



**Full Length Article**

## Genetic Basis of Variation for Fiber Quality and Quality Related Biochemical Traits in Bt and non-Bt Colored Cotton

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### Abstract

Colored cotton is an environment friendly raw material for textile industry and in the recent past rapid adoption of Bt cotton by the farmers is provoking the researchers to develop a resilient breeding strategy for superior non-Bt and Bt colored cottons. We studied the genetic basis for boll weight, fiber quality, cellulose and flavonoids contents using combination of line × tester (8 × 3), genotype-by-traits, correlation and path coefficient analyses. Plant population was developed by manual hybridization of eight white and three colored cotton genotypes. The study revealed the non-additive gene action *i.e.*, low  $G^2_{gca}$  and narrow sense heritability for all traits except flavonoid contents supported the adoption of heterosis breeding coupled with rigorous selection for superior colored cotton development. In parents selection, combine or separate use of genotype-by-trait analysis and *gca* effects revealed the good performance of MNH-886 and FH-142 for boll weight and fiber quality traits and their high *gca* effects. Best specific combination for boll weight, fiber length and fiber strength was observed in Bt green cotton hybrids VH-303 × BZUG2. While an interspecific green hybrid GIZA-7 × BZUG1 had maximum significant *sca* effects for fiber length and fiber uniformity. Significant positive association of cellulose contents with boll weight and fiber quality traits in the presences of negative direct and indirect effects of flavonoid contents on fiber quality and boll weight suggested that rigorous efforts are needed for the development of elite colored cotton genotypes. © 2018 Friends Science Publishers

**Keywords:** Coefficient of correlation; Gene action; Genotype-by-trait analysis; **General and specific combining ability**

### Introduction

Natural dyes have been used for fabric dyeing since ancient times, but their limited and narrow color range arose the demand of synthetic dyes. The massive applications of synthetic dyes resulted in huge production of industrial effluents, irrational utilization of energy and ultimately high cost of textile products (Dickerson *et al.*, 1996). Moreover, the effluents of textile industries have heavy metals like copper, lead, cadmium and mercury threatened biodiversity around the globe (Kant, 2012). In this scenario, naturally colored cotton is an attractive proposition for textile industry to mitigate the harmful effects of fabric dyeing process (Waghmare and Koranne, 1998). Colored cotton is available in different colors including green, blue, brown and white. However poor fiber quality has hampered the use of naturally colored cotton for manufacturing of textile products (Dutt *et al.*, 2004). It is observed that fiber quality of green, brown and white cotton is highly dependent upon quantity of cellulose and its biosynthesis (Cannell and Thornley, 2000; Hua *et al.*, 2007; Yuan *et al.*, 2013). In colored cotton cellulose and pigment biosynthesis occur simultaneously during the process of fiber development (Hua *et al.*, 2007; Xiao *et al.*, 2007; Zhang *et al.*, 2011) and

the utilization of same source of energy for pigment as well as for cellulose synthesis lead to the poor fiber quality (Zhao, 2003). Good fiber quality is imperative for the textile industry because it directly affects processing performance, productivity, yarn quality, and the marketing of textile goods. The advancement in spinning and weaving technologies and introduction of Bt gene in cotton mandated the plant breeders and geneticists to develop Bt and non-Bt colored cotton genotypes with good fiber quality.

The choice of any breeding procedure for the improvement of plant materials depends upon the knowledge of inheritance of important traits (Esmail, 2007). Line×tester analysis is considered as an efficient biometrical approach to select the desired parental lines and hybrids, and has been widely used by the cotton breeders (Ahuja and Dhayal, 2007; Basbag *et al.*, 2007; Basal *et al.*, 2009; Anandan, 2010). It provides better understanding about the general combining ability (*gca*) and specific combining ability (*sca*) of selected parental lines and also depicts the nature and magnitude of gene action involved in the inheritance of different traits that helps the breeders in the development of cultivars and hybrids (Pradhan *et al.*, 2006). Moreover different biometrical techniques are

available to compute the index of transmissibility of traits to next generations and the heritability estimates of a trait guide plant breeders to predict behavior as well as response of selection in the succeeding generations (Haq *et al.*, 2008).

The performance-based characterization of the collected and newly developed plant materials is very important for reliable results and can be achieved using genotype by trait analysis (Malik *et al.*, 2013). Further, phenotype of studied plant is the result of complex nature of association/interaction among genes involved in the inheritance of traits and it is very much difficult to have harmonious combination of desired traits in plants (Naveed *et al.*, 2004). Most of previous studies had reported the existence of negative association among fiber quality and different agronomic traits (Ulloa, 2006; Feng *et al.*, 2011; Malik *et al.*, 2013) and especially in colored cotton, it is challenging to improving fiber color and quality simultaneously (Du and Shi, 2002). Furthermore, fiber yield and quality are complex traits and depend upon several components of traits. Therefore direct selection for these traits is often not fruitful. Correlation and path coefficient are proved to be effective tools to probe direct and indirect relationship among different traits (Guler *et al.*, 2001; Basal *et al.*, 2009). The current study was designed to choose the best genotypes and to develop a breeding strategy for the improvement of fiber yield without compromising the fiber quality in colored cotton.

## Materials and Methods

### Plant Materials

Five non-Bt cotton genotypes (CRSM-38, MNH-814, MNH-552, White exotic and GIZA-7), and three Bt cotton (MNH-886, VH-303 and FH-142) were used as female parents (lines). Three colored cotton genotypes *i.e.*, BZUG1 (green), BZUG2 (green) and BZUB (brown) were used as male parent (tester). Among parents only GIZA-7 belongs to *Gossypium barbadense* L. having extra long and good fiber traits. While rest of lines and testers were from *Gossypium hirsutum* L. and belongs to different cotton research institutes. The CRSM-38, MNH-814 and MNH-552 are non-Bt high yielding with good fiber quality traits and developed by the Cotton Research Institute, Multan. The white exotic is exotic cotton genotype with good fiber quality. The selected Bt cotton genotypes MNH-886, VH-303 and FH-142 have considerable tolerance against CLCuV and developed by Cotton Research Institute, Multan, Cotton Research Station, Vehari and Cotton Research Station, Faisalabad respectively. These genotypes are dominantly being cultivated in cotton growing areas of Pakistan. The colored cotton genotypes BZUG1, BZUG2 and BZUB have average fiber quality traits and were collected from the germplasm maintained at the experimental farm of Department of Plant Breeding and Genetics, Bahauddin Zakariya University, Multan Pakistan.

## Development of Breeding Population and Field Evaluation

Seed of eight female (lines) and three males (testers) were sown in 25 cm × 35 cm earthen pots, containing nutrient rich soil. Inside the glasshouse temperature 30 ± 5°C was maintained during the day and 25 ± 5°C at night using automatic cooling and heating systems. In earthen pots 3-5 de-linted seeds were sown at a depth of about 15 mm and covered by a thin layer of fine soil. Twelve days after the germination of seeds, seedlings were thinned to two plants per pot. At the onset of flowering, crosses were attempted manually in line × tester fashion (8 × 3) to obtain F<sub>0</sub> seed for growing F<sub>1</sub> generation. During next normal growing season *i.e.*, mid of May the intraspecific, interspecific F<sub>1</sub> hybrids and their parents were grown in the experimental field following randomized complete block design having three replications. The plant-to-plant and row-to-row distance was 30 cm and 75 cm respectively. Recommended cultural and plant protection practices were adopted from sowing to harvesting of crop to have good crop stand.

### Analysis of Boll Weight and Fiber Quality Traits

Each parental and F<sub>1</sub> generation was represented by 15 plants in each replication. Ten plants of parents and F<sub>1</sub> generation from each replication were selected for sampling and 30 mature bolls from upper, middle and lower parts of each plant were handpicked. The dried and cleaned seed cotton samples were ginned using single roller gin and analysis of fiber quality traits *i.e.*, fiber length (mm), fiber strength (g/tax), fiber fineness (µg/inch), fiber maturity (%) and fiber uniformity (ratio) was carried out using High Volume Instrument (HVI-1000 USTER, America). The boll weight was calculated using following formula:

$$\text{Boll weight (g)} = \frac{\text{Seedcotton weight per sample}}{\text{Number of bolls in a sample}}$$

### Analysis of Biochemical Traits

The separated fiber from the mature bolls of interspecific, intraspecific hybrids and their parents were used for the analysis of cellulose and flavonoids contents.

### Cellulose Contents

Extraction of cellulose contents (%) was done following the method described by Pan *et al.* (2010) with little modifications. In brief, 2 g of fiber sample and 350 mL of 95% ethanol were taken in 500 mL flask of Soxhlet apparatus and distillation was carried out for 6 h. After heating, the fiber was then taken out using forceps and dried in an oven at 60 ± 5°C for 12 h. The dried fiber was weighed by electric balance and the difference in fiber weight was calculated as cellulose contents.

## Total Flavonoids

Amount of total flavonoids (mg/g) in mature cotton fibers was determined following Dutt *et al.* (2004) with little modification. Briefly, 2 g fiber sample along with 100 mL solution of HNO<sub>3</sub> and ethanol (1:3 v/v) was distilled for 4 h until the color of cotton fiber was faded to white. The solvent was filtered twice using filter paper and required amount of HNO<sub>3</sub> and ethanol (1:3 v/v) was added to maintain the final volume up to 100 mL. The total flavonoid concentration was determined using Rutin (R5143, Sigma-Aldrich) as a standard curve (Wang *et al.*, 1999).

## Statistical Analysis

The data were analyzed following line × tester method described by Kempthorne (1957) as explained by Singh and Chaudhary (1999). The sum of squares for genotypes was subdivided into variation among parents, hybrids and parents × hybrids. The sum of squares for parents was also subdivided into variation among lines, variation in testers and variation among lines × testers. The mean sum of squares, covariance of full sib and half sib were used to calculate the variances for *gca* and *sca*. The estimates of narrow sense heritability were computed by given formula:

$$\text{Narrow sense heritability } (h^2) = \frac{\sigma_A^2}{\sigma_P^2}$$

Genotype-by-trait analysis (Ogunbayo *et al.*, 2005) was done using XLSTAT statistical software. Co-efficient of correlation and frequency distributions were calculated by R-package using mean values for each trait. The path coefficient analysis described by Dewey and Lu (1959) was performed to estimate the possible casual linkage between studied variables using SPSS-AMOS version-20.

## Results

### Genetic Effects

Significant variation caused by treatments, parents, crosses and parents vs. crosses for most of the studied traits (Table 1) was found. The estimates of  $\sigma^2_{gca}$  (variance due to general combining ability) were lower than  $\sigma^2_{sca}$  (variance due to specific combining ability) for all traits except flavonoid contents. The results revealed that combining ability ratios ( $\sigma^2_{sca}/\sigma^2_{gca}$ ) were more than 1 for all the traits excluding flavonoid contents having less than 1 degree of dominance ( $\sigma^2_D/\sigma^2_A$ )<sup>1/2</sup>. The amount of narrow sense heritability ranged from 1.88% to 65.18% and maximum amount of heritability was for flavonoid contents. The proportional contribution in-term of total variance of lines (female) was more than tester and line × tester interaction for boll weight and fiber fineness. Similarly, the male parents (testers) contributed more for fiber strength and flavonoid contents (Table 2).

## Combining Ability Estimates

The estimates of *gca* effects of 11 parents (lines and testers) significantly varied for boll weight, fiber quality, cellulose and flavonoids contents. The line MNH-886 showed maximum positive *gca* effects for boll weight and fiber maturity. While cotton genotype FH-142 was better for fiber strength and fiber fineness. GIZA-7 (*G. barbadense*) had maximum *gca* effects for fiber length. The data also revealed that among lines white exotic had maximum *gca* effects for cellulose contents followed by VH-303 and FH-142. Among testers a brown cotton genotype BZUB was better general combiner as it showed positively significant maximum *gca* effects for boll weight, fiber strength, fiber uniformity and flavonoid contents. The tester BZUG1 also showed positive *gca* effects for boll weight, fiber strength, fiber maturity, fiber uniformity and cellulose contents (Table 3). Different crosses have significant *sca* effects in desirable direction for different traits in intraspecific and interspecific hybrids. The intraspecific Bt green cotton hybrids VH-303 × BZUG2 showed maximum positive and significant *sca* effects for boll weight (0.33), fiber length (2.17) and fiber strength (2.01). Similarly Bt green cotton FH-142 × BZUG1 occupied second position with medium *sca* effects (0.28) for boll weight. While interspecific non-Bt green cotton hybrid GIZA-7 × BZUG1 had maximum significant *sca* effects (2.64) for fiber length and fiber uniformity. In the case of fiber strength, intraspecific Bt- green cotton hybrid MNH-886 × BZUG1 was found best specific combiner with significant maximum *sca* effects (2.68) followed by another intraspecific Bt green hybrid VH-303 × BZUG2 (2.01). Among all hybrids, intraspecific hybrids MNH-814 × BZUB (non Bt brown cotton) had significant positive *sca* effects (3.19) for cellulose contents which is followed by Bt green cotton hybrid FH-142 × BZUG1 having value 1.55 (Table 4).

### Genotype-by-Trait Analysis

The biplot revealed that parental lines MNH-886, MNH-552 and FH-142 were best for fiber fineness, fiber strength, boll weight and cellulose contents. Similarly, CRSM-38 and VH-303 showed better performance for fiber maturity, fiber uniformity and length. However, green cotton genotype BZUG1 was best in flavonoid contents. Among the hybrids Bt brown cotton hybrids FH-142 × BZUB and VH-303 × BZUB were best for fiber fineness, fiber strength, boll weight and cellulose contents and non-Bt interspecific brown hybrid GIZA-7 × BZUB performed well for fiber maturity, uniformity and length. Trait vectors for fiber fineness, strength, uniformity and length depicted more variation as compared to others because of their more length from the origin of biplot (Fig. 1).

**Table 1:** Mean squares for boll weight and fiber quality in colored cotton

Sources	D.f	Boll weight	Fiber length	Fiber strength	Fiber fineness	Fiber maturity	Fiber uniformity	Cellulose	Flavonoids
Replication	2	0.0	3.95	2.88	0.24	4.53	6.99	12.38**	0.00
Treatment	34	0.78**	17.73**	50.73**	1.30**	16.03**	58.87**	17.81**	0.37**
Parent	10	1.40**	18.10**	133.93**	2.05**	18.12**	123.34**	43.40**	0.79**
Parent vs. crosses	1	0.15	5.79	16.46	2.53**	0.09	161.36**	5.47*	2.03**
Crosses	23	0.54**	18.09**	16.04**	0.92**	15.81**	26.38**	7.23**	0.11**
Lines	7	1.25**	21.64	11.59	1.15*	13.63	20.61	10.80	0.12**
Tester	2	0.85*	33.32	79.35**	3.68**	46.31*	101.83*	3.72	0.87**
Line× Tester	14	0.15*	14.14**	9.22	0.40**	12.55**	18.49**	5.94**	0.01**
Error	68	0.07	2.43	7.77	0.10	5.48	7.85	0.80	0.00

**Table 2:** Estimates of genetic components, narrow sense heritability and per cent contribution of lines, testers and line × tester to the total variation for boll weight and quality parameters in colored cotton

Genetic components	Boll weight (g)	Fiber length (mm)	Fiber strength (g/tax)	Fiber fineness (µg/inch)	Fiber maturity (%)	Fiber uniformity (ratio)	Cellulose (%)	Flavonoids (mg/g)
$\sigma^2_{gca}$	0.01	0.09	0.16	0.01	0.08	0.18	0.03	0.00
$\sigma^2_{sca}$	0.02	3.90	0.49	0.10	2.36	3.55	1.71	0.00
$\sigma^2_{sca}/\sigma^2_{gca}$	2.59	42.92	3.09	8.52	31.39	19.51	57.89	0.80
$\sigma^2_{gca}/\sigma^2_{sca}$	0.39	0.02	0.32	0.12	0.03	0.05	0.02	1.24
$\sigma^2_A$	0.02	0.18	0.31	0.02	0.15	0.36	0.06	0.01
$\sigma^2_D$	0.02	3.90	0.49	0.10	2.36	3.55	1.71	0.00
$(\sigma^2_D/\sigma^2_A)^{1/2}$	1.14	4.63	1.24	2.06	3.96	3.12	5.38	0.63
$h^2(n.s)$	15.64	2.79	3.66	10.41	1.88	3.09	2.30	65.18
Contribution of lines	69.94	36.40	21.99	38.25	26.23	23.78	45.48	31.66
Testers	13.69	16.02	43.01	34.91	25.46	33.56	4.48	64.81
Line × Tester	16.36	47.58	35.00	26.84	48.31	42.66	50.04	3.53

$\sigma^2_{gca}$  = Variance of general combining ability,  $\sigma^2_{sca}$  = Variance of specific combining ability,  $\sigma^2_A$  = Additive genetic variance,  $\sigma^2_D$  = Dominant genetic variance,  $(\sigma^2_D/\sigma^2_A)^{1/2}$  = Degree of dominance,  $h^2(n.s)$  = Narrow sense heritability  
 \*, \*\* = Significant at 5% and 1% level of probability respectively, D.f = Degree of freedom

**Table 3:** General combining ability effects of parental genotypes

Lines	Boll weight (g)	Fiber length (mm)	Fiber strength (g/tax)	Fiber fineness (µg/inch)	Fiber maturity (%)	Fiber uniformity (ratio)	Cellulose (%)	Flavonoids (mg/g)
White exotic	-0.09	-1.90**	0.83	-0.02	-1.47	-3.20**	1.26	0.18**
CRSM-38	-0.09	0.16	-1.78**	-0.50	-1.55*	2.02**	-1.94**	-0.11**
MNH-814	-0.07	1.01	-0.83	-0.38	-0.65	0.35	-1.24	-0.05**
MNH-552	0.02	0.49	0.33	0.09	-0.32	0.95	-0.07	0.01*
MNH-886	0.58	-0.27	0.35	0.23	2.04**	0.001	0.01	0.12**
VH-303	-0.17	-1.63*	0.72	0.27	0.75	-0.55	0.93	-0.04
FH-142	0.44	-0.79	1.53	0.56	0.46	0.59	0.76	0.06
GIZA-7	-0.62**	2.93**	-1.17	-0.24	0.75	-0.17	0.26	-0.17
SE( <i>gi</i> )	0.09	0.52	0.93	0.11	0.78	0.93	0.30	0.01
SE( <i>gi-gj</i> )	0.13	0.73	1.31	0.15	1.10	1.32	0.42	0.01
Testers	Boll weight (g)	Fiber length (mm)	Fiber strength (g/tax)	Fiber fineness (µg/inch)	Fiber maturity (%)	Fiber uniformity (ratio)	Cellulose (%)	Flavonoids (mg/g)
BZUB	0.12*	0.52	2.09**	0.45	1.34	1.59*	-0.19	0.21**
BZUG2	-0.22**	-1.35	-1.23	-0.17	-1.44	-2.33**	-0.27	-0.03**
BZUG1	0.09*	0.83	-0.86	-0.28	0.10	0.74	0.45	-0.17**
SE( <i>gj</i> )	0.06	0.32	0.57	0.07	0.48	0.57	0.18	0.01
SE( <i>gi-gj</i> )	0.08	0.45	0.80	0.09	0.68	0.81	0.26	0.01

\*, \*\* = Significant at 5% and 1% level of probability respectively, SE(*gi*) = Standard error (*gca* effects for lines), SE(*gi-gj*) = Standard error (between *gca* effects of two lines), SE(*gj*) = Standard error (*gca* effects for testers), SE(*gi-gj*) = Standard error (between *gca* effects of two testers)

**Correlation and Path Coefficient Analyses**

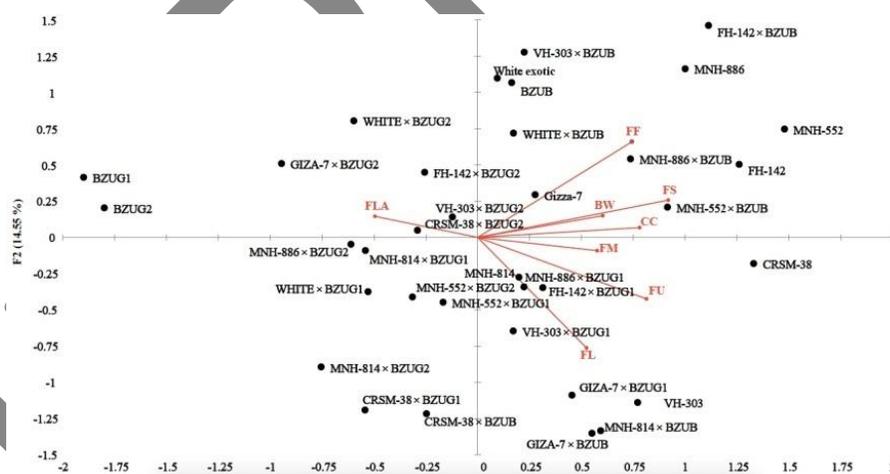
Co-efficient of correlations revealed the negative association between flavonoid contents and all other traits with significant maximum negative value between cellulose contents and flavonoid contents. However, the correlation between cellulose contents and other studied traits was positive and significant. The maximum

positive and highly significant correlation was observed between fiber strength and fiber fineness followed by fiber strength and cellulose contents. Frequency distributions indicated the comparatively more continuous variation for boll weight, fiber length and its maturity (Fig. 2). Path coefficient analysis revealed negative direct effect of flavonoid contents on fiber quality traits with maximum value for cellulose contents except

**Table 4:** Specific combining ability effects of colored cotton hybrids

Hybrids	Boll weight (g)	Fiber (mm)	length Fiber (g/tax)	Fiber strength (μg/inch)	Fiber fineness (%)	Fiber maturity (ratio)	Fiber uniformity (%)	Cellulose (%)	Flavonoids (mg/g)
WHITE ×BZUB	0.02	-0.52	-0.09	-0.04	-1.13	1.05	-0.82	-0.01	
WHITE ×BZUG2	0.14	0.92	-0.11	0.35	2.25**	-0.91	-0.23	0.05**	
WHITE ×BZUG1	-0.16	-0.40	0.19	-0.31	-1.12	-0.14	1.05	-0.04*	
CRSM-38 ×BZUB	0.14	0.92	-2.31**	-0.53	-2.65**	0.56	-0.21	0.05**	
CRSM-38 ×BZUG2	0.02	-0.35	1.84*	0.56	1.02	1.24	0.47	-0.05**	
CRSM-38 ×BZUG1	-0.16	-0.56	0.47	-0.03	1.62*	-1.80*	-0.25	0.00	
MNH-814 ×BZUB	-0.04	1.57*	0.24	-0.35	1.25	2.02**	3.19**	0.03*	
MNH-814 ×BZUG2	-0.13	0.84	-1.44	-0.13	-0.38	1.34	-0.73	-0.06**	
MNH-814 ×BZUG1	0.17	-2.41**	1.19	0.48	-0.88	-3.36**	-2.45**	0.03*	
MNH-552 ×BZUB	0.24	1.49	1.08	0.35	-0.58	-1.30	0.02	0.03*	
MNH-552 ×BZUG2	0.00	-0.14	-1.11	-0.30	0.65	2.39**	1.10	-0.05**	
MNH-552 ×BZUG1	-0.23	-1.35	0.03	-0.05	-0.07	-1.09	-1.12	0.02	
MNH-886 ×BZUB	0.12	-0.82	-1.07	0.14	1.16	1.31	0.44	0.01	
MNH-886 ×BZUG2	-0.17	0.65	-1.62*	-0.24	-0.76	-0.24	-0.73	0.01	
MNH-886 ×BZUG1	0.05	0.17	2.68**	0.10	-0.40	-1.07	0.30	-0.03*	
VH-303 ×BZUB	-0.39*	-3.70**	-0.31	0.17	-0.72	-2.58**	-0.48	-0.04*	
VH-303 ×BZUG2	0.33*	2.17**	2.01**	-0.08	0.69	0.21	0.10	0.06**	
VH-303 ×BZUG1	0.06	1.53	-1.69*	-0.10	0.02	2.37**	0.38	-0.02	
FH-142 ×BZUB	-0.10	-0.60	1.88**	0.42	-0.99	-0.51	-1.31	-0.06**	
FH-142 ×BZUG2	-0.17	0.20	0.53	-0.33	-1.99**	0.01	-0.23	0.05**	
FH-142 ×BZUG1	0.28	0.39	-2.41**	-0.09	2.98**	0.50	1.55*	0.02	
GIZA-7 ×BZUB	0.02	1.65*	0.58	-0.18	3.65**	-0.55	-0.81	-0.01	
GIZA-7 ×BZUG2	-0.02	-4.28**	-0.11	0.18	-1.48	-4.04**	0.27	-0.01	
GIZA-7 ×BZUG1	0.01	2.64**	-0.47	0.001	-2.16**	4.59**	0.55	0.02	
S.E(ij)	0.16	0.90	1.61	0.18	1.35	1.62	0.52	0.02	
S.E (sij-skl)	0.22	1.27	2.28	0.26	1.91	2.29	0.73	0.02	

\*, \*\*= Significant at 5% and 1% level of probability respectively,  $SE(ij)$  = Standard error (*sc*a effects for crosses),  $SE(sij-ski)$  = Standard error (between *sc*a effects of two crosses)

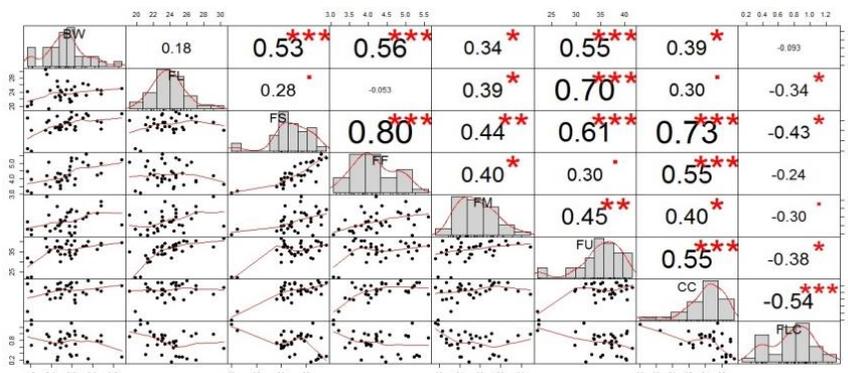


**Fig. 1:** Biplot developed by genotype-by-trait analysis based on mean performance of eight lines, three testers and their hybrids for various traits. BW=boll weight, FL=fiber length, FS=fiber strength, FF=fiber fineness, FM=fiber maturity, FU=fiber uniformity, CC=cellulose contents, FLC=flavonoids contents

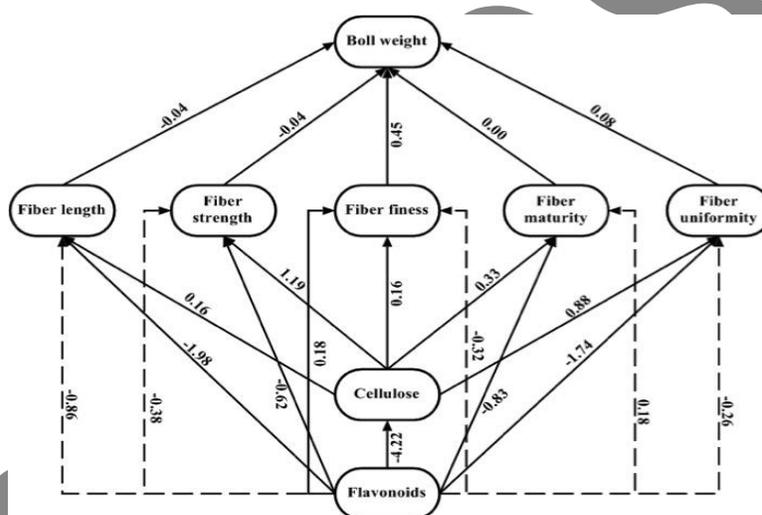
fiber fineness. However, cellulose contents had positive direct effects for all fiber quality traits with maximum value for fiber strength. Cellulose contents also possessed positive indirect effects on boll weight via fiber fineness, its maturity and uniformity. Among these, indirect effect of cellulose on boll weight via fiber fineness has maximum positive value. However, indirect effects of cellulose contents on boll weight via fiber strength and length were negative (Fig. 3).

## Discussion

The present study used line × tester analysis to identify the genetic basis for a useful colored cotton breeding program. In our study, significant mean square values of parents and crosses for studied traits were evident for prevalence of substantial amount of genetic variability.



**Fig. 2:** Co-efficient of Correlations, traits relationships and frequency distributions for all studied traits using R-package. The values are based on the mean data over all treatments. The font size for the value of correlation depicted the intensity of association among the traits.\*\*\*, \*\* and \* = Significance levels 0.001, 0.01 and 0.05 respectively, BW=boll weight, FL=fiber length, FS=fiber strength, FF=fiber fineness, FM=fiber maturity, FU=fiber uniformity, CC=cellulose contents, FLC=flavonoids contents



**Fig. 3:** Path coefficient analysis of flavonoid, cellulose and fiber quality traits as independent and boll weight as dependent variable. Continuous lines (—) and (---) depicted the direct and indirect effects of different traits on boll weight

Similarly, significant mean square values of parents vs. crosses and line  $\times$  tester indicated the presence of non-allelic interactions and non-additive mode of inheritance (Basal *et al.*, 2009; Shashikumar *et al.*, 2011). The values of  $\sigma^2_{sca}/\sigma^2_{gca}$  ratio and  $(\sigma^2_D/\sigma^2_A)^{1/2}$  were greater than unity along with less amount of  $\sigma^2_{gca}$  as compared to  $\sigma^2_{sca}$  for all traits except flavonoids contents suggested the preponderance of non-additive genetic effects. In addition to this, low narrow sense heritability estimates for boll weight, cellulose contents and fiber quality traits further confirmed the involvement of non-additive mode of inheritance. These finding suggested that direct selection based on these traits would not give encouraging results as phenotype is not only influence by genotype but also by environment and genotype  $\times$  environment interaction (Abid *et al.*, 2016). Above mentioned contradictory mode of inheritance for

flavonoid contents and other studied traits suggested that one of the effect way to improve boll weight, cellulose contents and fiber quality in colored cotton is heterosis breeding coupled with rigorous selection from the early segregation generations. Similar results have been reported by Feng *et al.* (2011).

The *gca* effects of parental line are mainly due to accumulation of genes as a result of recurrent selection process and these can be inherit to next generation (Hallauer and Miranda, 1988; Kang, 1994). Positive *gca* effects for boll weight, fiber quality, cellulose and flavonoid contents exhibited by different parental lines indicated that these lines could serve as potential source for contributing the desire alleles of studied traits (Khan *et al.*, 2009; Abid *et al.*, 2016). Among parental lines, maximum *gca* effects of GIZA-7 (*G. barbadense*) for fiber length confirmed its

ability to inherit the favorable alleles to the next generations. Similarly, Bt cotton lines MNH-886 and FH-142 with maximum *gca* effects for boll weight and fiber strength respectively, unveiled their potential to be used in breeding program for the development of Bt cotton genotypes having more boll weight and fiber strength. However, at the same time these genotypes are poor general combiner either for other fiber quality traits, suggested that three way crosses, modified back crosses or recurrent selection procedure could be fruitful for the improvement of fiber yield and quality traits simultaneously (Coyle and Smith, 1997). Genotype by traits analysis also confirmed that both Bt lines MNH-886 and FH-142 were best for boll weight and fiber strength. These finding further suggested that combined use of both *gca* effects and genotype by trait analysis can be potential tool to identify best general combiner. Previously it is also reported that suitable combiner can be selected on the basis of mean performance and it is not important to estimate the *gca* effects for parents selection (Singh *et al.*, 1974).

In present study, desired significant *sca* effects suggested ample scope for the exploitation of hybrid vigor for different studied traits (Ahuja and Dhayal, 2007). Among hybrids, Bt green hybrid VH-303 × BZUG2 and non-Bt brown hybrid MNH-814 × BZUB with maximum *sca* effects but negative *gca* effects for both parental lines suggested the best specific combiners were not always obtained from the lines having positive and high *gca* effects (Basal *et al.*, 2009). The hybrids with negative × negative general combining ability suggested the preponderance of non-additive type of epistasis. Further, it was also evident by genotype by traits analysis that performance of hybrids not necessarily indicates the *sca* effect (Singh and Bhutani, 1974). Two cotton hybrids FH-142 × BZUG1 (Bt green cotton) and GIZA-7 × BZUG1 (interspecific green cotton) with positive and high or medium *gca* effects for both parental lines suggested that additive × additive type of gene-interactions responsible for their high *sca* effects in the case of boll weight and fiber length respectively. These findings suggested that biparental progeny selection could be fruitful breeding strategy to get the transgressive segregants for boll weight and fiber length (Andrus, 1963).

The positive and significant co-efficient of correlations between cellulose contents and other studied traits including boll weight and fiber quality indicated that cellulose is key contributing factor for fiber quality and boll weight. However, negative and significant correlations between flavonoids and fiber quality and boll weight suggested that accumulation of pigment in cotton fiber is might be one of the cause of low boll weight and fiber quality (Pan *et al.*, 2010). According to breeding prospects, negative correlation of flavonoids with cellulose and other studied traits is an indication of negative linkage among the genes controlling these traits and this negative linkage can be broken by selection of plants from populations generated by random mating (Miller and Rawlings, 1967). Path analysis

could also be useful in prediction of the correlation responses to directional selection and in the identification of some characters that may have no importance themselves, but can be used as precursors of more important ones under study (Rasheed *et al.*, 2009). Path coefficient analysis showed significant positive direct effects of cellulose contents on all the fiber traits. These finding indicated the importance of cellulose contents in the development of cotton fiber and again confirmed that cellulose contents are principle-contributing trait for fiber quality (Pan *et al.*, 2010). While discussing the breeding strategy for high boll weight the negative indirect effects of cellulose contents via fiber length and fiber strength and positive direct effect of cellulose via other fiber quality traits suggested the complex mode of inheritance for boll weight. Similarly the negative direct and indirect effects of flavonoids contents on fiber quality as well as on boll weight respectively suggested, for simultaneous improvement of boll weight and fiber quality in colored cotton demands arduous breeding efforts. Ahuja and Dhayal (2007) also found similar kind of direct and indirect effects between boll weight and fiber quality.

## Conclusion

The preponderance of non-additive genetic effects for boll weight, fiber quality and cellulose contrary to the additive genetic effects for flavonoids contents depicted the considerable difficulty to develop the economically and technically superior colored cotton genotypes. In addition to this, the negative association between flavonoid with cellulose contents and their direct and indirect effects on fiber quality as well as on boll weight respectively also confirmed the complex mode of inheritance with difficulty for concurrent improvement of these traits. In this scenario, breeding scheme aimed at disrupting gene pools followed by a strategic selection method in addition to rigorous testing for color intensity, fiber quality traits and biochemical are expected to yield good results.

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## References

- Abid, M.A., W. Malik, A. Yasmeen, A. Qayyum, R. Zhang, C. Liang, S. Guo and J. Ashraf, 2016. Mode of inheritance for biochemical traits in genetically engineered cotton under water stress. *AoB Plant*, 8: doi:10.1093/aobpla/plw008
- Ahuja, S.L. and L.S. Dhayal, 2007. Combining ability estimates for yield and fibre quality traits in 4 × 13 line × tester crosses of *Gossypium hirsutum*. *Euphytica*, 153: 87–98

- Anandan, 2010. Environmental impact on the combining ability of fiber traits and seed-cotton yield in cotton. *J. Crop Improv.*, 24: 31–323
- Andrus, C.F., 1963. Plant Breeding systems. *Euphytica*, 12: 205–208
- Basal, H., A. Unay, O. Canavar and I. Yavas, 2009. Combining ability for fiber quality parameters and within-boll yield components in intraspecific and interspecific cotton population. *Span. J. Agric. Res.*, 7: 364–374
- Basbag, S., R. Ekinci and O. Gencer, 2007. Combining ability and heterosis for earliness characters in line × tester population of *Gossypium hirsutum* L. *Hereditas*, 144: 185–190
- Cannell, M.G.R. and J.H.M. Thornley, 2000. Modelling the components of plant respiration: some guiding principles. *Ann. Bot.*, 85: 45–54
- Coyle, G.G. and C.W. Smith, 1997. Combining ability for with in boll yield components in cotton *Gossypium hirsutum* L. *Crop Sci.*, 37: 1118–1122
- Dewey, D.R. and K.H. Lu, 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agron. J.*, 51: 515–518
- Dickerson, D.K., E.F. Lane and D.F. Rodriguez, 1996. *Evaluation of Selected Performance Characteristics of Naturally Colored Cotton Knit Fabrics*. California Agricultural Technology Institute Research Publications No: 961003
- Du, X.M. and Y.Z. Shi, 2002. Features of colored cotton fiber and its exploitation (in Chinese). *Knitting Ind.*, 1: 29–33
- Dutt, Y., X.D. Wang, Y.G. Zhu and Y.Y. Li, 2004. Breeding for high yield and fibre quality in colored cotton. *Plant Breed.*, 123: 145–151
- Esmail, R.M., 2007. Genetic analysis of yield and its contributing traits in two intra-specific cotton crosses. *J. Appl. Sci. Res.*, 3: 2075–2080
- Feng, C.H., M. Ulloa, M.C. Perez and J.M.D. Stewart, 2011. Distribution and Molecular Diversity of Arborescent *Gossypium* Species. *Botany*, 89: 615–624
- Guler, M., M.S. Adak and H. Ulukan, 2001. Determining relationships among yield and some yield components using path coefficient analysis in chickpea (*Cicerarietinum* L.). *Ent. J. Agron.*, 14: 161–166
- Hallauer, A.R. and J.B. Miranda, 1988. *Quantitative Genetics in Maize Breeding*. Iowa State University Press, Ames, IA
- Haq, W.U., M.F. Malik, Rashid, M. Munir and Z. Akram, 2008. Evaluation and estimation of heritability and genetic advancement for yield related attributes in wheat lines. *Pak. J. Bot.*, 40: 1699–1702
- Hua, S.J., X.D. Wang, S.N. Yuan, M.Y. Shao, X.Q. Zhao, S.J. Zhu and L.X. Jiang, 2007. Characterization of pigmentation and cellulose synthesis in colored cotton fibers. *Crop Sci.*, 47: 1540–1547
- Kang, M.S., 1994. *Applied quantitative genetics*. Kang, Baton Rouge, LA
- Kant, R., 2012. Textile dyeing industry an environmental hazard. *Nat. Sci.*, 4: 22–26
- Kemphorne, O., 1957. *An Introduction to General Statistics*. John Wiley and Sons. Inc., New York, USA
- Khan, N.U., G. Hassan, M.B. Kumbhar, K.B. Marwat, M.A. Khan, A. Parveen, U. Aiman and M. Saeed, 2009. Combining ability analysis to identify suitable parents for heterosis in seed cotton yield, its components and lint % in upland cotton. *Indust. Crop Prod.*, 29: 108–115
- Malik, W., A.A. Khan and B. Sadia, 2013. *In situ* characterization of coloured cotton genotypes. *Aust. J. Crop Sci.*, 7: 299–304
- Miller, P.A. and J.O. Rawlings, 1967. Breakup of initial linkage blocks through intermating in a cotton breeding population. *Crop Sci.*, 7: 199–204
- Naveed, M., F.M. Azhar and A. Ali, 2004. Estimates of heritabilities and correlations among seed cotton yield and its components in *G. hirsutum* L. *Int. J. Agric. Biol.*, 6: 712–714
- Ogunbayo, S.A., D.K. Ojo and R.G. Guei, 2005. Phylogenetic diversity and relationship among forty rice accessions using morphological and RAPD technique. *Afr. J. Biotechnol.*, 4: 1234–1244
- Pan, Z., D. Sun, J.Z. Sun, Zhou, Y. Jia, B. Pang and X. Du, 2010. Effect of fibre wax and cellulose content on coloured cotton fibre quality. *Euphytica*, 173: 141–149
- Pradhan, S.K., L.K. Bose and J. Mehar, 2006. Studies on gene action and combining ability analysis in basmati rice. *J. Eur. Agric.*, 7: 267–272
- Rasheed, A., W. Malik, A.A. Khan, N. Murtaza, A. Qayyum and E. Noor, 2009. Genetic evaluations of fibre yield and yield components in fifteen cotton (*Gossypium hirsutum*) genotypes. *Int. J. Agric. Biol.*, 11: 581–585
- Shashikumar, K.T., M. Pitchaimuthu, D.P. Kumar and R.D. Rawal, 2011. Heterosis and combining ability for resistance to powdery mildew in adult melon plants. *Plant Breed.*, 130: 383–387
- Singh, F., R.K. Singh and V.P. Singh, 1974. Combining ability studies in pearl millet (*Pennisetum typhoides* (Burn.) S. & H.). *Theor. Appl. Genet.*, 44: 106–110
- Singh, K.R.K. and R.D. Bhutani, 1974. Combining ability studies in tomato (*Lycopersicon esculentum* Mill.). *Theor. Appl. Genet.*, 44: 358–363
- Singh, R.K. and B.D. Chaudhary, 1999. *Biometrical methods in quantitative genetics analysis*. Kalyani Publisher, New Delhi
- Ulloa, M., 2006. Heritability and correlations of agronomic and fiber traits in okra-leaf upland cotton population. *Crop Sci.*, 46: 1508–1514
- Waghmare, V.N. and K.D. Koranne, 1998. Coloured cotton: present status, problems and future potentials. *Ind. J. Genet. Plant Breed.*, 58: 1–15
- Wang, G.Q., C.H. Xu and J.M. Wei, 1999. Photosynthesis, respiration, and carbohydrate metabolism. In: *Modern Guidance for Plant Physiology Experiments*, pp: 96-97. Tang, Z.C. (ed.). Science Press, Beijing
- Xiao, Y.H., Z.S. Zhang, M.H. Yin, M. Luo, X.B. Li, L. Hou and Y. Pei, 2007. Cotton flavonoid structural genes related to the pigmentation in brown fibers. *Biochem. Biophys. Res. Commun.*, 358: 73–78
- Yuan, S.N., W. Malik, N. Bibi, G.J. Wen, M. Ni and X.D. Wang, 2013. Modulation of morphological and biochemical traits using heterosis breeding in coloured cotton. *J. Agric. Sci.*, 151: 57–7
- Zhang, M., X. Zheng, S. Song, Q. Zeng, L. Hou, D. Li, J. Zhao, Y. Wei, X. Li and M. Luo, 2011. Spatiotemporal manipulation of auxin biosynthesis in cotton ovule epidermal cells enhances fiber yield and quality. *Nat. Bioethanol.*, 29: 453–458
- Zhao, X.Q., 2003. *Studies on Pigment Biosynthesis and Quality Formation in Coloured Cotton Fibers*. Dissertation, Zhejiang University

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