

## BREEDING AND GENETICS

### Neppiness in an Introgressed Population of Cotton: Genotypic Variation and Genotypic Correlation

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#### ABSTRACT

**Neps and seed coat fragments are impurities in ginned fibers causing severe problems in textile processing during spinning and dyeing. This study was designed to investigate genotypic variation of neps and seed coat fragments remaining in ginned fibers in an introgressed population. Two hundred lines of a population, i.e., JohnCotton (JC) germplasm, derived from multiple crosses between *Gossypium hirsutum* L. and *G. barbadense* L. and five cultivars were planted at two locations in 2006 and one location in 2007 with two replicates each. Neps and seed coat fragments were measured using Advanced Fiber Information System. Genotypic variations of nep count and seed coat nep (SCN) count in the JC germplasm were highly significant ( $P < 0.001$ ). Wide ranges of nep count (70 to 288 cnt g<sup>-1</sup>) and SCN count (2.7 to 16 cnt g<sup>-1</sup>) were identified among the 200 lines. The mean of the SCN in JC population was not significantly ( $P < 0.05$ ) different from the mean of the cultivars. Lint yield was negatively (favorably) correlated with nep count ( $r = -0.47$ ) and SCN ( $r = -0.31$ ). Unfavorable correlations were identified for nep count vs. fineness ( $r = -0.75$ ) and SCN count vs. fiber bundle strength ( $r = 0.60$ ). Nep count was unfavorably correlated to 2.5% span length ( $r = 0.56$ ), but not correlated to 50% span length ( $r = 0.10$ ). Two lines were selected with nep count less than 90 cnt g<sup>-1</sup> and fineness lower than 180 mg km<sup>-1</sup> and nine lines were selected with SCN less than 6 cnt g<sup>-1</sup> and strength greater than 230 kN m kg<sup>-1</sup>.**

**N**eppiness, i.e., neps and seed coat fragments, is the impurity in processed cotton causing severe problems in textile processing, affecting the quality of textiles. Neps are small fiber entanglements

that are formed during cultivation, harvesting, and ginning (Mangialardi, 1986). Seed coat fragment is “a portion of a cotton seed, usually black or dark brown in color, broken from a mature or immature seed, and to which fibers and linters may or may not be attached” (ASTM, 1985). In Advanced Fiber Information System (AFIS), the level of seed coat fragments is measured as seed coat neps (SCN) (Armijo et al., 2009). Seed coat neps are the seed coat fragments remaining with fibers or lint as detected by the method of AFIS and most likely would not be removed from fibers during processing, i.e., cleaning and carding (Baldwin et al., 1995; Krifa and Frydrych, 2002). There are three main components in the AFIS instrument: the lint stream, trash stream, and dust stream. The AFIS instrument sizes and counts neps and SCN in the lint stream. Large seed coat fragments without fibers attached are counted as trash in the trash stream while small seed coat fragments, i.e., the particles less than 0.5 mm, are counted as dust in dust stream (Baldwin et al., 1995).

Neps and seed coat fragments remaining in fibers severely affect textile processing during spinning and dyeing (Clegg and Harland, 1923; Pearson, 1933; Pearson, 1955; Jacobsen et al., 2001). Neps in raw fibers are the main source of yarn neps (Frydrych et al., 2001). They take up dye poorly and appear as “light spots,” or remain un-dyed and cause “white specks” when woven into fabrics (Pearson, 1933; Jacobsen et al., 2001; Altintas et al., 2007). Fiber nep count by AFIS in ten bales of cotton was significantly correlated to white speck occurrence on the finished dyed yarn (Altintas et al., 2007). Seed coat fragments are the main source of cotton yarn regularity defects (Pearson, 1955; Krifa and Gourlot, 2001). Because seed coat fragments remain in processed cotton attached to fibers, they can affect yarn quality and final fabrics by causing dyeing difficulties and holes in fabrics after “the dissolution of the seed coat tissue” (Pearson, 1955).

Reduction of neps and seed coat fragments can improve yarn performance and fabric color quality (Mangialardi and Lalor, 1990). Although seed coat fragments can be partially removed in lint cleaning,

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excessive cleaning aimed at eliminating seed coat fragments may increase short fiber content and neps, result in decreased yarn evenness and increased breaks during spinning, and reduce lint turn out (Mangialardi, 1981; Krifa and Frydrych, 2002). Genetic improvement of cotton cultivars for reducing neps and seed coat fragments during conventional ginning can be an important approach although there is a lack of related research currently. Significant differences in nep count in ginned lint were identified among six cultivars in a study by Mangialardi and Lalor (1990). Nep counts ranged from 140 to 292 neps  $g^{-1}$  lint and seed coat neps ranged from 6 to 22  $g^{-1}$  in ginned lint among 38 cultivars (Boykin, 2008). In contrast, no significant difference of seed coat fragments was identified among ten cotton cultivars in another study reported by Bolek et al. (2007). There is a general lack of information on genotypic variation of neppiness in cotton germplasm, especially in those exotic germplasm resources derived from crosses among species in *Gossypium*.

The need for high quality fibers has dramatically increased owing to the shift from domestic consumption of raw fibers to export and rapidly rising spinning speed in the surviving US textile industry. Meanwhile, the challenge for cotton breeders to continue improving lint yield in order to maintain profits for cotton growers remains high. Broadening the genetic base in upland cotton is essential for continuing genetic improvement of lint yield and fiber quality to meet these needs. Exploring exotic germplasm derived from interspecific crosses can be a good approach for broadening the genetic base although currently the application of this approach is limited due to problems such as lack of adaptability to local environments. Another common problem hindering the application of the exotic germplasm developed from inter-specific crosses is the existence of large amounts of aborted seeds which cause seed coat fragments in ginned lint. Previous evaluation of exotic germplasm populations of 'Species Polycross' (SP) and 'John Cotton' (JC), derived from multiple crosses among tetraploid species in *Gossypium*, identified significant genotypic variation for lint yield and fiber quality including fiber bundle strength, elongation, span lengths, short fiber content, and fineness in the populations (Zeng et al., 2007; Zeng and Meredith, 2009). However, the traits of neppiness were not investigated in these studies. Objectives of this study were investigating genotypic variation for neps and seed coat fragments in ginned fibers in JC

germplasm and analyzing interrelationships among neppiness and other fiber properties. Identification of significant genetic variation by exploration of existing germplasm will be the first step toward genetic improvement of the neppiness trait in cultivars. The determined interrelationships will help breeders identify traits that may be simultaneously improved in terms of neps and seed coat fragments, or compensated for in the selection for reduction in these traits.

## MATERIAL AND METHODS

The germplasm population was obtained from John Cotton (USDA-ARS at Las Cruces, NM) and was designated as JC for its originator, John Cotton. The development of this population was described in a previous study (Zeng and Meredith, 2009). The JC germplasm was derived from multiple crosses between *Gossypium barbadense* and Acala 1517 cultigens. Although the exact parents and crossing pattern were not clear, it was known that natural pollinations were allowed among parental plants and their hybrid progenies in a field at Las Cruces. The population underwent multiple generations of introgression between the 1970s and the 1990s at Las Cruces, NM and multiple generations of self - pollination between the 1990s and 2005 at Stoneville, MS after it was transferred. The sub-population was maintained and advanced by harvesting one boll from each plant and bulking the harvested bolls for planting the next generation. In 2005, 200 plants were randomly sampled from this population and fifteen to twenty bolls were collected from each plant. Seeds from each plant were planted as one line per row in 2006 for the evaluation trial.

Evaluation trials of this population were conducted in 2006 and 2007. The experiments including the experimental design, planting and harvest were described in a previous report (Zeng and Meredith, 2009). In details, 200 JC lines were grown with five cultivars, 'Deltapine 555BG/RR' (DP555BR; Delta Pine and Land Co., Scott, MS), 'Stoneville 4892BR' (ST4892BR; Stoneville Pedigree Seed, Stoneville, MS), 'FiberMax 960B2R' (FM960B2R, Bayer Crop Sciences, Research Triangle Park, NC), 'Phytogen 72' (PHY72, Phytogen Seed Co., Indianapolis, IN), and 'Paymaster 2167R' (PM2167R, Delta Pine and Land Co., Scott, MS). They were planted at two locations in 2006 and one location in 2007 with two replicates at each location. A randomized complete block

design was used in experiments with all 205 entries including 200 JC lines and 5 cultivars assigned randomly to each replicate in all environments. In 2006, the germplasm lines and cultivars were grown in single-row plots, each 4.6 m long with 1.0 m spacing. In 2007, the germplasm line and cultivars were grown in single-row plots, each 9.1 m long with 1.0 m spacing. The two field sites were located about 1000 m apart at the Delta Research Center, Stoneville, MS. Environments were differentiated between the field sites by soil types and planting dates. Soil type at the first field site, i.e., Location 1, was Beulah fine sandy loam (a coarse-loamy, mixed, active, thermic Typic Dystrudepts) and soil type at the second field site, i.e., Location 2, was Bosket fine sandy loam (a fine-loamy, mixed, active, thermic Mollic Hapludalf). Seeds were planted on 18 April 2006 at Location 1, 8 May 2006 at Location 2, and 30 April 2007 at Location 2. Standard conventional production practices were applied during the experiments at the two locations. The factor of location and year were combined as a factor of environment: Environment 1 for Field Location 1 in 2006; Environment 2 for Field Location 2 in 2006; Environment 3 for Field Location 2 in 2007. For the harvest in 2006, thirty bolls from each plot were sampled by hand, and the remaining bolls from each plot were also collected by hand to measure yield. For the harvest in 2007, fifty bolls were sampled by hand, and the remaining bolls were harvested by a mechanical picker for yield measurements. Samples from individual plots were ginned using a laboratory saw gin, and seed weight and lint weight from the boll sample of each plot were measured and recorded. Total seed cotton weight of each plot was the sum of seed cotton weight of the sampled bolls and the remaining bolls in that plot. Lint yield was calculated from seed cotton weight per plot and lint percent, and further converted to  $\text{kg ha}^{-1}$  for each line.

Fiber properties of nep size, nep count, seed coat nep (SCN) size, and SCN count were measured using Uster Advanced Fiber Information System (AFIS) (Uster AFIS, 1997). Other AFIS-measured properties were also measured such as short fiber content (SFCn) as the percentage by number of the fibers that were less than 12.7 mm, fineness (FN) as the weight per unit of length ( $\text{mg km}^{-1}$ ) where smaller values indicate a higher degree of fineness, and maturity ratio (MR) as the proportion of mature fibers to immature fibers. For these measurements, two samples of lint with 0.5 grams each were taken from each plot.

Twenty grams of lint from boll samples from each plot were submitted to StarLab (Knoxville, TN) for measurements of fiber bundle strength, micronaire, elongation, and lengths. Fiber bundle strength was measured by a stelometer as the force required for breaking a bundle of fibers; elongation was measured as the percentage of elongation at the point of break in strength determination; micronaire was measured in micronaire units using the Fibronaire instrument (Motion Control, Dallas, TX); and fiber span lengths were measured as mean length of the longest 50% or 2.5% of the fibers scanned.

The General Linear Model procedure of the Statistical Analysis System (SAS Institute, 2004) was used for analysis of variance in the experimental data. A mixed model was used with genotype as fixed effect and environment, genotype  $\times$  environment, and replication within environments as random effects. Means of lint yield and fiber properties were calculated across environments for all entries in the experiments. Means of the 200 JC lines were compared with the means of the five cultivars in t-test comparisons. Mean squares of the genotype  $\times$  environment interactions in JC population and the cultivars were used as variances in the t-tests. Genotypic correlations of the traits in neppiness with other fiber properties were calculated using the equation described by Kempthorne (1957):

$$R_g = \sigma_{gxy} / (\sigma_{g_x}^2 \sigma_{g_y}^2)^{1/2}, \sigma_{g_x}^2 = 1/r (V_x - V_{ex}), \\ \sigma_{g_y}^2 = 1/r (V_y - V_{ey}), \sigma_{gxy} = 1/r (Cov_{xy} - Cov_{exy})$$

Where  $\sigma_{gxy}$  is the genetic component of covariance between variable x and y;  $\sigma_{g_x}^2$  and  $\sigma_{g_y}^2$  are genetic components of variance for x and y, respectively;  $V_x$  and  $V_y$  are variances of x and y, respectively;  $V_{ex}$  and  $V_{ey}$  are errors for x and y, respectively; r is the number of replicates. Locations and years were treated as replicates for calculation of genetic correlation. Variance and covariance for traits were calculated by creating a matrix using MANOVA statement in PROC GLM procedures. Standard errors of the genotypic correlations were estimated using the method of multivariate restricted maximum likelihood analysis with Proc MIXED of SAS as described by Holland (2006). The codes of the procedure were illustrated in "APPENDIX A" of that report. Broad-sense heritability ( $h^2$ ) was calculated from variance components (Bernardo, 2002) using the equation described by Fehr (1987). Confidence intervals (90%) of heritability were calculated according to Knapp et al. (1985).

## RESULTS AND DISCUSSION

Two hundred lines of JC germplasm, a population derived from multiple crosses between *G. hirsutum* and *G. barbadense*, were evaluated for neps and seed coat fragments in ginned fibers and other AFIS-measured properties in two trials conducted in three environments in 2006 and 2007. Mean squares of most AFIS-measured properties except SCN size, dust count, and trash count were highly significant ( $P < 0.001$ ) for genotype (Table 1). The non-significant mean squares of genotype for dust count and trash count indicate that there were no differences of these two traits among the lines in this germplasm. Results for these two properties measured from boll samples collected manually during the experiments may not represent the situation when fibers are mechanically picked. In addition, obvious differences of morphological characters such as leaf shape, bract type, and pubescence were not observed among most JC lines although these morphological characters are known to contribute to genotypic effects on trash content in cotton fibers (Novick et al., 1991). For these reasons, these two properties were not further analyzed in this study. Although all AFIS-measured fiber properties except nep size were significantly ( $P < 0.001$ ) different among environments, the genotype  $\times$  environment interactions were not significant ( $P < 0.05$ ) for most properties except nep size and IFC (Table 1). Heritability was high, i.e., at least 0.79, for nep count, IFC, Lw, LwCV, UQL and SFCn, while moderate, i.e., 0.40, for SCN count (Table 1). The moderate heritability of SCN indicated that more than 50% of variation for the trait was due to environment. However, non-significant genotype  $\times$  environment of SCN indicated

that different genotypes performed similarly across environments for this trait, and therefore, general adaptability should be emphasized in breeding. The identified high heritability for nep count and other AFIS-measured properties in the JC germplasm indicates the potential of this germplasm for genetic improvement of these traits in breeding.

One of the objectives of this evaluation was to investigate seed coat fragments in ginned fibers among the 200 JC lines and determine if this trait in the germplasm is different from that in cultivars due to aborted seeds as commonly occurs in interspecific hybrids. The means of AFIS-measured properties in the five cultivars are given in Table 2. Lint yield and fiber bundle strength measured by a stelometer during the same evaluation trials were reported previously (Zeng and Meredith, 2009) and are presented in Table 2 only for purpose of comparison and study of their interrelationships with the neppiness. Lint yield of DP555BR, 1570 kg ha<sup>-1</sup>, was the highest among the five cultivars, but its fiber strength, 211 kN m kg<sup>-1</sup>, was the lowest among the five. Lint yield of PHY72 was the lowest, 933 kg ha<sup>-1</sup>, among the five with greatest fiber strength, 258 kN m kg<sup>-1</sup>. Nep counts were the highest for ST4892BR and PHY72 among the five cultivars, 92.2 and 108 cnt g<sup>-1</sup>, respectively, while the lowest for DP555BR, 84.7 cnt g<sup>-1</sup>. Similarly, SCN counts were the highest for ST4892BR and PHY72 among the five cultivars, 9.42 and 7.25 cnt g<sup>-1</sup>, respectively, while the lowest for DP555, 3.58 cnt g<sup>-1</sup>. These differences among the check cultivars in current study are consistent with previous reports when differences of nep content and seed coat fragments in ginned fibers were identified among cotton cultivars (Mangialardi and Lalor, 1990; Boykin, 2008). Mean of nep count in JC population was significantly ( $P < 0.05$ ) higher

**Table 1.** Mean squares, broad-sense heritability, and confidence intervals of heritability for the AFIS-measured properties in 200 JC lines.

Source	df	Nep size	Nep count $\times 10^{-3y}$	SCN size $\times 10^{-3}$	SCN count	Dust count $\times 10^{-3}$	Trash count $\times 10^{-3}$	IFC	Lw	LwCV	UQL	SFCn
Genotype (G)	199	1585****	7.5***	94	25***	108	2.5	5.5***	7.2***	17***	14***	67***
Environment (E)	2	2278	213***	364*	386***	7075***	32***	78***	25***	89***	62***	170***
G $\times$ E	398	1020*	1.6	96	15	106	2.6	0.73*	0.80	2.5	0.87	9.3
Replicate (E)	3	1663	36***	84	80**	3319***	187***	14***	9.2***	32***	8.6***	121***
Error	597	864	1.6	92	15	94	2.5	0.60	0.70	2.2	0.77	8.7
$h^2$		0.36	0.79	0.02	0.40	0.02	0.04	0.87	0.86	0.85	0.94	0.86
CI (90%)	Min	0.20	0.74	-0.25	0.27	-0.20	-0.27	0.84	0.86	0.82	0.92	0.83
	Max	0.47	0.83	0.16	0.51	0.20	0.15	0.89	0.91	0.88	0.95	0.89

<sup>y</sup> Values were decreased from real values  $\times 10^{-3}$ .

<sup>z</sup> Values followed by \*, \*\*, \*\*\*\* are significantly different at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , respectively.

than those in cultivars (Table 2). However, there was a wide range of nep count, 70 to 288 cnt g<sup>-1</sup> in ginned fibers. Distributions of nep count among the 200 JC lines indicated that most lines scored between 80 and 160 cnt g<sup>-1</sup> (Fig. 1). Some lines were scored with low counts, i.e., 5 lines with less than 75 cnt g<sup>-1</sup>. The means of SCN size and SCN count were not significantly different from the means of cultivars. Meanwhile, there was a wide range of SCN count, 2.7 to 16 cnt g<sup>-1</sup>, among the 200 JC lines. The distribution of SCN count among the 200 JC lines indicated that most lines scored between 4.2 and 9.5 cnt g<sup>-1</sup> (Fig. 2) and 6 lines scored with less than 3.5 cnt g<sup>-1</sup>. These results indicate that the JC germplasm was not different from the cultivars for seed coat fragments in ginned fibers. It is possible that the genomes of the hybrid progenies have stabilized after a long term of introgression and adaptation to the regional environments and therefore, embryonic growth during seed development in the population has been generally normal. Most of the JC lines were scored above 30 mm for UQL (Fig. 3), similar to the means of the cultivars, 29.9 mm. About one fourth of JC lines scored at 13.6% or lower for SFCn (Fig. 4), less than the mean of the cultivars, 16.3%. Most of the JC lines were scored below 174 mg km<sup>-1</sup> for fineness (Fig. 5), lower than the mean of the cultivars, 178 mg km<sup>-1</sup>. These results further confirmed the usefulness of this exotic germplasm regarding its normal seed coat fragments and high genotypic variation identified in other fiber properties.

Analysis of correlations among AFIS-measured properties may unravel the interrelationships between the neppiness and other AFIS-measured properties,

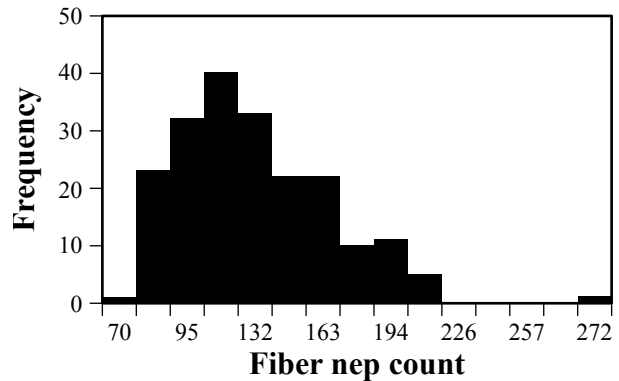


Fig. 1. Distribution of means for fiber nep count among 200 JC lines across environments.

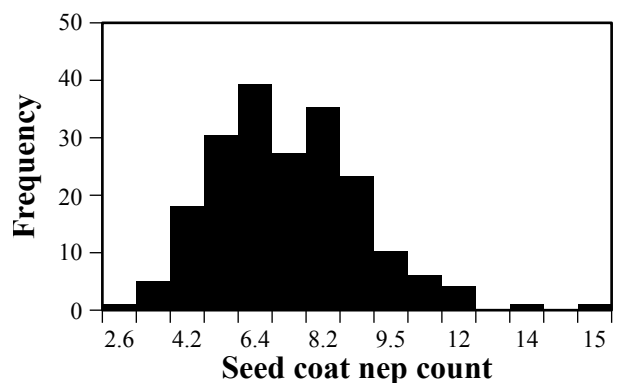


Fig. 2. Distribution of means for seed coat nep count among 200 JC lines across environments.

and thus help understand the variation of neps and seed coat fragments in the germplasm and facilitate selection for desirable combinations among these traits. First, nep counts measured in the lint stream of the AFIS instrument could include both fiber neps and SCN (Uster AFIS, 1997). Lack of correlation

Table 2. Means and ranges of the AFIS-measured properties in JC population and cultivars.

Entries	Lint <sup>y</sup> Yield kg ha <sup>-1</sup>	Strength kN m kg <sup>-1</sup>	Nep size μm	Nep count cnt g <sup>-1</sup>	SCN size μm	SCN count cnt g <sup>-1</sup>	IFC %	Lw mm	Lw CV %	UQL Mm	SFCn %	Fineness mg km <sup>-1</sup>	MR
DP555BR	1570	211	642	84.7	1029	3.58	4.25	25.3	31.2	31.1	20.0	180	0.968
ST4892BR	1083	215	688	92.2	1213	9.42	4.17	25.4	28.9	29.6	15.1	184	0.961
PM2167R	1139	211	650	88.8	1288	4.33	4.41	24.0	29.2	27.7	14.5	179	0.954
FM960B2R	1168	241	649	80.1	1258	4.92	4.28	26.5	30.0	31.4	17.1	177	0.969
PHY72	933	258	675	108.0	1251	7.25	3.98	26.4	30.3	31.0	15.1	172	0.974
Means cultivars	1179	227	661	91.0	1210	5.90	4.22	25.5	29.9	30.0	16.3	178	0.965
Means JC population	769 <sup>**</sup>	228	664	124 <sup>**</sup>	1178	6.87	4.71 <sup>*</sup>	25.5	30.1	29.9	16.3	173 <sup>*</sup>	0.948
Ranges JC population	Min 389 Max 1182	184 300	622 710	70 288	826 1506	2.7 16	2.8 6.8	23 28	27 34	26 33	9.3 25	155 200	0.88 1.00

<sup>y</sup> Means of lint yield, strength, and fineness were reported previously (Zeng and Meredith, 2009) and listed in this table only for purpose of comparisons.

<sup>z</sup> Values followed by \*, \*\* are significantly different at  $P < 0.05$ ,  $P < 0.01$ , respectively, in t test for comparisons between the means of JC populations and the means of the cultivars.

between nep count and SCN count in this study ( $r=0.05$ , Table 3) indicate these two properties were well distinguished in the lint stream and the nep counts estimated were mainly composed of fiber neps. Some fiber properties such as maturity and fineness can influence nep formation as reviewed by Van der Sluijs and Hunter (1999). High correlation between nep count and IFC ( $r=0.87$ ) (Table 3) and the higher IFC in JC lines than that in the five cultivars (Fig. 6) indicate the effect of maturity on nep content in ginned fibers. This is consistent with the phenotypic correla-

tion between nep count and micronaire ( $r=-0.85$ ) as reported previously by Mangialardi and Lalor (1990). It is believed that the relatively high fiber nep content in JC germplasm was due to the diversified maturity ratio among the 200 lines (Table 2). Positive correlations were also observed for nep count vs. length by weight ( $r=0.38$ ) and nep count vs. UQL ( $r=0.55$ ) although the correlations were only low to moderate. In contrast to nep count, SCN count was not correlated with most AFIS-measured properties except a low correlation to SFCn ( $r=-0.36$ ) (Table 3).

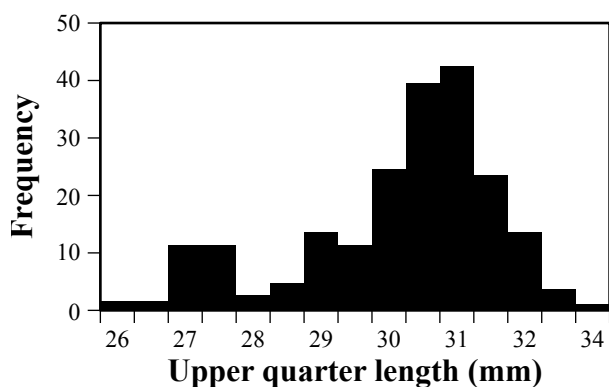


Fig. 3. Distribution of means for upper quarter length among 200 JC lines across environments.

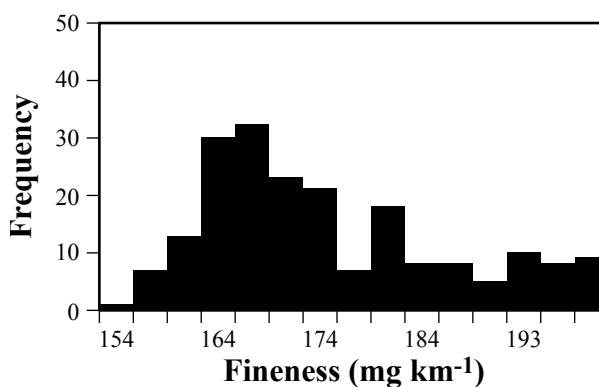


Fig. 5. Distribution of means for fineness among 200 JC lines across environments.

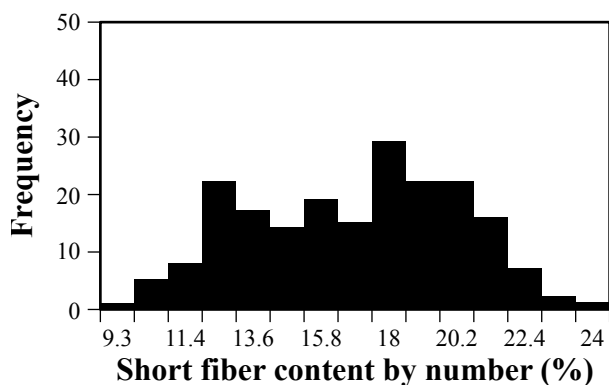


Fig. 4. Distribution of means for short fiber content by number among 200 JC lines across environments.

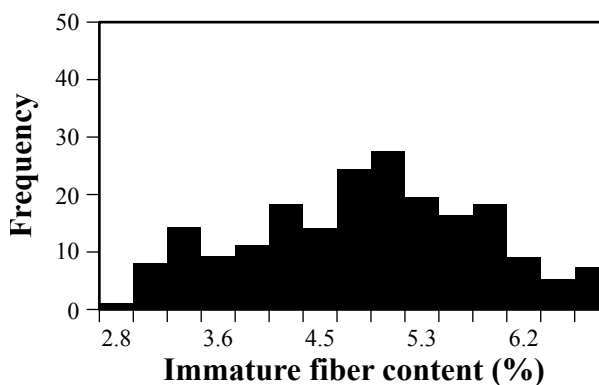


Fig. 6. Distribution of means for immature fiber content among 200 JC lines across environments.

Table 3. Genotypic correlation coefficients ( $r$ ) among AFIS-measured properties of JC germplasm

	Nep count	SCN size	SCN count	IFC	Lw	UQL	SFCn	Fineness	MR
Nep size	-0.21***	0.37	0.70***	-0.34***	-0.12	-0.25***	-0.47***	0.13	0.37***
Nep count		0.09	0.05	0.87***	0.38***	0.55***	0.74***	-0.75***	-0.86***
SCN size			0.14	-0.06	-0.09	-0.11	-0.11	0.06	0.04
SCN count				-0.13*	0.11*	0.00	-0.36***	-0.14**	0.14*
IFC					0.43***	0.61***	0.82***	-0.85***	-0.98***
Lw						0.96***	0.18***	-0.67***	-0.37***
UQL							0.44***	-0.74***	-0.55***
SFCn								-0.50***	-0.80***
Fineness									0.81***

<sup>z</sup> Values followed \*, \*\*, \*\*\* are significantly different at  $P<0.05$ ,  $P<0.01$ ,  $P<0.001$ , respectively.



Fineness was expected to affect nep content because finer fibers are more likely to bend and entangle than coarser ones (Van der Sluijs and Hunter, 1999). The negative (unfavorable) correlation between nep count and fineness ( $r=-0.75$ ) (Table 3) was consistent with this contention. However, the high heritability of nep count (0.79) indicates a large extent of genetic control in this trait. Conversely, the negative correlation between nep count and fineness does not necessarily mean a tight linkage between the genes controlling these two traits because the relationship between nep count and fineness was confounded by the relationships of nep count vs. maturity ratio ( $r=-0.86$ ) and fineness vs. maturity ratio ( $r=0.81$ ). These results implied that when fibers were more mature, neps were less common in ginned fibers and fiber became coarser. This situation is similar to that previously reported when the relationship between short fiber content and fineness was superimposed by their relations with maturity (Zeng and Meredith, 2009). These results indicate all three traits must be considered in selection for simultaneous improvement of nep count and fineness.

Genotypic correlation was also analyzed among AFIS-measured properties and other traits including lint yield and fiber properties measured by single-instruments, i.e., stelometer, fibrograph, micronaire, etc. (Table 4). Lint yield was negatively (favorably) correlated with nep count ( $r=-0.47$ ) and SCN count ( $r=-0.31$ ) although correlations were moderate or low. These results implied simultaneous improvement between lint yield and nep count was possible or at least there would be little compensation between the traits in selection. A low, but significant positive correlation was identified between nep count and elongation ( $r=0.32$ ) (Table 4). Fiber bundle strength was positively (unfavorably) correlated with SCN count ( $r=0.60$ ). A similar unfavorable correlation between strength and SCN ( $r=0.31$ ) was also observed in 38 cotton cultivars in a report by Boykin (2008). Nep count was unfavorably correlated to 2.5% span length ( $r=0.56$ ), but not correlated to 50% span length ( $r=0.10$ ). In our previous study, it was reported that 50% length was negatively (favorably) correlated to short fiber content ( $r=-0.40$ ) and 2.5% length positively (unfavorably) correlated to short fiber content ( $r=0.34$ ) (Zeng and Meredith, 2009). Close association between 50% length and short fiber content may contribute to the greater contribution of 50% length to fiber bundle strength than that of 2.5% length.

These results including the associations between nep count and fiber lengths identified in this study suggest a favor of 50% length over 2.5% in simultaneous improvement of neps, fiber length, short fiber content, and fiber bundle strength.

**Table 4. Genotypic correlation coefficients (r) of nep and seed coat nep (SCN) to other traits including lint yield and fiber properties measured by single-instruments.**

	Nep size	Nep count	SCN size	SCN count
Lint yield	-0.248 <sup>z</sup>	-0.467 <sup>***</sup>	-0.117	-0.313 <sup>***</sup>
Micronaire	0.193	-0.801 <sup>***</sup>	0.056	-0.051
Elongation	-0.001	0.318 <sup>***</sup>	0.046	0.191
Strength	0.480 <sup>***</sup>	-0.019	0.029	0.604 <sup>***</sup>
Length (50%)	0.175	0.098	0.001	0.354 <sup>***</sup>
Length (2.5%)	-0.229 <sup>*</sup>	0.557 <sup>***</sup>	-0.098	0.006

<sup>z</sup> Values followed \*, \*\*, \*\*\* are significantly different at  $P<0.05$ ,  $P<0.01$ ,  $P<0.001$ , respectively.

The unfavorable associations of nep count vs. fineness and SCN count vs. strength implies compensation in fineness and fiber strength when selections are made on nep content and seed coat fragment or vice versa, and thus warrants simultaneous selection among these traits. The relatively low nep count and SCN count as well as a relatively high fiber strength in FM960B2R (Table 2) demonstrated an example of how these traits can be simultaneously improved in breeding. In contrast, the high nep content and seed coat fragments in PHY72 with good fineness and fiber strength (Table 2) are consistent with the unfavorable interrelationships among these traits. In this study, eleven JC lines were selected based on two artificial selection criteria, i.e., (A) nep counts less than 90 cnt g<sup>-1</sup> and fineness lower than 180 mg km<sup>-1</sup> in matured fibers, arbitrarily defined as maturity ratio >0.95, and (B) SCN less than 6.0 cnt g<sup>-1</sup> and strength higher than 230 kN m kg<sup>-1</sup> (Table 5). There were two lines, JC26 and JC172, selected with nep count and fineness satisfying Criterion A and nine lines, JC30, JC35, JC51, JC69, JC90, JC119, JC153, JC171, JC182, selected with SCN and strength satisfying Criterion B. Because of unfavorable correlations between neps and fineness and between SCN and strength, it was not surprising that none was identified with desirable combination among all these properties. However, JC30 and JC171 are relatively desirable in combination among the properties listed in Table 5.

**Table 5. Means of lint yield, bundle strength, and the AFIS-measured properties in the 12 selected JC lines. The experiments were conducted at two locations in 2006 and one location in 2007.**

Entries	Lint Yield kg ha <sup>-1</sup>	Strength kN m kg <sup>-1</sup>	Length 50% mm	Length 2.5% mm	Nep size um	Nep count cnt g <sup>-1</sup>	SCN size um	SCN count cnt g <sup>-1</sup>	IFC %	UQL mm	SFCn %	Fineness mg km <sup>-1</sup>	MR
JC26	977	218	14.4	29.9	650	87.7	1237	3.67	4.43	31.3	15.7	178	0.960
JC30	956	235	14.2	28.4	654	76.2	1052	5.00	3.52	30.1	12.4	186	0.990
JC35	829	237	14.0	29.4	642	129	1000	3.67	4.88	30.4	17.1	167	0.945
JC51	473	231	13.8	28.0	651	103	949	5.50	4.85	29.5	15.3	170	0.955
JC69	622	258	14.3	28.8	649	114	989	5.83	5.18	30.1	13.7	163	0.930
JC90	905	241	14.5	31.4	641	135	988	5.17	4.68	32.7	16.4	173	0.958
JC119	743	236	14.3	27.9	646	99	1195	5.33	3.43	28.9	10.5	181	0.988
JC153	684	248	14.6	30.1	646	112	1312	5.17	4.17	30.3	15.0	169	0.963
JC171	447	257	13.8	26.3	672	94.3	1364	5.93	4.48	27.1	11.9	169	0.950
JC172	770	233	14.3	28.0	681	86.3	1400	12.10	4.13	28.8	12.1	179	0.962
JC182	877	232	13.7	26.4	677	70.5	1353	4.83	3.38	27.7	11.5	185	0.985

In summary, highly significant genotypic variation was identified for nep count and seed coat fragments in ginned fibers in JC germplasm. SCN count in JC germplasm was not significantly different from those in cultivars. Lint yield was negatively correlated to nep count and SCN count. Strength was positively correlated to SCN count. Although broad-sense heritability was determined to be high or moderate for nep count and SCN count in this study, it will be necessary to develop and analyze genetic populations to determine the correlated selection response and combining ability of these two traits with other fiber properties in selection. More importantly, the eventual effects of genetic improvement in neps and seed coat fragments in ginned fibers on yarn properties need to be determined in the future.

### DISCLAIMER

Mention of trade name or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation by the US Department of Agriculture.

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