	Bottom 10%	1.22
DBV- 153	Top 10%	1.21
DBG-206	Bottom 10%	1.20
DBV- 135	Bottom 10%	1.20
DBG-92	Top 10%	1.20
DBG-158	Bottom 10%	1.19
TAM 06 WE-621	FBS Check	1.19
DBG-250	Bottom 10%	1.15
CV %		3.60
LSD (P=.05)		0.05

* UHML values are different if separated by more than the LSD value at the base of the column.
† UHML= upper half mean length; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

Table 6. UHML† of GBB and FBB platform selections within the intraspecific population plus TAM B-182-33 ELS grown at College Station in 2019.

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBV- 35	Top 10%	1.38
DBV- 96	Bottom 10%	1.37
DBV- 69	Top 10%	1.37
DBV- 08	Top 10%	1.36
DBV- 16	Top 10%	1.35
DBV- 105	Top 10%	1.35
DBV- 118	Top 10%	1.34
DBG-218	Bottom 10%	1.34
DBV- 117	Top 10%	1.34
DBV- 78	Top 10%	1.34
DBV- 67	Top 10%	1.34
DBG-88	Top 10%	1.33
DBG-95	Top 10%	1.33
DBG-227	Top 10%	1.33
DBV- 86	Top 10%	1.33
DBV- 152	Top 10%	1.33
TAM B 182-33	UHML Check	1.32
DBV- 110	Top 10%	1.32
DBG-215	Bottom 10%	1.32
DBG-73	Top 10%	1.32
DBV- 45	Top 10%	1.32
DBV- 09	Top 10%	1.32
DBV- 64	Top 10%	1.32
DBG-204	Bottom 10%	1.31
DBG-202	Bottom 10%	1.31
DBG-09	Top 10%	1.31
DBG-07	Top 10%	1.31
DBV- 40	Bottom 10%	1.31
DBV- 116	Top 10%	1.31
DBG-190	Bottom 10%	1.31
DBV- 26	Bottom 10%	1.30
DBG-248	Bottom 10%	1.30
DBV- 01	Bottom 10%	1.30
DBG-45	Top 10%	1.30
DBV- 43	Top 10%	1.30

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBV- 142	Top 10%	1.30
DBG-79	Top 10%	1.30
DBV- 13	Bottom 10%	1.29
DBV- 29	Top 10%	1.29
DBG-87	Top 10%	1.29
DBG-241	Bottom 10%	1.29
DBG-212	Bottom 10%	1.29
DBG-214	Bottom 10%	1.29
DBV- 94	Top 10%	1.29
DBV- 112	Top 10%	1.29
DBG-03	Top 10%	1.28
DBG-39	Top 10%	1.28
DBG-80	Top 10%	1.28
DBV- 70	Bottom 10%	1.28
DBG-64	Bottom 10%	1.28
DBV- 49	Top 10%	1.28
DBV- 33	Top 10%	1.28
DBG-69	Top 10%	1.28
DBV- 07	Bottom 10%	1.28
DBV- 145	Bottom 10%	1.28
DBV- 65	Bottom 10%	1.28
DBG-242	Bottom 10%	1.27
DBG-61	Bottom 10%	1.27
DBG-81	Bottom 10%	1.27
DBV- 143	Top 10%	1.27
DBV- 22	Top 10%	1.27
DBG-46	Top 10%	1.27
DBG-49	Top 10%	1.27
DBG-196	Bottom 10%	1.27
DBG-168	Bottom 10%	1.27
DBG-38	Top 10%	1.27
DBG-201	Bottom 10%	1.27
DBG-194	Bottom 10%	1.26
DBV- 03	Top 10%	1.26
DBG-182	Bottom 10%	1.26
DBG-118	Bottom 10%	1.26

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBG-117	Bottom 10%	1.26
DBG-40	Top 10%	1.26
DBV- 155	Bottom 10%	1.26
DBG-166	Bottom 10%	1.25
DBG-76	Top 10%	1.25
DBG-23	Bottom 10%	1.25
DBG-60	Bottom 10%	1.25
DBV- 79	Bottom 10%	1.25
DBV- 62	Bottom 10%	1.25
DBG-36	Top 10%	1.24
DBG-236	Bottom 10%	1.24
DBV- 101	Top 10%	1.24
DBV- 14	Bottom 10%	1.24
DBV- 158	Bottom 10%	1.24
DBG-216	Bottom 10%	1.24
DBG-203	Bottom 10%	1.24
DBV- 135	Bottom 10%	1.24
DBV- 104	Bottom 10%	1.23
DBV- 54	Bottom 10%	1.23
DBG-47	Top 10%	1.23
DBV- 21	Bottom 10%	1.23
DBV- 80	Bottom 10%	1.23
DBG-92	Top 10%	1.23
DBG-53	Bottom 10%	1.23
DBV- 57	Bottom 10%	1.23
DBG-17	Top 10%	1.23
DBG-78	Top 10%	1.23
DBG-66	Top 10%	1.23
DBG-50	Top 10%	1.22
DBG-116	Bottom 10%	1.22
DBV- 137	Bottom 10%	1.22
DBG-89	Top 10%	1.22
DBV- 30	Bottom 10%	1.22
DBG-56	Top 10%	1.22
DBG-42	Top 10%	1.22
DBG-170	Bottom 10%	1.22

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBG-29	Bottom 10%	1.21
DBV- 148	Bottom 10%	1.21
DBV- 139	Bottom 10%	1.21
DBG-183	Bottom 10%	1.21
DBG-43	Top 10%	1.21
DBV- 133	Bottom 10%	1.21
DBG-93	Top 10%	1.20
DBV- 151	Bottom 10%	1.20
DBG-250	Bottom 10%	1.20
DBV- 153	Top 10%	1.20
DBG-02	Top 10%	1.20
DBV- 149	Top 10%	1.19
DBG-211	Bottom 10%	1.18
DBV- 156	Bottom 10%	1.17
DBV- 50	Bottom 10%	1.16
DBG-206	Bottom 10%	1.15
DBG-74	Top 10%	1.14
TAM 06 WE-621	FBS Check	1.14
DBG-205	Bottom 10%	1.14
DBG-158	Bottom 10%	1.13
CV %		4.16
LSD (P=.05)		0.08

* UHML values are different if separated by more than the LSD value at the base of the column.
† UHML= upper half mean length; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

However, one out of 36 $F_{3:5}$ progeny i.e. DBG-218, predicted to be in the bottom 10% for UHML based on the 11-SNP-GBB predictions also performed better (P=.05) than TAM B182-33. Eleven of the 28 $F_{3:5}$ progeny in Table 6 that were predicted to be in the bottom 10% for UHML based on the UHML of the founding F_3 IPS were not different (P=0.05) than the TAM B182-33 extra-long staple check in 2019. Twenty three of these 36 were predicted to be in the bottom 10% by the 11 SNPs used in the GBB population exhibited UHML of no statistical difference (P=.05) with TAM B182-33's UHML. Twenty-six of the total entries exhibited shorter UHML (P=.05) than TAM B182-33 in Table 6. and 10 out of these 26 entries belong to the top 10% sub-subpopulation, where 7 were predicted by the 11-SNP-GBB and 3 were selected based on the HVI UHML of the F_3 foundational plant. Five entries in 2018, DBV-16, DBV-69, DBV-152, DBV-09 and DBG 209, outperformed (p=0.05) TAM B182-33 for UHML with values of 1.40 inches, 1.40 inches, 1.39 inches, 1.37 inches and 1.37 inches, respectively, compared to TAM B 182-33's UHML of 1.32 inches. TAM B 182-33 has been shown to perform equal to or above the UHML of 1.37 inches in previous studies (Smith et al., 2009; Bhangu et al., 2017). The top 16 genotypes in 2019 included entries from the top and bottom sub-subpopulations that exhibited numerically superior UHML but with no statistical difference with the 1.32 inches of UHML of TAM B182-33. This deviation of TAM B 182-33 from its normally expressed UHML should be an environmental effect in both years and suggest that these entries could perform even better in more conducive environments.

Table 7. Average UHML† of GBB and FBB platform selections within the intraspecific population plus TAM B-182-33 ELS grown at College Station in 2018 and 2019.

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBV- 69	Top 10%	1.38
DBV- 16	Top 10%	1.37
DBV- 35	Top 10%	1.36
DBV- 152	Top 10%	1.36
DBG-218	Bottom 10%	1.35
DBV- 08	Top 10%	1.35
DBV- 105	Top 10%	1.35
DBV- 96	Bottom 10%	1.35
DBV- 67	Top 10%	1.35
DBV- 78	Top 10%	1.35
DBG-95	Top 10%	1.34
DBV- 09	Top 10%	1.34
DBG-45	Top 10%	1.34
DBG-227	Top 10%	1.34
DBV- 118	Top 10%	1.34
DBV- 40	Bottom 10%	1.33
DBV- 117	Top 10%	1.33
DBV- 110	Top 10%	1.33
DBV- 64	Top 10%	1.33
DBG-88	Top 10%	1.33
DBV- 26	Bottom 10%	1.33
DBV- 86	Top 10%	1.33
DBG-07	Top 10%	1.32
DBG-87	Top 10%	1.32
DBG-204	Bottom 10%	1.32
DBG-190	Bottom 10%	1.32
DBV- 45	Top 10%	1.32
DBG-03	Top 10%	1.32
TAM B 182-33	UHML Check	1.32
DBG-215	Bottom 10%	1.32
DBG-248	Bottom 10%	1.32
DBV- 116	Top 10%	1.31
DBG-79	Top 10%	1.31
DBV- 13	Bottom 10%	1.31

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBV- 49	Top 10%	1.31
DBG-39	Top 10%	1.31
DBV- 43	Top 10%	1.31
DBV- 01	Bottom 10%	1.31
DBV- 70	Bottom 10%	1.31
DBG-45	Top 10%	1.30
DBV- 142	Top 10%	1.30
DBG-202	Bottom 10%	1.30
DBG-214	Bottom 10%	1.30
DBV- 94	Top 10%	1.30
DBV- 112	Top 10%	1.30
DBG-50	Top 10%	1.30
DBV- 33	Top 10%	1.30
DBV- 07	Bottom 10%	1.29
DBG-49	Top 10%	1.29
DBV- 22	Top 10%	1.29
DBG-216	Bottom 10%	1.29
DBG-166	Bottom 10%	1.29
DBG-64	Bottom 10%	1.29
DBG-194	Bottom 10%	1.29
DBG-212	Bottom 10%	1.29
DBG-201	Bottom 10%	1.29
DBG-66	Top 10%	1.29
DBG-242	Bottom 10%	1.29
DBG-118	Bottom 10%	1.28
DBV- 29	Top 10%	1.28
DBG-61	Bottom 10%	1.28
DBG-196	Bottom 10%	1.28
DBV- 03	Top 10%	1.28
DBG-241	Bottom 10%	1.28
DBG-117	Bottom 10%	1.28
DBG-168	Bottom 10%	1.28
DBV- 79	Bottom 10%	1.28
DBV- 143	Top 10%	1.28
DBG-182	Bottom 10%	1.28
DBG-80	Top 10%	1.28

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBG-73	Top 10%	1.28
DBG-69	Top 10%	1.28
DBG-09	Top 10%	1.27
DBG-76	Top 10%	1.27
DBV- 155	Bottom 10%	1.27
DBG-81	Bottom 10%	1.27
DBV- 65	Bottom 10%	1.27
DBV- 14	Bottom 10%	1.27
DBV- 145	Bottom 10%	1.27
DBG-40	Top 10%	1.27
DBG-78	Top 10%	1.27
DBG-23	Bottom 10%	1.27
DBG-205	Bottom 10%	1.27
DBG-29	Bottom 10%	1.27
DBG-46	Top 10%	1.27
DBV- 158	Bottom 10%	1.26
DBG-38	Top 10%	1.26
DBG-60	Bottom 10%	1.26
DBG-236	Bottom 10%	1.26
DBG-17	Top 10%	1.26
DBV- 104	Bottom 10%	1.26
DBG-203	Bottom 10%	1.26
DBG-02	Top 10%	1.26
DBG-89	Top 10%	1.26
DBG-36	Top 10%	1.25
DBV- 137	Bottom 10%	1.25
DBV- 57	Bottom 10%	1.25
DBV- 21	Bottom 10%	1.25
DBV- 62	Bottom 10%	1.25
DBG-42	Top 10%	1.25
DBG-47	Top 10%	1.25
DBV- 148	Bottom 10%	1.25
DBV- 101	Top 10%	1.25
DBG-53	Bottom 10%	1.24
DBG-116	Bottom 10%	1.24
DBV- 54	Bottom 10%	1.24

$F_{4:5}$ Designation	Sub-subpopulation	UHML (inches)
DBV- 30	Bottom 10%	1.23
DBV- 80	Bottom 10%	1.23
DBG-170	Bottom 10%	1.23
DBV- 139	Bottom 10%	1.23
DBV- 151	Bottom 10%	1.22
DBG-93	Top 10%	1.22
DBV- 149	Top 10%	1.22
DBG-43	Top 10%	1.22
DBG-56	Top 10%	1.22
DBV- 133	Bottom 10%	1.22
DBV- 156	Bottom 10%	1.22
DBV- 135	Bottom 10%	1.22
DBG-183	Bottom 10%	1.21
DBG-92	Top 10%	1.21
DBV- 153	Top 10%	1.21
DBG-74	Top 10%	1.20
DBV- 50	Bottom 10%	1.20
DBG-211	Bottom 10%	1.20
DBG-206	Bottom 10%	1.17
DBG-250	Bottom 10%	1.17
TAM 06 WE-621	FBS Check	1.16
DBG-158	Bottom 10%	1.16
CV%		3.58
LSD (P=.05)		0.08

* UHML values are different if separated by more than the LSD value at the base of the column.

† UHML= upper half mean length; GBB=gene based breeding; FBB=field based breeding

**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

The regression and correlation data discussed above was targeted to determine if cotton breeding programs such as the CIL at Texas A&M AgriLife Research could select only within exceptionally and unique levels of UHML and FBS. Those results were somewhat equivocal. However, the ANOVA results for UHML are much more encouraging than the R^2 values from the regression analyses in that when averaged over the two years for presentation purposes, 15 of the longest 29 $F_{3:5}$ progeny exceeding the length check were so identified as F_3 IPS in the FBB protocol and 7 of the longest 29 were so identified by the 11 SNP GBB protocol (Table 7). These data are also more encouraging relative to the 11 SNP predicted UHML in the intraspecific population in that the R^2s for for the top 10% sub-subpopulation were essentially zero (Figure 5).

Intraspecific - FBS

The ANOVA data for top performing $F_{3:5}$ progeny for FBS was not as encouraging as the data for UHML (Table 5, 6, and 7). Only 18 out of the top 10% sub-subpopulations of $F_{3:5}$ progeny that were predicted by their FBB or GBB selection methods were not different (P=.05) than TAM 06WE-621 ESU parent and check in 2018 (Table 8). Fourteen entries belonging to the bottom 10% subpopulations (Table 7) exhibited a FBS (P=.05) with no statistical difference with TAM 06WE-621 where 9 were predicted by the 11-SNP-GBB and 5 were selected based on the HVI UHML of the F3 foundational plant. The results from 2019 (Table 9) for FBS in the $F_{3:5}$ showed a greater inconsistency than 2018 in separating the entries for the top 10% and bottom

Table 8. FBS† of GBB and FBB platform selections within the intraspecific population plus TAM 06WE-621 ESU grown at College Station in 2018.

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV- 143	Top 10%	38.40
DBG-182	Bottom 10%	38.20
DBG-42	Top 10%	38.15
TAM 06 WE-621	FBS Check	37.90
DBV- 149	Top 10%	37.90
DBV- 33	Top 10%	37.10
DBG-88	Top 10%	37.00
DBG-201	Bottom 10%	36.75
DBG-38	Top 10%	36.70
DBV- 22	Top 10%	36.70
DBG-81	Bottom 10%	36.70
DBV- 50	Bottom 10%	36.65
DBG-66	Top 10%	36.50
DBG-212	Bottom 10%	36.50
DBV- 117	Top 10%	36.45
DBV- 139	Bottom 10%	36.45
DBV- 110	Top 10%	36.25
DBV- 29	Top 10%	36.20
DBV- 148	Bottom 10%	36.10
DBG-87	Top 10%	36.05
DBG-227	Top 10%	35.95
DBG-205	Bottom 10%	35.90
DBG-93	Top 10%	35.85
DBV- 79	Bottom 10%	35.85
DBV- 152	Top 10%	35.75
DBG-194	Bottom 10%	35.75
DBG-53	Bottom 10%	35.70
DBV- 105	Top 10%	35.70
DBG-170	Bottom 10%	35.70
DBG-69	Top 10%	35.65
DBG-236	Bottom 10%	35.65
DBV- 80	Bottom 10%	35.60
DBV- 03	Top 10%	35.60
DBG-211	Bottom 10%	35.55
DBG-241	Bottom 10%	35.55

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-56	Top 10%	35.50
DBG-89	Top 10%	35.35
DBG-118	Bottom 10%	35.30
DBG-74	Top 10%	35.30
DBG-50	Top 10%	35.30
DBG-166	Bottom 10%	35.25
DBV- 151	Bottom 10%	35.25
DBG-196	Bottom 10%	35.25
DBV- 94	Top 10%	35.10
DBG-47	Top 10%	35.00
DBV- 49	Top 10%	35.00
DBV- 137	Bottom 10%	34.95
DBG-76	Top 10%	34.90
DBG-206	Bottom 10%	34.90
DBG-214	Bottom 10%	34.85
DBG-204	Bottom 10%	34.75
DBV- 156	Bottom 10%	34.75
DBG-80	Top 10%	34.75
DBV- 67	Top 10%	34.75
DBV- 96	Bottom 10%	34.75
DBV- 14	Bottom 10%	34.70
DBG-45	Top 10%	34.70
DBV- 43	Top 10%	34.70
DBV- 104	Bottom 10%	34.70
DBG-73	Top 10%	34.65
DBG-116	Bottom 10%	34.60
DBV- 133	Bottom 10%	34.60
DBV- 142	Top 10%	34.55
DBG-17	Top 10%	34.55
DBG-92	Top 10%	34.55
DBV- 09	Top 10%	34.55
DBG-216	Bottom 10%	34.50
DBG-242	Bottom 10%	34.50
DBV- 112	Top 10%	34.50
DBG-39	Top 10%	34.50
DBV- 62	Bottom 10%	34.50

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-183	Bottom 10%	34.50
DBV- 101	Top 10%	34.45
DBV- 57	Bottom 10%	34.40
DBV- 153	Top 10%	34.35
DBG-190	Bottom 10%	34.35
DBG-07	Top 10%	34.35
DBG-95	Top 10%	34.35
DBV- 16	Top 10%	34.35
DBV- 21	Bottom 10%	34.35
DBV- 08	Top 10%	34.35
DBV- 07	Bottom 10%	34.35
DBG-40	Top 10%	34.30
DBG-168	Bottom 10%	34.30
DBV- 64	Top 10%	34.30
DBV- 78	Top 10%	34.30
DBG-60	Bottom 10%	34.25
DBG-36	Top 10%	34.25
DBG-29	Bottom 10%	34.25
DBV- 118	Top 10%	34.15
DBV- 35	Top 10%	34.00
DBV- 40	Bottom 10%	33.90
DBG-45	Top 10%	33.90
DBG-02	Top 10%	33.85
DBV- 86	Top 10%	33.85
DBV- 30	Bottom 10%	33.80
DBV- 45	Top 10%	33.80
DBG-03	Top 10%	33.80
DBV- 69	Top 10%	33.80
DBG-215	Bottom 10%	33.70
DBG-117	Bottom 10%	33.70
DBV- 70	Bottom 10%	33.65
DBG-78	Top 10%	33.65
DBG-61	Bottom 10%	33.60
DBG-218	Bottom 10%	33.60
DBV- 13	Bottom 10%	33.50
DBG-49	Top 10%	33.45

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-23	Bottom 10%	33.15
DBV- 26	Bottom 10%	33.15
DBG-46	Top 10%	33.05
DBG-43	Top 10%	33.05
DBG-158	Bottom 10%	32.90
DBG-79	Top 10%	32.80
DBV- 158	Bottom 10%	32.75
DBG-202	Bottom 10%	32.70
DBG-250	Bottom 10%	32.50
DBV- 65	Bottom 10%	32.45
DBV- 116	Top 10%	32.45
DBV- 01	Bottom 10%	32.40
DBG-64	Bottom 10%	32.40
DBV- 54	Bottom 10%	32.35
DBG-203	Bottom 10%	32.25
TAM B 182-33	UHML Check	32.25
DBG-248	Bottom 10%	32.10
DBG-09	Top 10%	32.10
DBV- 155	Bottom 10%	31.65
DBV- 145	Bottom 10%	31.65
DBV- 135	Bottom 10%	30.10
CV %		4.21
LSD (P=.05)		2.31

* FBS values are different if separated by more than the LSD value at the base of the column.

† FBS= fiber bundle strength; GBB=gene based breeding; FBB=field based breeding

**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

Table 9. FBS† of GBB and FBB platform selections within the intraspecific population plus TAM 06WE-621 ESU grown at College Station in 2019.

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV- 33	Top 10%	44.70
DBV- 149	Top 10%	44.30
DBV- 143	Top 10%	44.15
DBG-201	Bottom 10%	44.10
DBG-242	Bottom 10%	43.90
DBV- 29	Top 10%	43.35
DBV- 03	Top 10%	43.00
DBV- 67	Top 10%	42.65
DBG-50	Top 10%	42.60
DBV- 142	Top 10%	42.60
DBV- 139	Bottom 10%	42.30
DBG-236	Bottom 10%	42.20
DBV- 94	Top 10%	42.10
DBG-76	Top 10%	42.05
DBV- 110	Top 10%	41.95
DBG-69	Top 10%	41.90
DBV- 105	Top 10%	41.85
DBG-38	Top 10%	41.85
DBG-211	Bottom 10%	41.80
DBG-92	Top 10%	41.75
DBV- 152	Top 10%	41.75
DBV- 16	Top 10%	41.65
DBV- 22	Top 10%	41.60
DBV- 79	Bottom 10%	41.45
DBV- 40	Bottom 10%	41.30
DBG-241	Bottom 10%	41.15
TAM 06 WE-621	FBS Check	41.15
DBG-227	Top 10%	41.10
DBG-88	Top 10%	41.00
DBG-182	Bottom 10%	40.95
DBG-170	Bottom 10%	40.95
DBG-212	Bottom 10%	40.90
DBG-117	Bottom 10%	40.80
DBG-39	Top 10%	40.80
DBG-89	Top 10%	40.75

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV- 30	Bottom 10%	40.75
DBG-204	Bottom 10%	40.65
DBV- 43	Top 10%	40.65
DBG-118	Bottom 10%	40.65
DBG-02	Top 10%	40.65
DBV- 64	Top 10%	40.55
DBV- 62	Bottom 10%	40.50
DBV- 117	Top 10%	40.45
DBG-53	Bottom 10%	40.40
DBV- 21	Bottom 10%	40.25
DBV- 104	Bottom 10%	40.25
DBV- 49	Top 10%	40.20
DBG-95	Top 10%	40.20
DBG-196	Bottom 10%	40.15
DBV- 112	Top 10%	40.15
DBG-93	Top 10%	40.10
DBG-80	Top 10%	40.10
DBG-116	Bottom 10%	40.10
DBG-29	Bottom 10%	40.05
DBV- 96	Bottom 10%	40.00
DBG-42	Top 10%	39.90
DBG-03	Top 10%	39.85
DBV- 09	Top 10%	39.85
DBG-166	Bottom 10%	39.75
DBG-214	Bottom 10%	39.75
DBG-194	Bottom 10%	39.70
DBV- 57	Bottom 10%	39.65
DBV- 133	Bottom 10%	39.55
DBV- 13	Bottom 10%	39.45
DBG-60	Bottom 10%	39.30
DBV- 137	Bottom 10%	39.30
DBV- 148	Bottom 10%	39.20
DBV- 14	Bottom 10%	39.15
DBV- 145	Bottom 10%	39.10
DBG-87	Top 10%	39.10
DBV- 08	Top 10%	39.10

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-47	Top 10%	39.00
DBG-07	Top 10%	38.95
DBV- 50	Bottom 10%	38.90
DBV- 101	Top 10%	38.85
DBG-79	Top 10%	38.85
DBG-66	Top 10%	38.70
DBG-17	Top 10%	38.65
DBV- 35	Top 10%	38.60
DBV- 70	Bottom 10%	38.55
DBG-215	Bottom 10%	38.45
DBV- 153	Top 10%	38.35
DBG-206	Bottom 10%	38.30
DBG-183	Bottom 10%	38.25
DBV- 86	Top 10%	38.25
DBG-250	Bottom 10%	38.20
DBV- 156	Bottom 10%	38.15
DBG-36	Top 10%	38.10
DBV- 80	Bottom 10%	38.10
DBV- 69	Top 10%	37.95
DBG-61	Bottom 10%	37.95
DBG-45	Top 10%	37.90
DBG-74	Top 10%	37.90
DBG-23	Bottom 10%	37.90
DBG-09	Top 10%	37.85
DBG-56	Top 10%	37.80
DBG-218	Bottom 10%	37.80
DBV- 45	Top 10%	37.70
DBV- 151	Bottom 10%	37.70
DBG-49	Top 10%	37.55
DBG-40	Top 10%	37.55
DBG-43	Top 10%	37.50
DBG-203	Bottom 10%	37.45
DBG-216	Bottom 10%	37.45
DBV- 158	Bottom 10%	37.35
DBV- 118	Top 10%	37.30
DBV- 116	Top 10%	37.20

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-81	Bottom 10%	37.20
DBG-205	Bottom 10%	37.20
DBG-168	Bottom 10%	37.05
DBG-190	Bottom 10%	37.00
DBV- 26	Bottom 10%	36.75
DBV- 65	Bottom 10%	36.75
DBV- 78	Top 10%	36.75
DBG-46	Top 10%	36.75
DBG-78	Top 10%	36.65
DBG-73	Top 10%	36.50
DBV- 07	Bottom 10%	36.40
DBG-158	Bottom 10%	36.10
DBG-202	Bottom 10%	35.90
DBV- 54	Bottom 10%	35.90
DBG-64	Bottom 10%	35.40
TAM B 182-33	UHML Check	35.35
DBV- 01	Bottom 10%	35.25
DBV- 135	Bottom 10%	34.95
DBV- 155	Bottom 10%	34.55
DBG-248	Bottom 10%	34.00
CV %		5.60
LSD		3.73

* FBS values are different if separated by more than the LSD value at the base of the column.
† FBS= fiber bundle strength; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

10% sub-subpopulation based on the FBB and GBB selection methods. One-hundred three entries exhibited a FBS with no difference (P=.05) in comparison to TAM 06WE-621 ESU, out of which 48 entries were predicted to be among the bottom 10% sub-subpopulation for FBB or GBB. Table 8 and 9 showed a similar pattern among the lower performing entries where more genotypes, that were predicted to be in the bottom 10% sub-subpopulations for either FBB or GBB selection protocol, exhibited a shorter FBS value in comparison the numerically superior FBS values of the top performing genotypes.

For presentation purposes, the combined data for both years show only 19 of the 29 top performing $F_{3:5}$ progeny were predicted by their F_3 FBB or GBB selection methods (Table 10). None were stronger (p=0.05) than the TAM 06WE-621 ESU parent and check. Of the strongest 29, two were predicted by their F_3 HVI performance to be in the lowest 10% sub-subpopulation and eight were predicted by the 11 SNPs used in the GBB platform to be in the lowest FBS sub-subpopulation. Eleven entries belonging to the F3:5 pogeny that were predicted to be in the top 10% sub-subpopulations of and FBB and 11-SNP-GBB selections were found to exhibit the least FBS in the trial over two years. Further disturbing data from Table 10 is that only 13 of the strongest $F_{3:5}$ progeny were so predicted by the FBS of their foundational F_3 IPS within the FBB protocol. DBV-143 and six other entries numerically outperformed but were not different (p=0.05) than TAM 06 WE-621ESU with a difference of only1.8 g/tex. No significant differences were observed in the top 29 $F_{3:5}$ progeny genotypes.

Table 10. Average FBS† of GBB and FBB platform selections within the intraspecific population plus TAM 06WE-621 ESU grown at College Station in 2018 and 2019.

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV- 143	Top 10%	41.28
DBV- 149	Top 10%	41.10
DBV- 33	Top 10%	40.90
DBG-201	Bottom 10%	40.43
DBV- 29	Top 10%	39.78
DBG-182	Bottom 10%	39.58
TAM 06 WE-621	FBS Check	39.53
DBV- 139	Bottom 10%	39.38
DBV- 03	Top 10%	39.30
DBG-38	Top 10%	39.28
DBG-242	Bottom 10%	39.20
DBV- 22	Top 10%	39.15
DBV- 110	Top 10%	39.10
DBG-42	Top 10%	39.03
DBG-236	Bottom 10%	38.93
DBG-69	Top 10%	38.78
DBV- 105	Top 10%	38.78
DBV- 152	Top 10%	38.75
DBV- 67	Top 10%	38.70
DBG-212	Bottom 10%	38.70
DBG-211	Bottom 10%	38.68
DBV- 79	Bottom 10%	38.65
DBV- 94	Top 10%	38.60
DBV- 142	Top 10%	38.58
DBG-227	Top 10%	38.53
DBG-76	Top 10%	38.48
DBV- 117	Top 10%	38.45
DBG-241	Bottom 10%	38.35
DBG-88	Top 10%	38.33
DBG-170	Bottom 10%	38.33
DBG-92	Top 10%	38.15
DBG-53	Bottom 10%	38.05
DBG-89	Top 10%	38.05
DBV- 16	Top 10%	38.00

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-93	Top 10%	37.98
DBG-118	Bottom 10%	37.98
DBV- 50	Bottom 10%	37.78
DBG-50	Top 10%	37.73
DBG-194	Bottom 10%	37.73
DBG-204	Bottom 10%	37.70
DBG-196	Bottom 10%	37.70
DBV- 43	Top 10%	37.68
DBV- 148	Bottom 10%	37.65
DBG-39	Top 10%	37.65
DBG-66	Top 10%	37.60
DBV- 49	Top 10%	37.60
DBV- 40	Bottom 10%	37.60
DBG-87	Top 10%	37.58
DBG-166	Bottom 10%	37.50
DBV- 62	Bottom 10%	37.50
DBV- 104	Bottom 10%	37.48
DBG-80	Top 10%	37.43
DBV- 64	Top 10%	37.43
DBV- 96	Bottom 10%	37.38
DBG-116	Bottom 10%	37.35
DBV- 112	Top 10%	37.33
DBV- 21	Bottom 10%	37.30
DBG-214	Bottom 10%	37.30
DBV- 30	Bottom 10%	37.28
DBG-95	Top 10%	37.28
DBG-117	Bottom 10%	37.25
DBG-02	Top 10%	37.25
DBV- 09	Top 10%	37.20
DBG-29	Bottom 10%	37.15
DBV- 137	Bottom 10%	37.13
DBV- 133	Bottom 10%	37.08
DBV- 57	Bottom 10%	37.03
DBG-47	Top 10%	37.00
DBG-81	Bottom 10%	36.95
DBV- 14	Bottom 10%	36.93

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV- 80	Bottom 10%	36.85
DBG-45	Top 10%	36.83
DBG-03	Top 10%	36.83
DBG-60	Bottom 10%	36.78
DBV- 08	Top 10%	36.73
DBV- 101	Top 10%	36.65
DBG-07	Top 10%	36.65
DBG-56	Top 10%	36.65
DBG-17	Top 10%	36.60
DBG-74	Top 10%	36.60
DBG-206	Bottom 10%	36.60
DBV- 151	Bottom 10%	36.48
DBV- 13	Bottom 10%	36.48
DBV- 156	Bottom 10%	36.45
DBG-183	Bottom 10%	36.38
DBV- 153	Top 10%	36.35
DBG-205	Bottom 10%	36.33
DBV- 35	Top 10%	36.30
DBG-36	Top 10%	36.18
DBV- 70	Bottom 10%	36.10
DBG-215	Bottom 10%	36.08
DBV- 86	Top 10%	36.05
DBG-216	Bottom 10%	35.98
DBG-40	Top 10%	35.93
DBV- 69	Top 10%	35.88
DBG-79	Top 10%	35.83
DBG-61	Bottom 10%	35.78
DBV- 45	Top 10%	35.75
DBV- 118	Top 10%	35.73
DBG-218	Bottom 10%	35.70
DBG-168	Bottom 10%	35.68
DBG-190	Bottom 10%	35.68
DBG-23	Bottom 10%	35.53
DBV- 78	Top 10%	35.53
DBG-49	Top 10%	35.50
DBV- 145	Bottom 10%	35.38

$F_{4:5}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV- 07	Bottom 10%	35.38
DBG-250	Bottom 10%	35.35
DBG-43	Top 10%	35.28
DBG-73	Top 10%	35.27
DBG-78	Top 10%	35.15
DBV- 158	Bottom 10%	35.05
DBG-09	Top 10%	34.98
DBV- 26	Bottom 10%	34.95
DBG-46	Top 10%	34.90
DBG-203	Bottom 10%	34.85
DBV- 116	Top 10%	34.83
DBV- 65	Bottom 10%	34.60
DBG-158	Bottom 10%	34.50
DBG-202	Bottom 10%	34.30
DBV- 54	Bottom 10%	34.13
DBG-45	Top 10%	33.90
DBG-64	Bottom 10%	33.90
DBV- 01	Bottom 10%	33.83
TAM B 182-33	UHML Check	33.80
DBV- 155	Bottom 10%	33.10
DBG-248	Bottom 10%	33.05
DBV- 135	Bottom 10%	32.53
CV%		4.56
LSD (P=.05)		6.27

* FBS values are different if separated by more than the LSD value at the base of the column.

† FBS= fiber bundle strength; GBB=gene based breeding; FBB=field based breeding

**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

The ANOVA data from the intraspecific population were encouraging, especially for UHML. This would be expected since the UHML F_3 foundational plants were visually and HVI selected for length but were essentially random for FBS. While the regression and correlation data suggested, or was equivocal at best, that breeders can't effectively select within such populations as the intraspecific population used in this study, the ANOVA suggest that breeders could and should be successful using the FBB, especially for UHML, and would encounter some success using either FBB or GBB for FBS.

Interspecific Population-Means Separation Analyses- 2018 and 2019.

Interspecific - UHML

ANOVA results of the interspecific trial showed a lack of significance for the genotype by year effect suggesting that expression of UHML was consistent for both years. Ten of the 32 $F_{2:4}$ progeny in Table 11 that were predicted to be in top 10% sub-subpopulation for UHML based on the FBB selection protocol of the founding F_2 IPS in 2016 did outperform (P=.05) the TAM B182-33 in 2018. Two other entries outperformed TAM B182-33 with one genotype from the top 10% sub-subpopulation and another from the bottom 10% of GBB selection protocol. Among the entries in 2018 that exhibited UHML inferior to TAM B182-33 (P=.05), the FBB selections were better predicted for longer UHML than 11-SNP-GBB selections for the top 10% sub-subpopulation since 2 out of top 10% sub-subpopulation selections belonged to FBB compared to the 12 of 11-SNP-GBB model. Seven of the 32 $F_{2:4}$ progeny and one from

the 31 $F_{2:4}$ progeny (Table 12) that were predicted to be in the top 10% sub-subpopulation for UHML based on FBB and 11 SNP-GBB, respectively, were different (P=0.05) from the of TAM B182-33 extra-long staple check in 2019. Twenty-nine of the 52 $F_{2:4}$ progeny were predicted to be in the bottom 10% by the 11 SNPs used in the GBB and HVI UHML in FBB populations exhibited UHML of no difference (P=.05) with TAM B182-33's UHML of 1.31 inches (Table 12). Forty entries exhibited shorter UHML (P=.05) than TAM B182-33 in Table 11, and 23 out of these 40 entries belonged to the bottom 10% sub-subpopulations and 13 to the top 10% sub-subpopulations for FBB and GBB protocol. Two entries, DBV-11 in 2018 and DBV-26 in 2019 outperformed (p=0.05) TAM B182-33 for UHML with values of 1.44 inches and 1.48 inches, respectively. Tables 5 and 6 for the $F_{3:5}$ progeny and tables 11 and 12 for the $F_{2:4}$ progeny have shown a deviation for TAM B182-33 from its expressed UHML of 1.37 inches which was shown in the previous literature.

Table 11. UHML† of GBB and FBB platform selections within the interspecific population plus TAM B-182-33 ELS grown at College Station in 2018.

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-111	Top 10%	1.44
DBV-103	Top 10%	1.42
DBG-99	Top 10%	1.41
DBV-130	Top 10%	1.41
DBV-43	Top 10%	1.40
DBV-87	Top 10%	1.40
DBV-71	Top 10%	1.39
DBV-26	Top 10%	1.38
DBV-108	Top 10%	1.38
DBV-40	Top 10%	1.38
DBG-44	Bottom 10%	1.37
DBV-42	Top 10%	1.37
DBV-05	Top 10%	1.36
DBG-22	Bottom 10%	1.36
DBG-93	Top 10%	1.36
DBV-02	Top 10%	1.36
DBV-57	Top 10%	1.35
DBG-94	Top 10%	1.35
DBG-126	Top 10%	1.35
DBV-39	Bottom 10%	1.35
DBV-127	Top 10%	1.34
DBV-120	Top 10%	1.34
DBV-12	Top 10%	1.33
DBV-90	Bottom 10%	1.33
DBV-44	Top 10%	1.33
DBG-105	Bottom 10%	1.33
DBV-23	Top 10%	1.33
DBG-102	Top 10%	1.33
DBG-113	Top 10%	1.33
DBG-185	Bottom 10%	1.33
DBG-41	Bottom 10%	1.33
DBV-153	Bottom 10%	1.32
DBV-97	Top 10%	1.32
DBV-105	Top 10%	1.32
DBG-17	Bottom 10%	1.32

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-109	Bottom 10%	1.32
DBV-22	Top 10%	1.32
DBV-49	Top 10%	1.31
DBG-129	Top 10%	1.31
DBG-117	Top 10%	1.31
DBG-39	Bottom 10%	1.31
DBG-13	Bottom 10%	1.31
DBV-31	Top 10%	1.31
TAM B 182-33	UHML Check	1.30
DBV-126	Top 10%	1.30
DBG-46	Bottom 10%	1.30
DBG-08	Bottom 10%	1.30
DBV-20	Top 10%	1.30
DBG-136	Top 10%	1.29
DBG-101	Top 10%	1.29
DBG-63	Bottom 10%	1.29
DBV-77	Top 10%	1.29
DBV-24	Bottom 10%	1.29
DBV-78	Top 10%	1.29
DBG-211	Bottom 10%	1.29
DBG-118	Top 10%	1.28
DBG-144	Top 10%	1.28
DBG-61	Bottom 10%	1.28
DBG-200	Bottom 10%	1.28
DBG-222	Top 10%	1.28
DBV-148	Top 10%	1.28
DBV-154	Bottom 10%	1.28
DBG-15	Bottom 10%	1.28
DBV-66	Top 10%	1.28
DBG-40	Bottom 10%	1.27
DBV-151	Bottom 10%	1.27
DBV-48	Bottom 10%	1.27
DBG-122	Top 10%	1.27
DBG-07	Bottom 10%	1.27
DBG-124	Top 10%	1.27
DBV-155	Top 10%	1.26

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-34	Bottom 10%	1.26
DBG-137	Top 10%	1.26
DBG-163	Bottom 10%	1.26
DBV-75	Bottom 10%	1.25
DBG-116	Top 10%	1.25
DBG-75	Bottom 10%	1.25
DBG-20	Bottom 10%	1.25
DBV-76	Bottom 10%	1.24
DBV-29	Bottom 10%	1.24
DBG-223	Top 10%	1.24
DBG-127	Top 10%	1.24
DBG-96	Top 10%	1.24
DBG-95	Top 10%	1.24
DBV-88	Bottom 10%	1.23
DBG-134	Top 10%	1.23
DBG-62	Bottom 10%	1.23
DBG-98	Top 10%	1.23
DBG-138	Top 10%	1.23
DBV-95	Bottom 10%	1.23
DBG-48	Bottom 10%	1.23
DBG-106	Top 10%	1.22
DBV-27	Top 10%	1.22
DBG-52	Bottom 10%	1.22
DBG-135	Top 10%	1.22
DBV-102	Top 10%	1.21
DBG-165	Top 10%	1.21
DBV-45	Bottom 10%	1.21
DBV-17	Bottom 10%	1.21
DBG-186	Bottom 10%	1.20
DBG-111	Top 10%	1.20
DBV-11	Bottom 10%	1.20
DBV-106	Bottom 10%	1.20
DBG-115	Top 10%	1.20
DBV-149	Bottom 10%	1.19
DBG-54	Bottom 10%	1.19
DBV-121	Bottom 10%	1.19

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-104	Bottom 10%	1.19
DBV-92	Bottom 10%	1.19
DBG-104	Top 10%	1.18
TAM 06 WE-621	FBS Check	1.18
DBG-131	Top 10%	1.18
DBV-128	Bottom 10%	1.17
DBV-150	Bottom 10%	1.15
DBV-137	Bottom 10%	1.15
DBG-192	Bottom 10%	1.14
DBG-18	Bottom 10%	1.14
CV %		5.21
LSD (P=.05)		0.067

* UHML values are different if separated by more than the LSD value at the base of the column.
† UHML= upper half mean length; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

Table 12. UHML† of GBB and FBB platform selections within the interspecific population plus TAM B-182-33 ELS grown at College Station in 2019.

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-26	Top 10%	1.48
DBV-127	Top 10%	1.46
DBV-43	Top 10%	1.45
DBV-40	Top 10%	1.44
DBV-130	Top 10%	1.43
DBV-31	Top 10%	1.42
DBV-103	Top 10%	1.42
DBG-99	Top 10%	1.42
DBG-22	Bottom 10%	1.41
DBG-44	Bottom 10%	1.41
DBV-126	Top 10%	1.41
DBV-111	Top 10%	1.41
DBV-71	Top 10%	1.41
DBV-87	Top 10%	1.40
DBG-93	Top 10%	1.39
DBV-39	Bottom 10%	1.38
DBV-02	Top 10%	1.38
DBV-42	Top 10%	1.38
DBG-41	Bottom 10%	1.37
DBV-105	Top 10%	1.37
DBV-97	Top 10%	1.37
DBV-90	Bottom 10%	1.37
DBG-185	Bottom 10%	1.37
DBV-148	Top 10%	1.36
DBV-12	Top 10%	1.36
DBV-44	Top 10%	1.36
DBG-08	Bottom 10%	1.36
DBG-102	Top 10%	1.35
DBV-57	Top 10%	1.35
DBG-94	Top 10%	1.35
DBV-153	Bottom 10%	1.35
DBG-113	Top 10%	1.34
DBV-23	Top 10%	1.34
DBV-108	Top 10%	1.34
DBG-13	Bottom 10%	1.34

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
TAM B 182-33	UHML Check	1.34
DBV-05	Top 10%	1.33
DBV-20	Top 10%	1.33
DBV-155	Top 10%	1.33
DBV-78	Top 10%	1.33
DBV-77	Top 10%	1.33
DBV-49	Top 10%	1.33
DBG-17	Bottom 10%	1.33
DBV-109	Bottom 10%	1.33
DBG-39	Bottom 10%	1.33
DBG-144	Top 10%	1.32
DBG-129	Top 10%	1.32
DBV-106	Bottom 10%	1.32
DBV-34	Bottom 10%	1.32
DBG-63	Bottom 10%	1.32
DBG-118	Top 10%	1.31
DBG-122	Top 10%	1.31
DBG-136	Top 10%	1.31
DBG-46	Bottom 10%	1.31
DBG-40	Bottom 10%	1.31
DBG-200	Bottom 10%	1.31
DBG-07	Bottom 10%	1.31
DBG-101	Top 10%	1.30
DBV-22	Top 10%	1.30
DBV-66	Top 10%	1.30
DBG-15	Bottom 10%	1.30
DBG-105	Bottom 10%	1.30
DBG-211	Bottom 10%	1.29
DBG-124	Top 10%	1.29
DBG-137	Top 10%	1.29
DBG-61	Bottom 10%	1.29
DBV-29	Bottom 10%	1.28
DBG-126	Top 10%	1.28
DBV-151	Bottom 10%	1.28
DBV-88	Bottom 10%	1.28
DBG-54	Bottom 10%	1.27

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBG-134	Top 10%	1.27
DBG-98	Top 10%	1.27
DBG-135	Top 10%	1.27
DBV-75	Bottom 10%	1.26
DBV-120	Top 10%	1.26
DBG-75	Bottom 10%	1.26
DBG-48	Bottom 10%	1.26
DBG-96	Top 10%	1.26
DBV-104	Bottom 10%	1.26
DBG-117	Top 10%	1.25
DBG-222	Top 10%	1.25
DBV-154	Bottom 10%	1.25
DBG-95	Top 10%	1.25
DBV-11	Bottom 10%	1.25
DBG-116	Top 10%	1.24
DBV-27	Top 10%	1.24
DBV-95	Bottom 10%	1.24
DBG-138	Top 10%	1.24
DBG-106	Top 10%	1.23
DBG-127	Top 10%	1.23
DBV-76	Bottom 10%	1.23
DBG-62	Bottom 10%	1.23
DBV-128	Bottom 10%	1.23
DBG-186	Bottom 10%	1.23
DBV-45	Bottom 10%	1.22
DBV-48	Bottom 10%	1.22
DBG-20	Bottom 10%	1.22
DBG-223	Top 10%	1.22
TAM 06 WE-621	FBS Check	1.22
DBG-131	Top 10%	1.22
DBG-115	Top 10%	1.22
DBG-165	Top 10%	1.22
DBG-163	Bottom 10%	1.21
DBG-18	Bottom 10%	1.21
DBV-121	Bottom 10%	1.21
DBV-150	Bottom 10%	1.21

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-92	Bottom 10%	1.20
DBV-24	Bottom 10%	1.20
DBG-192	Bottom 10%	1.19
DBV-17	Bottom 10%	1.19
DBG-52	Bottom 10%	1.19
DBG-104	Top 10%	1.19
DBV-137	Bottom 10%	1.19
DBV-149	Bottom 10%	1.18
DBG-111	Top 10%	1.17
DBV-102	Top 10%	1.16
CV %		5.65
LSD (P=.05)		2.63

* UHML values are different if separated by more than the LSD value at the base of the column.
† UHML= upper half mean length; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

UHML of the 29 longest $F_{2:4}$ progeny, regardless of their sub-subpopulation selection origin within the interspecific population, are shown in Table 13 as averaged across years for presentation and discussion purposes. The origin of these progenies were similar to that reported above for the intraspecific $F_{3:5}$ progeny in that 23 of the 29, excluding the TAM B182-33 ELSU control, were predicted to have long UHML, 20 from the FBB protocol and three by the GBB protocol using 11 SNPs. Two progenies within this group were predicted to have shorter UHML based on the FBB protocol and

four were predicted to have shorter UHML based on the GBB protocol. This distribution of GBB versus FBB is a little surprising because the R^2 reported in Figures 7 and 11 and Table 1 were comparable in three of the four sub-subpopulations for the top 10% category; R^2 for the Top 10 % for GBB UHML was 0.34 while the others ranged from 0.49 to 0.56. These data do not support the conclusion from the regression analyses that the 11 SNPs used in GBB herein may be as good as FBB protocols even within the interspecific population.

Table 13. Mean values for UHML† of GBB and FBB selections, including top 10% and bottom 10% in the interspecific population and a UHML check, TAM B-182-33, grown at College Station in 2018 and 2019.

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-26	Top 10%	1.43
DBV-43	Top 10%	1.43
DBV-111	Top 10%	1.42
DBV-103	Top 10%	1.42
DBV-130	Top 10%	1.42
DBG-99	Top 10%	1.41
DBV-40	Top 10%	1.41
DBV-127	Top 10%	1.40
DBV-87	Top 10%	1.40
DBV-71	Top 10%	1.40
DBG-44	Bottom 10%	1.39
DBG-22	Bottom 10%	1.39
DBV-42	Top 10%	1.38
DBG-93	Top 10%	1.38
DBV-02	Top 10%	1.37
DBV-39	Bottom 10%	1.36
DBV-31	Top 10%	1.36
DBV-108	Top 10%	1.36
DBV-126	Top 10%	1.36
DBV-44	Top 10%	1.35
DBV-57	Top 10%	1.35
DBV-90	Bottom 10%	1.35
DBG-41	Bottom 10%	1.35
DBG-94	Top 10%	1.35
DBV-12	Top 10%	1.35
DBG-185	Bottom 10%	1.35
DBV-05	Top 10%	1.35
DBV-105	Top 10%	1.34
DBV-97	Top 10%	1.34
DBG-102	Top 10%	1.34
DBV-23	Top 10%	1.34
DBV-153	Bottom 10%	1.33
DBG-113	Top 10%	1.33
DBG-08	Bottom 10%	1.33

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBG-13	Bottom 10%	1.32
DBV-49	Top 10%	1.32
DBG-17	Bottom 10%	1.32
DBV-109	Bottom 10%	1.32
DBV-148	Top 10%	1.32
DBG-39	Bottom 10%	1.32
TAM B 182-33	UHML Check	1.32
DBG-129	Top 10%	1.32
DBG-105	Bottom 10%	1.31
DBG-126	Top 10%	1.31
DBV-20	Top 10%	1.31
DBV-78	Top 10%	1.31
DBV-77	Top 10%	1.31
DBV-22	Top 10%	1.31
DBG-46	Bottom 10%	1.30
DBG-63	Bottom 10%	1.30
DBG-136	Top 10%	1.30
DBG-144	Top 10%	1.30
DBV-120	Top 10%	1.30
DBG-118	Top 10%	1.30
DBV-155	Top 10%	1.30
DBG-101	Top 10%	1.29
DBG-200	Bottom 10%	1.29
DBG-122	Top 10%	1.29
DBG-211	Bottom 10%	1.29
DBV-34	Bottom 10%	1.29
DBG-40	Bottom 10%	1.29
DBV-66	Top 10%	1.29
DBG-07	Bottom 10%	1.29
DBG-15	Bottom 10%	1.29
DBG-61	Bottom 10%	1.28
DBG-117	Top 10%	1.28
DBG-124	Top 10%	1.28
DBV-151	Bottom 10%	1.27
DBG-137	Top 10%	1.27
DBG-222	Top 10%	1.27

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-154	Bottom 10%	1.27
DBV-29	Bottom 10%	1.26
DBV-75	Bottom 10%	1.26
DBV-106	Bottom 10%	1.26
DBV-88	Bottom 10%	1.25
DBG-98	Top 10%	1.25
DBG-75	Bottom 10%	1.25
DBG-134	Top 10%	1.25
DBG-96	Top 10%	1.25
DBG-116	Top 10%	1.24
DBG-95	Top 10%	1.24
DBV-48	Bottom 10%	1.24
DBV-24	Bottom 10%	1.24
DBG-48	Bottom 10%	1.24
DBG-135	Top 10%	1.24
DBV-76	Bottom 10%	1.24
DBG-20	Bottom 10%	1.23
DBG-127	Top 10%	1.23
DBG-163	Bottom 10%	1.23
DBG-138	Top 10%	1.23
DBV-95	Bottom 10%	1.23
DBG-54	Bottom 10%	1.23
DBG-223	Top 10%	1.23
DBV-27	Top 10%	1.23
DBG-62	Bottom 10%	1.23
DBG-106	Top 10%	1.23
DBV-104	Bottom 10%	1.22
DBV-11	Bottom 10%	1.22
DBV-45	Bottom 10%	1.21
DBG-186	Bottom 10%	1.21
DBG-165	Top 10%	1.21
DBG-115	Top 10%	1.21
DBG-52	Bottom 10%	1.20
DBV-17	Bottom 10%	1.20
TAM 06 WE-621	FBS Check	1.20
DBV-121	Bottom 10%	1.20

$F_{3:4}$ Designation	Sub-subpopulation	UHML (inches)
DBV-128	Bottom 10%	1.20
DBG-131	Top 10%	1.20
DBV-92	Bottom 10%	1.19
DBV-102	Top 10%	1.19
DBG-111	Top 10%	1.18
DBG-104	Top 10%	1.18
DBV-149	Bottom 10%	1.18
DBV-150	Bottom 10%	1.18
DBG-18	Bottom 10%	1.18
DBG-192	Bottom 10%	1.17
DBV-137	Bottom 10%	1.17
CV%		5.24
LSD (P=.05)		0.08

* UHML values are different if separated by more than the LSD value at the base of the column.
† UHML= upper half mean length; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

DBV-26 outperformed numerically and responded with a higher (p=0.05) UHML of 1.43 inches compared with TAM B 182-33's UHML of 1.32 inches. However, literature has shown genotypes derived from an interspecific population consists of higher UHML in comparison to intraspecific lines. Like the intraspecific population, TAM B 182-33 performed below expectations as a check since it has shown to perform equal to or above the UHML of 1.37 inches. The top 13 genotypes shown in Table 13, including DBV-26, were longer (p=0.05) in UHML than TAM B182-33. No differences were observed among the top percent and bottom percent for FBB and GBB progeny that were included in the best 29 progenies for UHML as well. DBG-44 (Bottom 10%) and DBG-99 (Top 10%) were not different (p=0.05) than the highest UHML exhibited by DBV-26. In numerical terms in Table 13, some of the entries exhibiting the lowest UHML over the two year trials included $F_{2:4}$ progeny from the top 10% sub-populations of FBB and 11-SNP-GBB platform.

Interspecific - FBS

The ANOVA data for the $F_{3:5}$ progeny for FBS indicates a significant interaction between the genotypes and year, which suggested a breakdown of the means separation analyses on yearly basis before presenting the means table on an average basis. Only 5 entries out of the top 10% sub-subpopulations of $F_{2:4}$ progeny that were predicted by the FBB selection method were different (P=.05) than the 34.8 g/tex of TAM 06WE-621 ESU parent and check in 2018 (Table 14). However, one entry from the bottom 10% sub-subpopulation of the GBB selection protocol did outperform TAM 06WE-621 ESU. Forty entries belonging to bottom 10% subpopulations (Table 14) exhibited a FBS (P=.05) with no statistical difference with TAM 06WE-621 where 22 were predicted by the 11-SNP-GBB and 19 were selected based on the HVI UHML of the F2 foundational plant. The results from 2019 (Table 15) for FBS in the $F_{2:4}$ showed a greater FBS value for TAM 06WE-621 ESU i.e. 41.85, a better representation of its true expression of FBS. The top five numerically superior entries belonging to the top 10 % sub-subpopulation for FBB prediction model and both sub-subpopulations of GBB exhibited a FBS not different than (P=.05) TAM 06WE-621 ESU. Table 15 results showed 2 out of 25 $F_{2:4}$ progenies, that were predicted to be in the bottom 10% sub-subpopulation for FBS based on the GBB of the founding F_2 IPS were among the strongest entries and were not different (P=0.05) than the TAM B182-33. The lower performing entries where more genotypes, that were predicted to be in the bottom 10% sub-subpopulations for either FBB or GBB selection protocol, exhibited a smaller FBS value in comparison

the numerically superior FBS values of the top performing genotypes. However, the lower performing entries did include selections from the top 10% sub-subpopulation.

The FBS means for the 29 best performing $F_{2:4}$ interspecific progeny were not as encouraging as the means data for interspecific UHML and the distribution was similar to that reported for the intraspecific progeny in Table 13 (Table 16). Only 19 of the top 29 performing $F_{2:4}$ interspecific progeny were predicted by their F_2 FBB or GBB selection

Table 14. FBS† of GBB and FBB platform selections within the interspecific population plus TAM 06WE-621 ESU grown at College Station in 2018.

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV-49	Top 10%	38.85
DBV-23	Top 10%	38.80
DBV-22	Top 10%	38.40
DBV-126	Top 10%	37.90
DBG-17	Bottom 10%	37.75
DBV-102	Top 10%	37.60
DBV-42	Top 10%	37.10
DBG-94	Top 10%	37.05
DBV-44	Top 10%	36.60
DBV-154	Bottom 10%	36.40
DBG-08	Bottom 10%	36.35
DBV-105	Top 10%	36.15
DBG-44	Bottom 10%	36.00
DBV-97	Top 10%	35.85
DBV-31	Top 10%	35.80
DBV-66	Top 10%	35.80
DBV-20	Top 10%	35.45
DBV-12	Top 10%	35.35
DBV-87	Top 10%	35.20
DBV-02	Top 10%	35.20
DBG-48	Bottom 10%	35.15

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV-95	Bottom 10%	35.15
DBG-61	Bottom 10%	35.10
DBG-163	Bottom 10%	35.10
DBG-40	Bottom 10%	35.05
DBG-62	Bottom 10%	35.00
DBV-76	Bottom 10%	35.00
DBG-126	Top 10%	34.95
DBG-41	Bottom 10%	34.90
DBV-77	Top 10%	34.90
DBG-138	Top 10%	34.90
DBG-117	Top 10%	34.90
DBV-150	Bottom 10%	34.90
DBV-43	Top 10%	34.85
TAM 06 WE-621	FBS Check	34.80
DBG-116	Top 10%	34.75
DBV-90	Bottom 10%	34.60
DBV-148	Top 10%	34.55
DBG-18	Bottom 10%	34.55
DBG-185	Bottom 10%	34.50
DBG-75	Bottom 10%	34.45
DBG-105	Bottom 10%	34.35
DBV-103	Top 10%	34.35
DBV-40	Top 10%	34.30
DBV-17	Bottom 10%	34.30
DBG-122	Top 10%	34.30
DBV-108	Top 10%	34.25
DBG-54	Bottom 10%	34.15
DBV-120	Top 10%	34.15
DBV-104	Bottom 10%	34.05
DBG-95	Top 10%	34.05
DBV-71	Top 10%	33.95
DBG-135	Top 10%	33.95
DBV-106	Bottom 10%	33.90
DBG-20	Bottom 10%	33.80
DBG-222	Top 10%	33.75
DBV-155	Top 10%	33.75

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-144	Top 10%	33.75
DBG-111	Top 10%	33.70
DBG-211	Bottom 10%	33.70
DBG-99	Top 10%	33.70
DBV-48	Bottom 10%	33.60
DBV-130	Top 10%	33.45
DBG-118	Top 10%	33.40
DBG-15	Bottom 10%	33.35
DBG-22	Bottom 10%	33.35
DBV-26	Top 10%	33.25
DBV-151	Bottom 10%	33.25
DBV-05	Top 10%	33.25
DBV-137	Bottom 10%	33.15
DBG-137	Top 10%	33.15
DBG-223	Top 10%	33.15
DBV-24	Bottom 10%	33.10
DBV-128	Bottom 10%	33.10
DBG-106	Top 10%	33.05
DBG-113	Top 10%	33.05
DBV-29	Bottom 10%	33.00
DBG-129	Top 10%	32.85
DBG-115	Top 10%	32.85
DBV-39	Bottom 10%	32.75
DBG-46	Bottom 10%	32.75
DBG-136	Top 10%	32.75
DBV-149	Bottom 10%	32.70
DBG-93	Top 10%	32.65
DBG-63	Bottom 10%	32.65
DBV-57	Top 10%	32.65
DBG-96	Top 10%	32.60
DBG-200	Bottom 10%	32.60
DBG-192	Bottom 10%	32.60
DBV-111	Top 10%	32.60
DBV-78	Top 10%	32.55
DBV-127	Top 10%	32.50
DBG-101	Top 10%	32.30

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV-34	Bottom 10%	32.25
DBV-121	Bottom 10%	32.20
DBG-165	Top 10%	32.15
DBV-109	Bottom 10%	32.05
DBG-98	Top 10%	32.05
DBG-07	Bottom 10%	32.00
DBG-13	Bottom 10%	31.95
DBG-134	Top 10%	31.80
DBV-27	Top 10%	31.75
DBG-52	Bottom 10%	31.75
DBG-102	Top 10%	31.70
DBG-127	Top 10%	31.60
DBV-153	Bottom 10%	31.60
DBV-45	Bottom 10%	31.35
DBG-186	Bottom 10%	31.30
DBG-131	Top 10%	31.30
DBG-104	Top 10%	31.20
DBV-11	Bottom 10%	31.20
DBG-124	Top 10%	31.10
TAM B 182-33	UHML Check	31.05
DBG-39	Bottom 10%	30.85
DBV-75	Bottom 10%	30.40
DBV-92	Bottom 10%	30.15
DBV-88	Bottom 10%	29.10
CV %		5.47
LSD (P=.05)		0.08

* FBS values are different if separated by more than the LSD value at the base of the column.
† FBS= fiber bundle strength; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

Table 15. FBS† of GBB and FBB platform selections within the interspecific population plus TAM 06WE-621 ESU grown at College Station in 2019.

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-17	Bottom 10%	44.65
DBV-22	Top 10%	44.10
DBV-102	Top 10%	42.90
DBG-48	Bottom 10%	42.15
DBV-154	Bottom 10%	41.90
TAM 06 WE-621	FBS Check	41.85
DBV-126	Top 10%	41.50
DBV-48	Bottom 10%	40.95
DBV-104	Bottom 10%	40.75
DBV-49	Top 10%	40.70
DBG-106	Top 10%	40.55
DBV-31	Top 10%	40.50
DBV-43	Top 10%	40.40
DBV-150	Bottom 10%	40.40
DBG-18	Bottom 10%	40.30
DBG-41	Bottom 10%	40.15
DBG-116	Top 10%	40.10
DBV-76	Bottom 10%	39.85
DBG-122	Top 10%	39.75
DBV-66	Top 10%	39.60
DBG-135	Top 10%	39.60
DBG-61	Bottom 10%	39.55
DBV-120	Top 10%	39.50
DBV-77	Top 10%	39.50
DBG-111	Top 10%	39.35
DBV-108	Top 10%	39.30
DBG-08	Bottom 10%	39.20
DBG-118	Top 10%	39.20
DBV-105	Top 10%	39.10
DBV-42	Top 10%	39.10
DBV-02	Top 10%	38.90
DBV-87	Top 10%	38.80
DBG-44	Bottom 10%	38.75
DBG-134	Top 10%	38.70
DBV-05	Top 10%	38.70

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-40	Bottom 10%	38.60
DBG-46	Bottom 10%	38.55
DBG-94	Top 10%	38.55
DBV-121	Bottom 10%	38.45
DBV-155	Top 10%	38.30
DBV-148	Top 10%	38.20
DBV-106	Bottom 10%	38.20
DBV-29	Bottom 10%	38.20
DBG-98	Top 10%	38.15
DBG-137	Top 10%	38.05
DBV-103	Top 10%	37.90
DBG-101	Top 10%	37.80
TAM B 182-33	UHML Check	37.70
DBG-165	Top 10%	37.70
DBG-52	Bottom 10%	37.65
DBV-12	Top 10%	37.60
DBG-144	Top 10%	37.60
DBV-23	Top 10%	37.60
DBG-62	Bottom 10%	37.55
DBV-97	Top 10%	37.50
DBV-44	Top 10%	37.30
DBV-40	Top 10%	37.20
DBV-27	Top 10%	37.00
DBG-126	Top 10%	36.95
DBG-163	Bottom 10%	36.90
DBG-185	Bottom 10%	36.80
DBG-211	Bottom 10%	36.70
DBG-138	Top 10%	36.65
DBG-192	Bottom 10%	36.60
DBV-151	Bottom 10%	36.55
DBG-75	Bottom 10%	36.55
DBG-136	Top 10%	36.55
DBV-127	Top 10%	36.50
DBG-39	Bottom 10%	36.50
DBG-117	Top 10%	36.45
DBG-124	Top 10%	36.40

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV-128	Bottom 10%	36.35
DBV-111	Top 10%	36.30
DBV-90	Bottom 10%	36.30
DBV-34	Bottom 10%	36.30
DBG-222	Top 10%	36.20
DBV-26	Top 10%	36.10
DBV-71	Top 10%	36.10
DBV-11	Bottom 10%	36.10
DBV-137	Bottom 10%	36.05
DBV-17	Bottom 10%	36.00
DBG-115	Top 10%	35.85
DBV-149	Bottom 10%	35.80
DBG-223	Top 10%	35.75
DBG-20	Bottom 10%	35.65
DBG-15	Bottom 10%	35.40
DBV-78	Top 10%	35.40
DBV-109	Bottom 10%	35.40
DBG-95	Top 10%	35.40
DBG-63	Bottom 10%	35.30
DBV-45	Bottom 10%	35.30
DBG-186	Bottom 10%	35.20
DBG-93	Top 10%	35.15
DBG-105	Bottom 10%	35.15
DBG-104	Top 10%	35.00
DBV-95	Bottom 10%	35.00
DBG-96	Top 10%	34.85
DBG-54	Bottom 10%	34.85
DBG-131	Top 10%	34.80
DBG-129	Top 10%	34.80
DBV-24	Bottom 10%	34.80
DBG-113	Top 10%	34.80
DBV-57	Top 10%	34.70
DBG-127	Top 10%	34.60
DBG-99	Top 10%	34.55
DBG-13	Bottom 10%	34.50
DBG-22	Bottom 10%	34.50

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-07	Bottom 10%	34.25
DBV-130	Top 10%	34.10
DBG-102	Top 10%	34.10
DBV-75	Bottom 10%	34.10
DBV-92	Bottom 10%	34.00
DBG-200	Bottom 10%	33.95
DBV-20	Top 10%	33.80
DBV-88	Bottom 10%	33.25
DBV-39	Bottom 10%	33.20
DBV-153	Bottom 10%	33.10
CV %		6.45
LSD (P=.05)		3.28

* FBS values are different if separated by more than the LSD value at the base of the column.
† FBS= fiber bundle strength; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

Table 16. Mean values for FBS† of GBB and FBB selections, including top 10% and bottom 10% in the interspecific population and an FBS check, TAM 06 WE-621, grown at College Station in 2018 and 2019.

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV-22	Top 10%	41.25
DBG-17	Bottom 10%	41.20
DBV-102	Top 10%	40.25
DBV-49	Top 10%	39.78
DBV-126	Top 10%	39.70
DBV-154	Bottom 10%	39.15
DBG-48	Bottom 10%	38.65
TAM 06 WE-621	FBS Check	38.33
DBV-23	Top 10%	38.20
DBV-31	Top 10%	38.15
DBV-42	Top 10%	38.10
DBG-94	Top 10%	37.80
DBG-08	Bottom 10%	37.78
DBV-66	Top 10%	37.70
DBV-150	Bottom 10%	37.65
DBV-105	Top 10%	37.63
DBV-43	Top 10%	37.63
DBG-41	Bottom 10%	37.53
DBG-116	Top 10%	37.43
DBV-76	Bottom 10%	37.43
DBG-18	Bottom 10%	37.43
DBV-104	Bottom 10%	37.40
DBG-44	Bottom 10%	37.38
DBG-61	Bottom 10%	37.33
DBV-48	Bottom 10%	37.28
DBV-77	Top 10%	37.20
DBV-44	Top 10%	37.07
DBV-02	Top 10%	37.05
DBG-122	Top 10%	37.03
DBV-87	Top 10%	37.00
DBG-40	Bottom 10%	36.83
DBV-120	Top 10%	36.83
DBG-106	Top 10%	36.80
DBG-135	Top 10%	36.78

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBV-108	Top 10%	36.78
DBV-97	Top 10%	36.68
DBG-111	Top 10%	36.53
DBV-12	Top 10%	36.48
DBV-148	Top 10%	36.38
DBG-118	Top 10%	36.30
DBG-62	Bottom 10%	36.28
DBV-103	Top 10%	36.13
DBV-106	Bottom 10%	36.05
DBV-155	Top 10%	36.03
DBG-163	Bottom 10%	36.00
DBV-05	Top 10%	35.98
DBG-126	Top 10%	35.95
DBG-138	Top 10%	35.78
DBV-40	Top 10%	35.75
DBG-144	Top 10%	35.68
DBG-117	Top 10%	35.68
DBG-46	Bottom 10%	35.65
DBG-185	Bottom 10%	35.65
DBV-29	Bottom 10%	35.60
DBG-137	Top 10%	35.60
DBG-75	Bottom 10%	35.50
DBV-90	Bottom 10%	35.45
DBV-121	Bottom 10%	35.33
DBG-134	Top 10%	35.25
DBG-211	Bottom 10%	35.20
DBG-98	Top 10%	35.10
DBV-95	Bottom 10%	35.08
DBG-101	Top 10%	35.05
DBV-71	Top 10%	35.03
DBG-222	Top 10%	34.98
DBG-165	Top 10%	34.93
DBV-151	Bottom 10%	34.90
DBV-17	Bottom 10%	34.87
DBV-149	Bottom 10%	34.77
DBG-105	Bottom 10%	34.75

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-20	Bottom 10%	34.73
DBV-128	Bottom 10%	34.73
DBG-95	Top 10%	34.73
DBG-52	Bottom 10%	34.70
DBV-26	Top 10%	34.68
DBG-136	Top 10%	34.65
DBV-20	Top 10%	34.63
DBV-137	Bottom 10%	34.60
DBG-192	Bottom 10%	34.60
DBG-54	Bottom 10%	34.50
DBV-127	Top 10%	34.50
DBV-111	Top 10%	34.45
DBG-223	Top 10%	34.45
TAM B 182-33		34.38
DBG-15	Bottom 10%	34.38
DBV-27	Top 10%	34.38
DBG-115	Top 10%	34.35
DBV-34	Bottom 10%	34.28
DBG-99	Top 10%	34.13
DBG-63	Bottom 10%	33.98
DBV-78	Top 10%	33.98
DBV-24	Bottom 10%	33.95
DBG-22	Bottom 10%	33.93
DBG-93	Top 10%	33.90
DBG-129	Top 10%	33.83
DBV-130	Top 10%	33.78
DBG-124	Top 10%	33.75
DBG-96	Top 10%	33.73
DBV-109	Bottom 10%	33.73
DBG-39	Bottom 10%	33.68
DBV-57	Top 10%	33.68
DBV-11	Bottom 10%	33.65
DBG-113	Top 10%	33.63
DBV-45	Bottom 10%	33.33
DBG-200	Bottom 10%	33.28
DBG-186	Bottom 10%	33.25

$F_{3:4}$ Designation	Sub-subpopulation	FBS (g/tex)
DBG-13	Bottom 10%	33.23
DBG-07	Bottom 10%	33.13
DBG-104	Top 10%	33.10
DBG-127	Top 10%	33.10
DBG-131	Top 10%	33.05
DBV-39	Bottom 10%	32.98
DBG-102	Top 10%	32.90
DBV-153	Bottom 10%	32.35
DBV-75	Bottom 10%	32.25
DBV-92	Bottom 10%	32.08
DBV-88	Bottom 10%	31.18
CV%		5.42
LSD (P=.05)		5.18

* FBS values are different if separated by more than the LSD value at the base of the column.
† FBS= fiber bundle strength; GBB=gene based breeding; FBB=field based breeding
**DBV selections belong to the top and bottom sub-subpopulations of FBB platform; DBG selections belong to the top and bottom sub-subpopulations of GBB platform.

methods. None were stronger (p=0.05) than the TAM 06WE-621 ESU parent and check. Of the strongest 29, 10 were predicted by their F_2 IPS HVI or their 11 SNPs to be in the lowest 10 % sub-subpopulations. Like the UHML selections among F2:4 progeny, the lowest FBS exhibiting entries also feature in top 10% of the sub-subpopulations of FBB and 11-SNP-GBB platform. This inconsistency among FBS in the selection protocol, especially on the GBB platform side, can be partly due to the varying environmental factors in both years of the trial as indicated by the ANOVA results in Table 3. Again, similar to the intraspecific population, further disturbing data from Table 15 is that only 14 of the strongest $F_{2:4}$ progeny were so predicted by the FBS of their foundational F_2 IPS within the FBB protocol, again supporting the difficulty that breeders face in selecting high strength plants and progeny.

ANOVA Summary

Based on the summary statistics in Table 16, the FBB protocol was clearly the best predictor of both UHML and FBS in both the intraspecific and interspecific populations. However, in the intraspecific population, the GBB protocol using only 11 SNPs were associated with about half of best 29 progenies as the FBB. The GBB and FBB protocols misclassified about the same number within these 29 for UHML. For FBS, the R^2 comparisons suggested almost no association between foundational plant performance, either GBB or FBB protocols, and progeny performance. However, FBB protocols were essentially twice as good at predicting the best performing progeny for

FBS. It is interesting to note in the summary table 8 that from 21 to 38 % of the predicted worst performing IPS plants were actually in the best performing 29 progenies.

Table 17. Summary of high performing 29 progeny and their GBB or FBB predicted sub-subpopulation status.

	Predicted to be top 10%		Predicted to be bottom 10%		
Trait - population	GBB	FBB	GBB	FBB	Total
UHML - Intraspecific	7	15	4	3	29
UHML - Interspecific	3	20	4	2	29
FBS - Intraspecific	6	13	8	2	29
FBS - Interspecific	3	14	7	4	29

While the efficacy of these GBB SNPs can be improved in future studies, yet the cost benefit analysis cannot be ignored when comparing GBB with FBB protocols. The utility of SNP markers has achieved success in the recent past due to rapid developments of sequencing technologies, increasing read length, and more available reference genomes (You et al., 2019), however the progress has been slow in polyploids because of the complexity of the genome and inheritance of marker associated traits (Clevenger et al., 2015). The cost of SNP genotyping used in this study is well under a dollar per individual plant sample when compared with 2.70 dollars per sample for HVI measurements for fiber quality traits. Notably, HVI fiber properties are also determined for GBB IPS, therefore in an overall comparison with FBB, GBB protocols would produce results at a higher cost when making individual plant selections for superior fiber quality traits. A valid comparison for a future study will rests upon a cost-benefit analysis on the cost of number of field-based workers visually determining superior fiber

quality IPS in the field with the same sample size as the GBB based genotyping protocol conducted by one molecular breeder.

Though FBB was effective and a better breeding strategy than GBB in identifying superior fiber quality plants in this study, however the potential success of GBB strategy will also aid in a quicker turnout for high performing varieties of improved fiber length and strength. The perception of cotton as a fabric is widely appreciated for being soft, natural, environment friendly and comfortable when compared with other fabrics (Cotton Inc, 2020). However, the competition from synthetic fibers has eaten into the market share of cotton products especially in the apparel industry. Aksoy and Beghin (2004) reported that based on a total fiber consumption level, annual consumption rate of manmade fibers grew at 4.8% compared with the growth rate of 1.8% of cotton from 1960 until 2002 in the world. The growth of cotton consumption can be misleading since the per capita consumption has remained stagnant even with the rise of population rate (Aksoy and Beghin, 2004). The rise and production of synthetic fiber consumption can be attributed to its durability, tenacity, its specifically engineered fiber measurements and advanced spinning technologies, which cotton is devoid of due to its limitation of being a natural fiber. The challenge presented by synthetic fibers to the cotton industry is real and can be dealt with if cotton industry can match the superior fiber standards of the manmade fiber industry. From the surveys of Cotton Incorporated as well as the recent findings of marine pollution being caused by the microplastics present in the synthetic fibers (Cesa et al, 2017), it is even more imperative for cotton breeders produce superior fiber cotton varieties. A novel approach

such as the GBB used in this study is one of the tools that are being studied to address the ongoing and a renewed interest in environment friendly fiber i.e. cotton.

The scope of the GBB markers used in this study is yet to be tested. The RIL populations used to develop these SNP-based genes were grown in College Station, thereby mitigated the impact of environmental factors in the expression of these genes. The limitation, however, in using these GBB markers will be seen if a different environment or a multiple environment testing model is applied. A multi-environment trial can produce a different result for the additive or the dominance effects of these fiber quality alleles, where a different environment can lead to unintended effects of genes (Crossa et al., 2012). These fiber quality SNP-based genes are certainly present irrespective of the environment; however, the effect is yet to be tested and seen in another environment other than College Station. Moreover, the development of these SNP-based genes accounted for a multi-year testing which included trials grown in College Station in 2009, 2010, 2011 (Pers comm., Zhang). Thus, it would not be surprising to see the varying effects of these genes on a year to year basis. The consecutive yearly variation seen in this study could help explain the inconsistency of R^2 values found between the foundational IPS with its next generation progeny from both populations.

The segregation of the SNP markers located within these fiber quality genes cannot be ruled out as a possible impact in measuring the prediction accuracy of GBB when compared with FBB. The original selections of 2016 were subjected to a screening for the presence of favorable and unfavorable alleles, which were

homozygous, and the rest of the SNPs that were heterozygous. Since cotton is not an outcrossing crop, some of these heterozygous markers might have shifted their makeup because selections move towards homozygosity when grown after each successive generation in Cotton (Smith and Cothren, 1999). This move towards homozygosity could have compounded the prediction accuracy by either estimating an increase or a decrease for UHML and FBS because an $F_{2:4}$ and an $F_{3:5}$ population was analyzed for associating the fiber quality measurements with original selections of 2016. Therefore, out of the 11 SNPs that were used in this study, if one of them was heterozygous, they could have segregated to homozygosity in the $F_{2:4}$ and $F_{3:5}$ progenies of 2018 and 2019. The segregation to homozygosity could have impacted in identifying the wrong selections for the top and the bottom 10% sub-population.

Previous studies in cotton breeding research has been successful in developing SNPs associated with fiber quality and agronomic traits and yield. Few studies such as Su et al. (2016) identified favorable SNP alleles and candidate genes for early maturity traits in upland cotton and Hulse-Kemp et al. (2015) developed a 63-K SNP array in cotton and high-density mapping of intraspecific and interspecific populations of Gossypium spp.. The utility of these SNP markers in Su et al. (2016) and Hulse-Kemp et al. (2015) has not been checked and therefore lack of successive applicability in an actual cotton breeding program is still to be tested. The GBB platform tested in this study offered an alternative approach to most of the marker assisted or gene assisted selection whereby individual plants in an interspecific or intraspecific population were

selected based of their SNP makeup of allele specific fiber UHML and fiber bundle strength.

Karie Hugie et al. in 2016 indicated that SSR markers if validated in larger number of environments could potentially serve its purpose in selecting UHML and FBS selection for improved fiber quality, however the efficacy of these SSR markers were dependent upon the type of population. This study embarked on the same journey but with gene-based SNPs that were utilized in a replicated study to predict fiber quality measurements of IP in two different populations. The results were unfortunate like Hugie et al. where 11-SNP-GBB model failed to significantly perform better than FBB model and discriminate between high and low selection groups for UHML and FBS in F_2 and F_3 populations as indicated by the means separation analyses of sub-populations for top and bottom 10% selections in GBB platform.

A similar study, related to the results of this research, also suggested a lack of robustness of marker assisted selection platforms in publicly available breeding programs. Ullrich et al. (2018) had utilized SSR markers to screen the same F_3 intraspecific population and another interspecific population in predicting superior UHML and FBS individual plants but the result was inconclusive in separating the top and bottom 10% sub-subpopulations for UHML and FBS. Though SSR markers used in Ullrich et al. study (2018) are not comparable to the allele specific SNPs of GBB, yet its juxtaposition with this study suggests that more markers can provide more power in selecting bottom and top performing lines in different populations. A larger genome

wide SNP coverage has been propagated as a potential advancement in to capture the genetic the variation in fiber quality alleles (Islam et al., 2014; Zheng et al., 2016).

The means separation analyses with separate years as well as combined for both years demonstrates that FBB selection protocol selects superior fiber UHML in individual plant nursery for both types of populations. The selection protocol for FBS under the 11-SNP-GBB protocol needs to be reexamined since inconsistencies are at a larger rate in separating the top and bottom sub-subpopulations in both intraspecific and interspecific. A larger set of genes may provide better R^2 values for establishing the relationship between the 11-SNP-GBB predictions against their HVI UHML. The regression analyses and the ANOVA suggest inconclusiveness of GBB prediction accuracy in two different sets of populations, while this study maintains the robustness FBB selection protocol in comparison to GBB in selecting individual plants for superior fiber quality in UHML and FBS.

CHAPTER V

CONCLUSIONS

The R^2 or correlation values from the simple regression analyses suggested that in three of the four sub-subpopulation comparisons that GBB using only 11 of the current 226 gene-based SNPs identified by the Zhang Lab is comparable to FBB or phenotypic protocols for UHML. The one outlier was the top 10 % sub-subpopulations for GBB and FBB. Regression and correlation suggested poorer relationships between the foundational IPS and progeny performance for FBS within either the GBB or FBB protocols for FBS. Analysis of variance and mean separation indicated the FBB protocol was the best predictor of both UHML and FBS in both the intraspecific and interspecific populations. The means separation analysis of the subpopulations determined FBB as the most effective and consistent in selecting the top 10 % and bottom 10% sub-subpopulations in intraspecific and intraspecific. In the same analysis, GBB protocol was inconclusive in predicting the top and the bottom 10% sub-subpopulations for UHML and FBS in either of the populations. In the intraspecific population, the GBB protocol using only 11 SNPs were associated with about half as many of the best 29 progenies as the FBB. However, FBB protocols were essentially twice as good at predicting the best performing progeny for FBS.

From a proof of concept model, the cotton breeders would be unable to predict progeny performance from gene-based breeding protocol due to the inconsistent predictions of fiber quality measurements grown over two years as indicated by poor R^2

correlation data. The only redeeming performance that was comparable to FBB can be attributed to progenies of the top and bottom 10% sub-subpopulation of GBB protocol in the interspecific population. Though the correlation data was below the expected R^2, the phenotypic predictive data of FBB protocol was significantly better than GBB when selecting within a narrow range of UHML or FBS elite quality.

Apart from the correlation data, the breakdown of ANOVA results into a means separation analyses identified the FBB protocol in separating the foundational IPS for the top and bottom 10% sub-subpopulations; whereas, the GBB protocol failed to separate the superior and low performing lines from each other. The preliminary results from this study suggests that GBB protocol may be well suited for an interspecific population than an intraspecific population. Moreover, an ideal population developed with a large proportion of individual plants consisting of high and the low end of fiber quality will probably gather better results within the limitations of GBB protocol. Also, the sample size of this study is not indicative of a large population size, therefore the applicability of GBB protocol is yet to fully exploited. The inclusion of different types of populations from different environments and more gene-based SNPs may provide better picture in the near future to assess the utility of the GBB protocol when predicting progeny performance for superior fiber length and strength.

11 gene-based SNPs proved to be ineffective in identifying superior UHML and FBS among individual plants in a F_2 and F_3 breeding nursery. At its current state in a cotton breeding program, the early season applicability of gene-based SNPs as a

promising tool in fiber quality predictions will be dependent upon the numbers of gene-based SNPs, sample size, multiyear testing, and multiple environment testing.

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