

GENETIC DIVERSITY AMONG *Bt* COTTON (*GOSSYPIUM HIRSUTUM* L.) GERMPLASM ASSESSED THROUGH MORPHOLOGICAL AND WITHIN-BOLL YIELD ATTRIBUTES

B. Riaz^{1,2*}, A. Saced¹, S. Fiaz^{1,3*} and A. Riaz^{1,4}

¹Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan

²Institute of Crop Science, Graduate School of Chinese Academy of Sciences, Beijing, 100081, China

³State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, 310006, China

⁴Biotechnology Research Institute, Graduate School of Chinese Academy of Sciences, Beijing, 100081, China

*Corresponding author Email: bisma.riaz75@gmail.com; sajidfiaz50@yahoo.com

ABSTRACT

An experiment was conducted to evaluate the genetic diversity of *Bt* cotton germplasm through morphological and within-boll yield attributes at the experimental area of the University of Agriculture, Faisalabad, Pakistan, during 2015-16 crop season. The experiment was carried out in randomized complete block design (RCBD) with two replications. Genetic diversity assessment among 60 *Bt* cotton genotypes is studied using principle component analysis (PCA). The coefficient of variance, genetic advance, and heritability have been estimated for each trait. Analysis of variance showed the significant variation among the genotypes for all the traits under investigation. Highest heritability and genetic advance were recorded for bolls per plant and lint mass per boll. In PCA, first five PCs exhibited more than one Eigen value. In PC-I, sympodial branches, monopodial branches, bolls per plant and seed cotton yield per plant were the most important traits contributing 26.15% in the total variation obtained. The variation of 18.87% in PC-II was mainly contributed by node number for the first fruiting branch, node number for first effective boll formation, node height up to first fruiting branch, boll weight and the lint mass per boll. The high genetic variability, heritability and high to moderate genetic advance indicated that germplasm contains genetic potential to be utilized for the future breeding program to develop high yielding cultivars.

Key words: ECV, GCV, genetic advance, heritability, PCA, PCV.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important fiber crop and the second important oilseed crop after soybean (Freeland *et al.*, 2006). Fiber quality in cotton has supreme importance as similar to yield, which involves growers, ginners and the textile industry simultaneously (Yaqoob *et al.*, 2016). In Pakistan, cotton yield is low in comparison to average production of other cotton growing countries. This is due to the lack of resistant varieties against abiotic and biotic stresses such as high temperature, cotton leaf curl virus (CLCuV) disease, pest attack and the improper production technology (Panni *et al.*, 2012). To overcome this situation, several possibilities can be employed, including increasing inputs, use of pesticides and genetic improvement of elite varieties. In future, sustainable production of cotton will depend upon the development of elite cotton varieties with high yield, better quality of seed cotton, and resistant to biotic and abiotic stresses (Ahmad *et al.*, 2012).

Due to its importance, cotton crop has attracted plant breeders to improve the plant genetic architecture (Ahmad *et al.*, 2016). These efforts led to the evolution of

high yielding cultivars with better yield potential and fiber quality attributes (Khan *et al.*, 2017).

For the successful breeding program, it is very important to have enough knowledge and understanding regarding genetic diversity present in the available crop germplasm, which will be helpful for plant breeders to select the best parental material that will be useful in generating high yielding cultivars (Esmail *et al.*, 2008).

The aim of this study was to evaluate the genetic diversity in *Bt* germplasm of upland cotton using PCA. Genotypic, phenotypic, environmental variances, coefficients of variance, heritability and genetic advance were also calculated to determine heritable and non-heritable part of variability. The obtained results will be utilized in the improvement of existing cotton varieties or development of new cultivars.

MATERIALS AND METHODS

Sixty *Bt* cotton genotypes were evaluated under field condition during summer 2015-2016 (Table 1). Delinted seeds of all genotypes were grown in RCBD design with two replications at the University of Agriculture, Faisalabad, Pakistan. Plant to plant and row to row distances were kept at 75 and 30 cm, respectively.

The crop was raised to maturity with standard production practices. On maturity, the data were recorded for morphological characters *viz.*, node number of first fruiting branch, node number of first effective boll formation, node height up to first fruiting branch, plant height, monopodial branches per plant, sympodial branches per plant, bolls per plant, seed cotton yield per plant and within-boll yield components like boll weight, seeds per boll, lint mass per boll, lint percentage (%), seed cotton per seed and lint per seed, from randomly selected five plants.

Table 1. Experimental material used in the study.

No.	Name of accessions	No.	Name of accessions
1	C-26	31	VH-283
2	VH-282	32	VH-295
3	VH-259	33	AS-01
4	A.A-802	34	VH-329
5	MNH-886	35	FH-175
6	MNH-886	36	FH-187
7	IR-3071	37	FH-177
8	CRS-2007	38	CIM-599
9	FH-113	39	CIM-602
10	SB-149	40	CIM-598
11	NS-131	41	MG-6
12	KZ-181	42	MNH-586
13	KZ-189	43	FH-941
14	FH-169	44	VH-324
15	FH-172	45	VH-325
16	KZ-191	46	VH-333
17	A.A-703	47	VH-339
18	FH-154	48	VH-228
19	FH-142	49	FH-182
20	FH-170	50	FH-171
21	VH-148	51	FH-159
22	CRS-456	52	FH-183
23	FH-118	53	FH-158
24	NS-121	54	Lalazar
25	FH-114	55	VH-338
26	NIAB-820	56	VH-337
27	IR-3	57	VH-341
28	IR-901	58	VH-330
29	FH-4243	59	IUB-222
30	S-3	60	IUB-212

Statistical Analysis: Replicated mean data of all the characters were subjected to the analysis of variance (ANOVA) technique as outlined by Steel *et al.* (1997). Data were further analyzed by PCA as described by Neyman and Pearson (1928) using statistical software Statistica.

The ANOVA was performed in Statistics 8.1 statistical software. The detailed procedure for all kinds of statistical data analysis has been given below:

Components of variances were calculated by the following formulas:

$$\text{Genotypic variance } (\sigma_g) = V_g = (MS_g - MS_e) / r$$

$$\text{Environmental variance } (\sigma_e) = V_e = (MS_e)$$

$$\text{Phenotypic variance } (\sigma_p) = V_p = V_g + V_e$$

The coefficients of variation on environmental, genotypic and phenotypic basis were determined as described by Burton (1952) and revealed by Singh and Narayanan (2000).

$$\text{Genotypic coefficient of variance (GCV)} = (\sigma_g / \text{trait mean}) \times 100$$

$$\text{Environmental coefficient of variance (ECV)} = (\sigma_e / \text{trait mean}) \times 100$$

$$\text{Phenotypic coefficient of variance (PCV)} = (\sigma_p / \text{trait mean}) \times 100$$

The PCV and GCV were classified as suggested by Sivasubramanian and Madhavamenon (1973) and are given below:

Low: less than 10%

Moderate: 10-20%

High: more than 20%

Broad-sense heritability was calculated by the formula given by Lush (1940).

$$h^2 (\text{b.s}) \% = (V_g / V_p) \times 100$$

Categorization of broad-sense heritability was made according to Johnson *et al.* (1955) and has been given below:

Low: less than 30%

Moderate: 30-50%

High: more than 50%

Genetic advance and genetic advance as a percentage of the mean were calculated by the following formula given by Johnson *et al.* (1955).

$$\text{Genetic advance (G.A.)} = K \times \{V_g / (V_p)^{1/2}\}$$

$$\text{Genetic advance (G.A.) \%} = (\text{Genetic Advance} / \text{Trait Mean}) \times 100$$

Where;

$(V_p)^{1/2}$ = phenotypic standard deviation

K = selection differential, and its value at selection intensity of 10% is 1.76 Falconer and Mackay (1996).

The classification of genetic advance as a percentage of mean was made as suggested by the Johnson *et al.* (1955) and is given below:

Low: less than 10%

Moderate: 10-20%

High: more than 20%

RESULTS AND DISCUSSION

ANOVA for each character showed that mean squares for studied genotypes were significant, suggesting differences among all 60 genotypes for all characters under investigation. Several researchers also reported similar results for all these characters (Siddique *et al.*, 2007; Baraiya *et al.*, 2011; Shakeel *et al.*, 2012; Imran *et al.*, 2012; Iqbal *et al.*, 2013; Tang and Xiao,

2013; Baloch *et al.*, 2014; Saeed *et al.*, 2014). The variations among cotton genotypes for these traits influenced by genetic as well as environmental factors (Khodarahmpour *et al.*, 2010). Therefore, the genotypic variance (Vg), genotypic coefficient of variance (GCV), phenotypic variance (Vp), phenotypic coefficient of

variance (PCV), environmental variance (Ve), and environmental coefficient of variance (ECV), broad sense heritability (h^2), genetic advance (GA) and genetic advance as a percentage of the mean (GA%) for all traits are calculated and given below in Table 2.

Table 2. Mean square of various morphological and within-boll yield traits in 60 cotton genotypes

Traits	Vg	GCV%	Vp	PCV%	Ve	ECV%	h^2	GA	GA%
Node no. for the 1 st fruiting branch	0.6925	12.094	0.988	12.572	0.296	7.912	70.02	1.226	17.81
Node height up to 1 st fruiting branch	1.5258	9.332	2.772	12.578	1.246	8.435	55.03	1.604	12.11
Node 1 st effective boll formation	1.5178	13.322	3.263	19.534	1.745	14.287	46.51	1.47	15.89
Monopodial branches	0.1687	31.555	0.276	40.337	1.107	25.12	61.19	0.562	43.19
sympodial branches	6.8687	14.54	11.737	19.006	4.868	12.24	58.52	3.508	19.46
Bolls per plant	175.95	31.459	34.098	34.373	210.05	13.85	83.77	21.246	50.38
Plant height	239.11	13.219	311.17	15.08	72.065	7.26	76.84	23.72	20.27
Seed cotton	414.29	22.31	517.54	24.945	103.24	11.14	80.05	31.86	34.94
Yield per plant									
Boll weight	0.0839	12.957	0.118	15.381	0.034	8.287	70.96	0.427	19.1
Seeds per boll	3.3106	8.799	4.775	10.569	1.465	5.853	69.33	2.65	12.82
Lint mass per boll	0.0033	34.365	0.0045	40.267	0.001	20.99	72.84	0.086	51.33
Seed cotton per seed	0.0002	13.324	0.0004	18.119	0.0002	12.281	54.065	0.019	17.14
Lint %age	3.679	4.659	5.102	5.486	1.423	2.897	72.12	2.85	6.92
Lint per seed	0.0000001	14.8192	0.0000002	18.908	0.0000001	11.744	61.42	0.0005	20.32

GV= Genetic Variance; GCV= Genetic coefficient of Variance; PV= Phenotypic variance; PCV= Phenotypic coefficient of Variance; EV= Environmental variance; ECV= Environmental coefficient of Variance; h^2 = Heritability; G.A. = Genetic Advance.

For a particular trait, if the phenotypic coefficient of variance is more than the genotypic coefficient of variance it shows that environmental influence is more than genetic component and vice versa. From Table 2 it is clear that the highest (34.37) genotypic coefficient of variance recorded for lint mass per boll while the highest (40.34) phenotypic coefficient of variance for the monopodial branches per plant. Estimation of heritability helps the breeders while making the selection. However, Johnson *et al.* (1955) reported that alone heritability estimates do not give the clear idea about expected gain in the next generation but only in conjunction with genetic advance. Genetic advance gives the magnitude of expected genetic gain obtained by one cycle of selection (Idahosa *et al.*, 2010). The estimation of genetic components showed high heritability for seed cotton yield per plant (80%) and bolls per plant (83 %). The node number of first effective boll formation showed lower heritability about 46% as

compared to other traits. Genetic advance for lint mass per boll was recorded as highest (51%) whereas the lint percentage exhibited lowest (6.9%).

PCA: The mean data were further analyzed by PCA using Statistica software. The data matrix of 14 × 60 was prepared for the analysis. Out of 14 PCs, first five PCs exhibited more than one Eigen value. The PC-I showed maximum variation (26.15%) which was mainly due to the sympodial branches, monopodial branches, bolls per plant and seed cotton yield per plant. In PC-II, node number of first fruiting branch, node number for first effective boll formation, node height sup to first fruiting branch, boll weight and the lint mass per boll were the most important traits contributing about 18.87% to the variation. The PC-III explained variation (12.94%) of the total variation mainly contributed by lint percentage, seeds per boll and lint per seed. In PC-IV, node number for first effective boll formation, node height up to the

first fruiting branch and the seeds per boll were the most important traits contributing to the variation which amounted for about 10.25%. Whereas, PC-V exhibited 6.94% of the total variation which was mainly caused by node number for first effective boll formation, node height up to the first fruiting branch and seeds per boll (Table 2).

Biplot analysis: A principal component biplot (Figure 1) showed that variables were super imposed on the plot as vectors. The distance of each variable with respect to PC-

I and PC-II showed the contribution of this variable in the variation of germplasm. Different parameters including bolls per plant, seed cotton yield per plant, boll weight, node number of first fruit branch and node height up to first fruiting branch showed more differences (as represented in biplot) together in PC-I and PC-II, while lint percentage, sympodial branches, lint per seed and seeds per boll had minimum differences in PC-I and PC-II.

Table3. PCA for 14 characters in 60 *Bt* germplasm lines of *G. hirsutum* L.

Variables	PC-I	PC-II	PC-III	PC-IV	PC-V
Plant Height	-0.563455	-0.383145	0.118730	0.170069	0.347123
Sympodial branches	-0.673425	0.187292	0.225339	-0.078826	0.487360
monopodial branches	-0.609106	-0.031683	0.052797	0.129960	-0.619140
Bolls per plant	-0.736143	0.345000	0.171165	0.354958	-0.028165
Seed cotton yield per plant	-0.835743	-0.002981	0.272066	0.206863	0.041962
Node no. for 1 st fruiting branch	0.434488	-0.572124	0.235541	0.361894	-0.035669
Node no. for 1 st effective boll formation	0.340218	-0.525938	-0.15394	0.458341	0.134098
Node height up to 1 st fruiting branch	0.119371	-0.648156	0.160286	0.464240	0.181972
Boll weight	-0.520593	-0.665693	0.147303	-0.344195	-0.120687
Lint %age	0.078984	0.100924	0.588373	0.354886	-0.400152
Seeds per boll	-0.133697	-0.355513	0.647483	-0.513656	0.071263
Seed cotton per seed	-0.598057	-0.435737	-0.449271	-0.053085	-0.205881
Lint per seed	-0.423162	-0.457144	-0.698234	-0.040712	-0.033370
Lint mass per boll	0.359515	-0.580759	0.243970	-0.382098	-0.138486

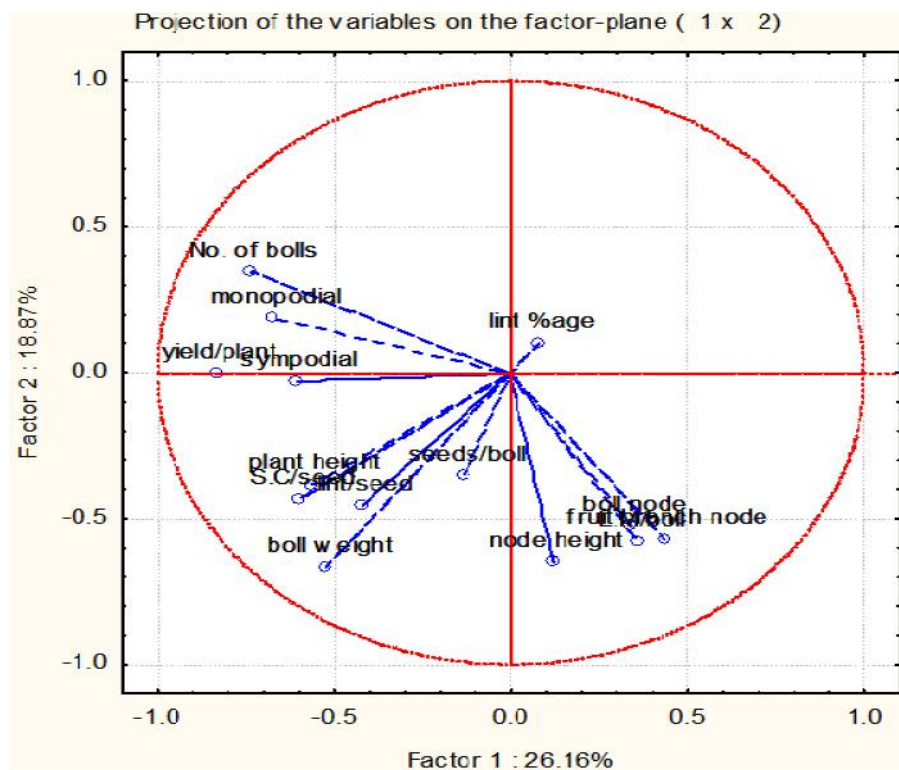


Figure 1:Principal component biplot of 60 genotypes of *G. hirsutum* L.

The distribution of morphological traits around the vectors and the angles of vectors in biplot display provides multi-trait selection associated with yield. The evaluation of genotypes for further improvement of cotton seed yield and its related components is essential in breeding programs for developing high yielding cotton varieties (McCarty *et al.*, 2005).

The genetic variations among cotton genotypes for sympodial and monopodial branches, bolls per plant, seed cotton yield per plant, boll weight and lint percentage have also been reported in previous studies (Khan *et al.*, 2017; Nazir *et al.*, 2013). The genetic diversity studies for agronomic essential traits using principal component analysis ultimately lead to the identification of phenotypic variations in cotton germplasm (Li *et al.*, 2008).

Ahmed *et al.* (2012) employed PCA to determine the extent of genetic diversity in cotton germplasm, which might be helpful for selection of parents for a successful breeding program. Mohammadi and Prasanna (2003) focused on the use of statistical tools and methods of genetic ranges. They analyzed that cluster analysis and PCA are the most frequently employed and seemed predominantly valuable.

Conclusion: The components of variances and PCA have revealed the valuable variations among cotton accessions for all the yield related traits. The germplasm used in this study also exhibited the high broad-sense heritability and moderate to high genetic advance. These findings indicate that germplasm exhibits potential for being utilized in the future breeding programs for the improvement of seed cotton yield by selecting the genotypes having desirable traits.

REFERENCES

- Ahmad, M.Q., S.H. Khan and F.M. Azhar (2012). Decreasing level of genetic diversity in germplasm and cultivars of upland cotton (*Gossypium hirsutum* L.) in Pakistan. *J. Agric. Soc. Sci.* 8 (3): 92-96.
- Ahmad, S., S. Fiaz., A. Riaz., I. Bashir and A. Zeb (2016). Correlation analysis of morphological and fiber quality traits in upland cotton (*Gossypium hirsutum* L.). *Int. J. Biol.* 9(4): 200-208.
- Baraiya, B.R., S.K. Brade and R.K. Chhapare (2011). Screening of cotton genotypes for the drought tolerance in rain fed conditions. *J. Cotton. Res. Dev.* 25: 50-54.
- Baloch, M.J., N.U. Khan, M.A. Rajput, W.A. Jatoti, S. Gul, I.H. Rind and N.F. Veesar (2014). Yield related morphological measures of short duration cotton genotypes. *The J. Anim. Plant Sci.* 24: 1198-1211.
- Burton, G.W (1952). Quantitative inheritance in grasses. *Proceedings of the 6th International Grassland Congress*, 1: 227-283.
- Esmail, R.M., J.F. Zhang and A.M. Abdel-Hamid (2008). Genetic diversity in the elite cotton germplasm lines using field performance and RAPDs markers. *World J. Agri. Sci.* 4: 15-25.
- Falconer, D. S., and T.F.C. Mackay (1996). *Introduction to Quantitative Genetics*. Ed 4. Longmans Green, Harlow, Essex, UK.
- Freeland, J.T.B., B. Pettigrew, P. Thaxton and G.L. Andrews (2006). *Agro meteorology and cotton production*. In: (3rd Ed). *Guide to Agricultural Meteorological Practices*, pp. 1-17.
- Idahosa, D. O., J. E. Alikaand A. U. Omoregie (2010). Genetic variability, heritability and expected genetic advance as indices for yield and yield components selection in cowpea (*Vigna unguiculata*L.). *Acad. Arena.* 2(5): 22-26.
- Imran, M., A. Shakeel, F.M. Azhar, J. Farooq, M.F. Saleem, A. Saeed, W. Nazeer, M. Naeem and A. Javaid (2012). Combining ability analysis for within-boll yield components in upland cotton (*Gossypium hirsutum* L.). *Genet. Mol. Res.* 11: 2790-2800.
- Iqbal, M., M. Naeem, M. Rizwan, W. Nazeer, M.Q. Shahid, U. Aziz, T. Aslam, and M. Ijaz (2013). Studies of genetic variation for yield related traits in upland cotton. *Am-Euras. J. Agric. Environ. Sci.* 13: 611-618.
- Johnson, H.W, H.F Robinson and R.E Comstock (1955). Estimation of genetic and environmental variability in soybeans. *Agron. J.* 47: 314-318.
- Khan, A.M., S. Fiaz, I. Bashir, S. Ali, M. Afzal, K. Kettener, N. Mehmood and M. Manzoor (2017). Estimation genetic effects controlling different plant traits in cotton (*Gossypium hirsutum* L.) under CLCuV epidemic condition. *Cer. Agro. Mol.* 1(169): 47-58.
- Khodarahmpour, Z., R. Choukan, M. Bihamta, and E. Majidi Hervan (2010). Determination of the best heat stress tolerance indices in maize (*Zea mays* L.) inbred lines and hybrids under Khuzestan province conditions. *J. Agri. Sci. Tech.* 13: 111-121.
- Li, Z., X. Wang, Y. Zhang, G. Zhang, L. Wu, J. Chi, and Z. Ma (2008). Assessment of genetic diversity in glandless cotton germplasm resources by using agronomic traits and molecular markers. *Front. Agri. China* 2(3): 245-252.
- Lush, J.L (1940). Intra-sire correlations or regressions of offspring on the dam as a method of estimating heritability of characteristics. *Proc. Am. Soc. Anim. Prod.* 33: 293-301.
- McCarty J. C., J.N. Jenkins, and J. Wu (2005). Primitive accession derived germplasm by cultivar crosses

- as sources for cotton improvement. *Crop Sci.* 44:1231-1235.
- Mohammadi, S.A. and B.M. Prasanna (2003). Analysis of genetic diversity in crop plants, salient statistical tools, and considerations. *Crop Sci.* 43: 1235-1248.
- Nazir, A., F. Jehanzeb, M. Abid, S. Muhammad, and R. Muhammad (2013). Estimation of genetic diversity for CLCuV, earliness and fiber quality traits using various statistical procedures in different crosses of *Gossypium hirsutum* L. *Vestnik Orel GAU.* 4(43): 2-9.
- Neyman, J. and E.S. Pearson (1928). The use and interpretation of certain test criteria for purpose of statistical inference. *Biometrika* 20: 175-240.
- Panni, M.K., N.U. Khan, Fitmawati, S. Batool and M. Bibi (2012). Heterotic studies and inbreeding depression in F₂ populations of upland cotton. *Pakistan J. Bot.* 44: 1013-1020.
- Saeed, F., J. Farooq, A. Mahmood, T. Hussain, M. Riaz and S. Ahmad (2014). Genetic diversity in upland cotton for cotton leaf curl virus disease, earliness and fiber quality. *Pakistan J. Agric. Res.* 27: 226-236.
- Shakeel, A., S. Ahmad, M. Naeem, T.A. Malik, M.F. Saleem and S. Fareed (2012). Assessment of best parents and superior cross combinations for earliness related traits in upland cotton. *The J. Anim. Plant Sci.* 22: 722-727.
- Shaukat, S., T.M. Khan, A. Shakeel and S. Ijaz (2013). Estimation of best parents and superior cross combinations for yield and fiber quality related traits in upland cotton (*Gossypium hirsutum* L.). *Sci. Tech. Dev.* 32: 281-284.
- Siddique, M.H., F.C Oda and U.A. Buriro (2007). Response of cotton cultivars to varying irrigation regimes. *Asian J. Plant Sci.* 6: 153-157.
- Singh, P. and S.S. Narayanan (2000). *Biometrical Techniques in plant breeding.* 2nded. Kalyani Publ. Ludhiana, India.
- Sivasubramanian, V. and P. Madhavamenon (1973). Path analysis of yield and yield components in rice. *Madras Agric. J.* 60: 1217-21.
- Steel, R.G.D., J.H. Torrie and D.A. Dickey (1997). *Principles and procedures of statistics. A biometrical approach.* Singapore: 3rd Ed. McGraw Hill Book Co. Inc.
- Tang, F. and W. Xiao (2013). Genetic effects and heterosis of within-boll yield components in upland cotton (*Gossypium hirsutum* L.). *Euphytica* 194: 41-51.
- Yaqoob, M., S. Fiaz and B. Ijaz (2016). Correlation analysis for yield and fiber quality traits in upland cotton. *Commun. Plant Sci.* 6 (3): 55-60.