

# Correlation Analysis of stem hardness traits with fiber and yield-related traits in core collections of *Gossypium hirsutum*

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## Research

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# Abstract

**Background** Stem hardness is one of the major influencing factors for plant architecture in upland cotton (*Gossypium hirsutum* L.). Evaluating hardness phenotypic traits is very important for the selection of elite lines for resistance to lodging in *Gossypium hirsutum* L. Cotton breeder are interested in using diverse genotypes to enhance fiber quality and high-yield. The research for hardness and its relation with fiber quality and yield were very few. This study was designed to find the relationship of stem hardness traits with fiber quality and yield contributing traits of upland cotton.

**Results** Experiments were carried out to measure the bending, acupuncture, and compression properties of the stem from a collection of upland cotton genotypes, comprising 237 accessions. The results showed that the genotypic difference in stem hardness was highly significant among the genotypes, and the stem hardness traits (BL, BU, AL, AU, CL, and CU) have a positive association with fiber quality traits and yield-related traits. Statistical analyses of the results showed that bending (BL, BU) has a maximum coefficient of variance and trait fiber length and fiber strength have less coefficient of variance among the genotypes. Principal component analysis (PCA) reduced quantitative characters into nine principal components. The first nine principal components (PC) with Eigenvalues >1 explained 86% of the variation among 237 accessions of the cotton crop. Both 2017& 2018, PCA results indicated that BL, BU, FL, FE, and LI variables contributed their variability in PC1 and BU, AU, CU, FD, LP, and FWPB have shown their variability in PC2.

**Conclusion** We describe here, to the best of our knowledge, the systematic study of the mechanism involved in the regulation of enhancing fiber quality and yield by stem bending strength, acupuncture, and compression properties of *Gossypium hirsutum* crop.

## Introduction

Cotton is one of the most important cash crops and the only major fiber crop in the world. The contribution of cotton to the total fiber used worldwide is about 35 percent (Zhang et al. 2014). Upland cotton (*Gossypium hirsutum* L.) accounts for more than 90 percent of the world cotton cultivated area, which reflects widespread adaptability and high yield production characteristics (Wendel, 1989; Chen et al. 2007). *Gossypium hirsutum* L. is allotetraploid ( $2n = 4x = 52$ ), and is composed of two ancestral genomes that are designated as At from *Gossypium arboreum* and Dt from *Gossypium raimondii* (Al-Ghazi et al. 2009). Due to long-term natural selection and artificial breeding, several cotton germplasm resources for sustainable genetic improvement have been created under varied climatic and cultivating conditions. In the National Gene Bank for Cotton, China, 7 712 *G. hirsutum* accessions are present. All these accessions were collected from cotton-producing countries around the world since 1865 when the United States introduced upland cotton (Dai et al. 2016). To efficiently use these resources, various efforts have been made to investigate and evaluate cotton diversity (Fang et al. 2017; Huang et al. 2017; Sun et al. 2017; Wang et al. 2017; Ma et al. 2018).

The yield and quality of the products are the most important factors for all crops (Fang et al. 2017). Stem hardness is a basic characteristic in the plant architecture of cotton and supports a large cotton load under adverse conditions. Stem hardness may have a relationship with yield and fiber quality. The physical characteristics contributing to the strength of stem are the bending force, puncture force, and compression force. The stem bending force is the force at which the trunk bends or breaks under a particular load. The basal portion of the culm internode plays a crucial role in ensuring that the plant remains upright (Peng et al. 2014). The greater carbohydrate accumulation in the base stem could increase the force need to bend the stem (Ishimaru et al. 2008). Stem thickness is a biological indicator of green or dry biomass. The strength (force, stress) and energy requirements are the compressive properties. Therefore, the selection of genotypes with increased stem strength is a useful field indicator (Beeck et al. 2006). Compression properties of stem depend on species, variety, stalk structure, stalk diameter, maturity, moisture, and cell structure (Persson 1987). A physical quantitative measurement may enhance selection effectiveness and boost genetic gain, such as penetrometer sorghum measurement (Pedersen & Toy 1999). Therefore, a greater understanding of these parameters provides a theoretical basis to enhance the physical strength of the stem and basal part of the culm internode, to obtain higher yield and good fiber quality of cotton.

Amorphous fibrils, lignin, and pectin, present in the cell wall, are also known to enhance the strength and hardness of the stem (Mohsenin 1986). Lignin or cellulose generally determines physical strength, as low content of lignin or cellulose causes a brittle culm (Tanaka et al. 2003). In the wheat plant the mechanical strength of the stem provided by cellulose and lignin to the lodging resistance in plants (Cai et al. 2019). The selection of elevated stalk strength and resistance to the corn borer (genus: *ostrinia*, species: *O. nubilalis*) increases the elements of cell walls in the breeding program (Li et al. 2016). Cotton has a high biomass output and a high cellulose and lignin proportion. In mature cotton fiber, the secondary cell wall (SCW) includes over 90% cellulose and it differs from all other known species of plant by having the only known source of cellulose in such high concentration and purity. By contrast, typical SCWs contain 40-50% cellulose in dicotyledonous stem xylem (Huang et al. 2016). An exhaustive study of stem hardness in cotton might, therefore, allows the cell wall to be modified to improve fiber quality and quantity because plant cell wall has a close association with mechanical and biochemical strength of stem parameters.

Principal component analysis (PCA) has been used extensively in the plant sciences for variable reduction and genotype grouping. This is the most prevalent statistical multivariate method used in environmental studies (Tahri et al. 2005; Yongming et al. 2006). PCA commonly used in the analysis of the relationships between observed variables and in the extraction of a small number of autonomous factors (major component) (Tokalioglu S et al. 2006). It commences with the correlation matrix, it describes the dispersion of the original variables and extracts eigenvalues and eigenvectors (Astel et al. 2008). The eigenvector is a list of coefficients that multiply the original correlated variants to obtain new uncorrelated (orthogonal) principal components that are linearly weighted combinations of the original variables. The number of correlated variables can be reduced to a smaller set of orthogonal factors, which allows the interpretation of a specified multidimensional system by showing correlations between

the original variables. The analysis of the correlations also reflects a related response of a given character and also provides a good index for predicting the corresponding change in one character to the extent of the proportional change in the other. PCA was used by Kamara et al. (2003) to identify maize (*Zea mays* L.) traits which accounted for the majority of variance in the data. Granati et al. (2013) have used PCA to investigate the relationship between *Lathyrus* accessions. Žáková and Benková (2006) identified the traits of 106 Slovak barley accessions as the primary sources of variations in genetic diversity. PCA and cluster analysis were used by Cartea et al (2002), Salihu et al. (2006), respectively to group kale populations and genotypes of winter wheat. The current study examined the multivariate data analysis of agronomic and quality characteristics of a global collection of 237 genotypes.

Some studies have been conducted on stem strength behaviors of different plants; however, for stem hardness characteristics of cotton stalk, no data is reported. The present research, therefore, seeks to establish a relationship between stem hardness and yield characteristics and quality characteristics in *Gossypium hirsutum*. However, the yield is a complicated, multicomponent controlled character. Stem hardness components are less sensitive than yield per se to the environmental changes and are therefore comparatively more likely to improve with selection. Once the nature and extent of relations among these component characteristics and yield are understood, the effectiveness of choice in the segregated generation will improve. Therefore, the present research was carried out to assess the PCA and correlations of significant *Gossypium hirsutum* characteristics.

## **Materials And Methods**

### **2.1. Cotton Accessions**

From a set of 7 362 *G. hirsutum* accessions, preserved at the China National Gene Bank, Cotton Research Institute, Chinese Academy of Agriculture Sciences, Anyang, Henan, 237 cotton genotypes were selected. These accessions have various geographical origins including China, the United States, the former Soviet Union, Australia, Brazil, Pakistan, Mexico, Chad, Uganda, and Sudan, which are most of the world's largest cotton-growing areas.

### **2.2. Planting and phenotyping**

Phenotyping of stem hardness-related features was performed during the normal cotton-growing season (mid-April to late-October) at Cotton Research Institute, Anyang, Henan, China (Yellow River command area) for two years i.e., 2017 and 2018. Coordinates of the location are E 114.07° and N 35.85°, longitude, and latitude respectively. All accessions (237) were planted in a randomized complete block design with three replicates in the experimental field. Each entry plot had a dimension of 7m × 3m and row-to-row and plant-to-plant distance was 30cm and 76cm respectively. Field management practices were conducted according to the local management scheme. The scoring standards for phenotypic traits in both years were identical. Six stem hardness traits and 14 agronomic traits were characterized.

### **2.3. Sample preparation for stem hardness traits**

The stems were cut and separated from the branches after harvesting the cotton genotypes. Stem samples were air-dried for two months in the lab. At the time of hardness testing, the air-dried cotton stem had low humidity content. The stem was equally divided into two parts for the preparation of test samples: Upper and lower ((Additional file 2: Figure S1a).

## 2.4. Stem Hardness Traits

For each replicate, three plants were selected to test the hardness of the stem. These characteristics were the Breaking force of the Upper part (BU), the breaking point of the lower part (BL), the Compression force of upper and lower part (CU and CL), and acupuncture force of the upper and lower part (AU and AL). The YYD-1 SS testing system (TOP Instrument Co., Zhejiang, China) was used to measure all hardness characteristics of a 15 cm segment from the lower, and upper part of the stem (Additional file 2: Figure S2b). The tester was set perpendicular to the culm at the middle, under gradual loading, and the breaking force was measured when the culm was pushed to breaking point. The maximum force in Mega Newtons needed to break, puncture & compress the center of the two segments of the stem (upper and lower) was recorded.

## 2.5. Agronomic traits

Days to first flower opening, FD (days) were calculated from the date of sowing to the day when first flowers bloomed on 50 percent of the plants in each plot. Plant height (PH) recorded from the base of the plant above ground to the tip of the plant. Ten consecutive plants were selected for plant height in each plot. From each accession, 30 naturally opened bolls were harvested randomly to calculate boll weight (BW) in grams and to gin the fiber. The Seed Index (SI) was calculated after counting and weighing 100 cotton seeds. Fiber samples were separately weighed to calculate the Lint percentage (LP) and Fiber weight per boll (FWPB) in grams. The Lint index (LI) was calculated based on SI and LP data.

**See formula 1 in the supplementary files.**

Fiber samples were examined in the Cotton Quality Test Center in Anhui, China for fiber-quality characteristics using a high-volume instrument (HFT9000). Data on the fiber length (FL, mm), fiber strength (cN/tex), micronaire value (Mic,  $\mu\text{g}/\text{inch}$ ), elongation percentage (EP, %), Length uniformity (LU, %), Spinning Consistency Index (SCI) recorded. The average of the three replicates in the same year is defined to be phenotypic information per accession.

## 2.6. Statistical analysis

For the evaluation of phenotypic traits statistics, Minitab 18 and R were used. The primary impacts of the experimental variables and their relationships were analyzed by the analysis of variances (ANOVA). The significance level for ANOVA was set at  $p \leq 0.05$ . R software (package "corrplot") was used for calculating and plotting correlation. The principal component analysis was performed using Minitab 18.

# Results

## 3.1. Stem hardness variations among the genotypes

The ANOVA given in shows in Tab. 1. that genotypic difference in stem hardness was highly significant for traits like bending (BL and BU), and compression CU ( $p > 0.05$ ). Basic descriptive statistics (mean, standard deviation, minimum, maximum and coefficient of variance) of all the genotypes for morphological, yield and fiber traits were studied (Additional file 1: Table S1) It was observed that maximum coefficient of variance (26.67% & 20.40) was recorded in bending (BU & BL) respectively, which mean and SD was (0.08 & 0.19) and (0.02 & 0.04) followed by acupuncture upper and lower (17.81 & 15.76) compression upper and lower (14.96 & 13.45), seed index (14) and fiber length (14) with a mean and SD of (0.07, 0.08, 0.54, 0.82, 12.30 & 30.39) and (0.01, 0.01, 0.08, 0.11, 1.43 & 1.82) respectively. The traits like fiber elongation (1.47) and fiber strength (3.43) have comparably less coefficient of variance among the genotypes. A similar result was found in 2017 data.

The results showed (Additional file 1: Table S2) the variation among different varieties. Based on the bending trait values all 237 accessions of cotton were differentiated into two groups, higher stem hardness (HSH) because those varieties that have a higher value of bending trait and lower stem hardness (LSH) because those varieties have lower values of bending traits. In Table S2. Only mentioned in detail are six HSH genotypes and six LSH genotypes.

## 3.2. Principal component analysis

The principal component analysis was performed to only the Principal Component (PCs) with an eigenvalue higher than 1 according to the Kaiser (1960) criterion. Thus PC1, PC2, PC3, PC4, PC5 and PC6 (Tab. 2) were selected as these represented 23%, 14%, 12%, 8%, 8% and 6% of progeny variation, respectively, and accounted for 73% of the overall diversity. PC7, PC8 and PC9 variances represented a cumulative percentage of 78%, 82% and 86% respectively. Table 2 summarizes the PCs and the eigenvectors, which were estimated on the average of twenty variables. All twenty traits contributed to the total variation in PC1, but Fiber length (FL), uniformity percentage (UP), fiber elongation (FE), Lint index (LI), Bending lower (BL) and bending upper (BU) have contributed more in PC1. PC1 is a weighted average of these characters indicating that fiber quality traits have significant importance for this component. On the other hand, other traits are less important to PC1. While in PC2, all variables are significantly contributing the main contributors to variation were bending upper (BU), acupuncture upper (AU), compression upper (CU), days to flowering (FD), lint percentage (LP) and fiber weight per boll (FEPB) So Yield traits have more contribution in PC2. For PC3, bending lower (BL), compression lower (CL), compression Upper (CU) bending upper (BU), Maturity (M) and Spinning consistency index (SCI) is the most important trait, while multiple traits contributed to the other PCs in varying proportions and the same trend as found in the 2017 study result.

## 3.3. Stem hardness correlation with fiber quality traits

The result of the 2018 correlation of stem hardness indicated that bending lower (BL) has a positive association with fiber length, micronaire value, uniformity percentage, fiber elongation, spinning consistency index, and days to flowering (Fig. 1). Bending upper (BU) was a positive correlation with FL, UP, SCI, and FD. Compression lower (CL) was a positive correlation with FL, M, SCI, and FD, while compression upper (CU) was a positive correlation with LU, SCI, and FD. The same result was found in the 2017 correlation of stem hardness with fiber-related traits (Additional file 2: Figure S2).

### 3.4. Stem hardness correlation with yield-related traits and morphological traits

In Fig. 2, the 2018 correlation result showed that Bending lower (BL) was highly positively associated with PH, GP, LP, and FWPB, while bending upper (BU) was in positive association with PH, GP, and LI. Acupuncture lower (AL) showed a positive correlation with PH. Compression (CL and CU) has a positive correlation with PH, GP, BW, and LI. We also found the same correlation trend of all traits in 2017 data (Additional file 2: Figure S3).

## Discussion

In the last decade, there has been great progress in developing new cotton genotypes for better fiber quality and higher yield. The stem associated characteristics such as bending, acupuncture, and compression may be used to determine the yield and quality of the fiber. One factor influencing crop quality and yield is plant height (Tang JH 2007). The fiber quality parameters on which textile processing and the quality of the item rely, are fiber strength and length because those attributes receive premium pricing (Hussain K 2010).

Our breeding program goal for *G. hirsutum* was the identification of high-yield genotypes and some agronomic features that are easily evaluated and linked with these characteristics that could be used as markers (Biyun et al. 2014, Chen et al. 2007). In this study, we observed that the bending, compression, and acupuncture related to stem hardness have a positive and substantial correlation with the fiber length, spinning consistency, and flowering times. Pettigrew (2001 & 2008) reported that an increase in light and temperature also increased the strength, the difference was however not enough to cause a yield penalty. Our findings showed a positive association of length uniformity, micronaire values with stem hardness characteristics. Fiber fineness was positively associated with fiber length and fiber strength by Killi et al. (2005). There was a negative association of fiber fineness with a fiber strength and fineness with a fiber strength and fiber uniformity ratio. The fiber strength showed a positive correlation with fiber uniformity. Mature cotton fibers are approximately 95% cellulose with other polysaccharides such as arabinose, galactose, and xylose (Meinert and Delmer 1977) and pectin (Meinert and Delmer, 1977; Wang et al. 2010). These may enhance fiber strength by joining cellulose fibrils. A direct correlation between cellulose molecular weight and fiber strength was reported by Timpa and Ramey (1994). Though there may be an extra metabolic cost of these polysaccharides, (Amthor 2010), it is in such a small proportion to the cellulose that it, seems unlikely to be a yield drain for such small fractions of the fiber. There still exists the long-held negative association or trade-off between yield or mass of fibers and

quality components, such as strength, of individual fibers should be investigated further. Fiber diameter reduced from bottom to top, possibly because cell wall thickness decreased. Similarly, this can be explained by the fact that development in cell walls depends upon the accumulation of metabolism products (cellulose, hemicellulose, lignin, waxes, etc.) that rises with maturity (C. Ververis et al. 2003). The major requirement for increasing rice grain yield is to enhance the physical strength of the culm to enhance the breaking-type lodging resistance (Hirano et al. 2014). It has therefore been concluded that stem-related characteristics like bending stress have determined the morphology and the quality of the culm, such as cellulose, lignin, pectin inside the cell wall, which have a direct relationship to high yield and crop quality.

The Lint Index is the main feature and contributes significantly to the lint percentage increase. The promising cultivars showed a maximum lint percentage due to the close association of the lint index (LI) with the lint percentage. Our current findings have demonstrated an important correlation between the LI & growth period (GP) with the bending (BL and BU) and Compression (CL and CU). Bending lower (BL) was positively associated with lint yield features such as LI and GOT%. The GOT% has a positive and substantial correlation with the lint index, according to (Hussain K 2010). Positive observations were found by Scholl and Miller (1976), while Tyagi (1994) stated that the GOT% was negatively associated with the lint index. Positive associations between lint index and lint percentage suggest an increased cottonseed yield. This result indicates that the lint index and the lint percentage are significant elements for enhancing cotton yield and should be considered during the breeding program.

Plant height (PH) is one of the major morphological features, which plays a key part and closely associated with plant bolls (if there is no lodging) with the ultimate positive impact on cotton yield. Because of the lodging risk and appropriate for mechanical picking, cotton breeders are mostly interested in short-stature plants, but the plant height is strongly associated with bolls per plant and seed yields (Khan 2003). The present correlation results showed that Bending, Acupuncture & Compression have a positive correlation with PH. The stability and adaptability of *G. hirsutum* cultivars were studied by Meena et al. (2007) and reported varied values for yield components and plant height. The varieties of upland cotton were also evaluated by Suinaga et al. (2006) and it was found that the plant height was associated positively with the seed cotton yield and bolls per plant. The positive correlation between plant height and cotton yield seed was observed by Khan (2003), Soomro et al. (2005), and Taohúa and Haiping (2006), and their research showed that plant height contributed 70% of the total variability in seed cotton yield. Therefore, it is concluded that in cotton crops, the height of the plant is desirable if no lodging occurred.

Obtaining high seed yield per unit is one of the most important challenges in *G. hirsutum* breeding. Several agronomic traits are important for improving yield traits. Boll weight is the second major yield component and has a greater contribution to the enhancement of seed cotton yield. A similar proportion and variation for boll weight concerning the cottonseed yield was observed by Khan (2003) and Copur (2006). For yields and other economic characters, Taohua and Haipeng (2006) and Meena et al. (2007) assessed various *G. hirsutum* varieties, significant variations were observed for boll weight and the effect

on cottonseed yield was positive. The correlation result showed that Bending lower (BL) and compression upper (CU) have a positive correlation with BW, GP, and FEPB. Batool et al. (2010) and Makhdoom et al. (2010) also stated that boll weight was positive for yield and had a higher contribution to the yield improvement for upland cotton. Results also revealed that the boll weight following the bolls per plant had a positive effect on seed cotton yield. Therefore, it is concluded that boll weight is an important yield component and should be kept in mind while breeding for seed cotton yield.

Numerous characteristics affect the final yield of cotton as well as quality attributes. This study measured stem hardness and agronomic characters of a diverse set of cotton accessions to assess their variability and inter-relationships. Stem hardness may have a direct effect on yield by reducing stem lodging and an indirect effect on fibers because of possible shared pathways in strengthening cell walls.

## Conclusions

An evaluation of 237 accessions of the Chinese Cotton Germplasm Collection for stem hardness and agronomic characters showed sufficient variation to enable selection to improve several of these traits for cotton improvement. The data suggests inter-relationships among several traits and may simplify the improvement of multiple traits at once. Of most interest was the data suggesting relationships between stem hardness and fiber quality traits. Selection for stem hardness may improve yield by reducing losses due to stem lodging and improve fiber characteristics because of the possible sharing of pathways in strengthening cell walls of both structures. These analyses greatly simplify the breeder's selection of germplasm from a large collection and may speed up favorable gene accumulation in breeding schemes like recurrent selection for these traits. Also, the phenotypic data for stem hardness may be used in our subsequent genome-wide association studies for *G. hirsutum*.

## Declarations

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### Authors' contributions

Raza I, Du X designed the study. Raza I, Hu DW, Li H H, He SP, Nazir MF, Yasir M, Iqbal S, Wang XY, Jia YH, Pan Z, Geng XL, Wang LR and pang BY performed the experiment and collected data. Raza I. and Adeel A analyzed the data. Raza I wrote the manuscript. Du X, Adeel A, Hu DW review the manuscript. Supervision: Du X. All authors read and approved the final version of manuscript.

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### **Availability of data and materials**

No other data related to this study is available at this time.

### **Ethics approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable

### **Competing interests**

The authors declare that they have no competing interests

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## Additional Files

**Additional file1: Table S1.** Descriptive statistics of 20 traits in 2017 and 2018

**Additional file1: Table S2.** Stem hardness variation among *Gossypium hirsutum* genotypes

**Additional file2: Fig.S1a.** Parts of cotton dry stem

**Additional file2: Fig.S1b.** Plant stem strength meter

**Additional file2: Figure S2:** Frequency distribution of phenotypic variation of six stem hardness related traits BL: bending lower; AL: acupuncture lower; CL: compression lower; BU: bending upper; AU:

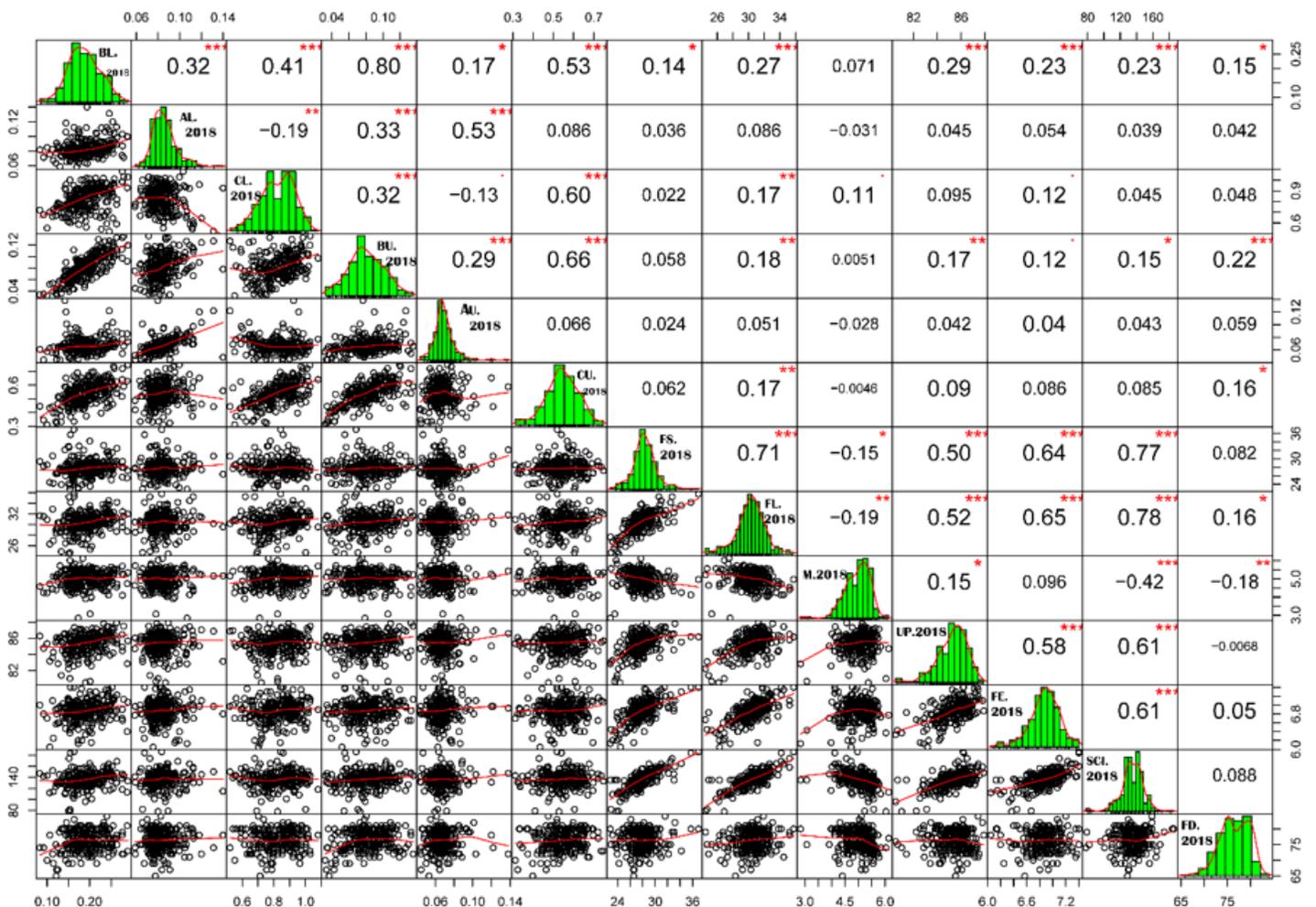
acupuncture upper; CU; compression upper: and 7 fiber-related traits and correlation coefficients among the traits in 237 accessions. FS: fiber strength; FL: fiber length; MI: micronaire Index; E: elongation ratio; UP: Uniformity percentage; FE: fiber elongation; SCI: spinning consistency index; FD: days to flowering. \*\*\* Indicates extremely significant difference at  $p = 0.05$  probability levels.

**Additional file2: Figure S3:** Frequency distribution of phenotypic variation of six-stem hardness related traits BL: bending lower; AL: acupuncture lower; CL: compression lower; BU: bending upper; AU: acupuncture upper; CU; compression upper: and seven yield-related traits and correlation coefficients among the traits in 237 accessions. PH: plant height; BW: boll weight; GP: growth period; LP: lint percentage; LI: lint index; SI: seed index index; FWPB: flower weight per boll. \*\*\* Indicates extremely significant difference at  $p = 0.05$  probability levels

## Tables

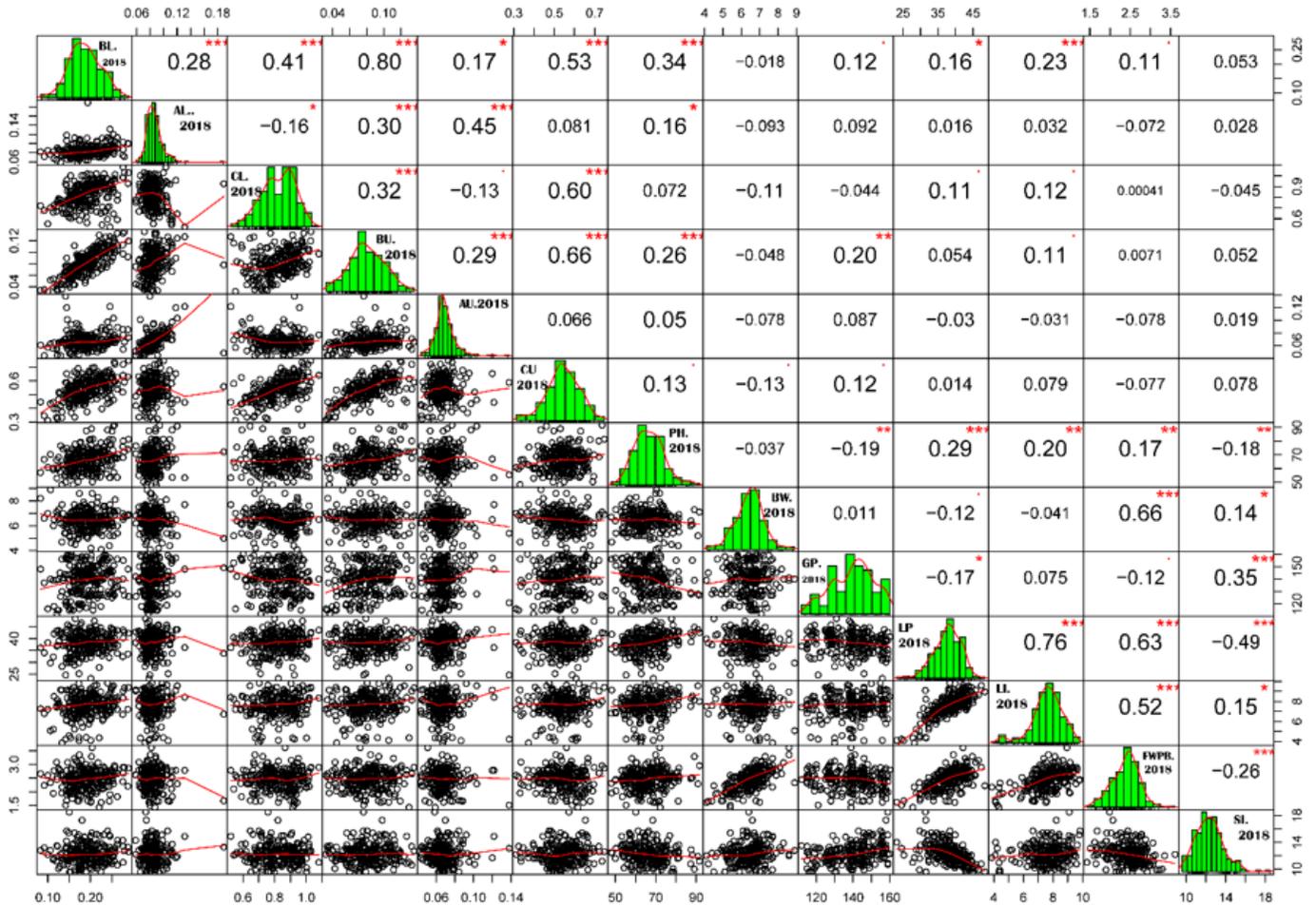
Due to technical limitations, the tables can only be accessed as a download in the supplementary files.

## Figures



**Figure 1**

Frequency distribution of phenotypic variation of six stem hardness related traits BL: bending lower; AL: acupuncture lower; CL: compression lower; BU: bending upper; AU: acupuncture upper; CU; compression upper: and seven fiber-related traits and correlation coefficients among the traits in 237 accessions. FS: fiber strength; FL: fiber length; MI: micronaire Index; E: elongation ratio; UP: Uniformity percentage; FE: fiber elongation; SCI: spinning consistency index; FD: days to flowering. ☒☒☒ Indicates extremely significant difference at p = 0.05 probability levels.



**Figure 2**

Frequency distribution of phenotypic variation of six-stem hardness related traits BL: bending lower; AL: acupuncture lower; CL: compression lower; BU: bending upper; AU: acupuncture upper; CU; compression upper: and seven yield-related traits and correlation coefficients among the traits in 237 accessions. PH: plant height; BW: boll weight; GP: growth period; LP: lint percentage; LI: lint index; SI: seed index index; FWPB: flower weight per boll. ☒☒☒ Indicates extremely significant difference at p = 0.05 probability levels.

## Supplementary Files

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