In situ characterization of coloured cotton genotypes

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Abstract

Knowledge about the genetic variation and association among the various plant traits has enabled the plant breeders to effectively utilize the available cotton germplasm for the development of elite genotypes. The current study was carried out for two consecutive years to find out genetic diversity and association among multiple morphological traits in 20 coloured and white cotton genotypes. The cluster and biplot analysis grouped coloured and white cotton genotypes into four clusters (I, II, III and IV) depending on 15 morphological traits. White cotton genotypes, except Cute-98 clustered in Cluster I. Cluster II and III had all the tetraploid coloured cotton genotypes except BWP-8 while Cluster IV had two diploid cotton genotypes. The differences were observed among the biplot generated using the cumulative data of all genotypes and two separate biplots analysis based on data of only coloured and white cotton genotypes. Biplot based on cumulative data of all cotton (coloured and white) genotypes showed positive significant correlation among fibre length, fibre strength, fibre uniformity, lint per seed and ginning out turn. However, biplot analysis based on data of only coloured genotypes revealed negative correlation between ginning out turn and fibre quality traits. Two separate biplot analysis for cumulative data and for coloured cotton data showed strong negative correlation between fibre colour and fibre quality traits. The genotype-by-traits comparison indicated that all white cotton genotypes had good combination of fibre yield and quality traits as compared to all coloured cotton genotypes. The information on genetic diversity, genotype-by-traits comparison and correlation among multiple morphological traits would be helpful in constructing the breeding populations with desired allelic combinations and implementing selection strategies.

Keywords: Biplot; correlation; fibre colour; fibre length; fibre strength; genetic diversity
Abbreviation: CV: coefficient of variability; GT: genotype-by-traits; MDS: Mahalonobis's D^2 statistics; PCA: principle component analysis; PCoA: principal coordinate analysis

Introduction

Genetic variation among the available germplasm resources has been a ground work for developing elite genotypes and enhancement of germplasm (Li et al., 2008). The variability among the germplasm has dual importance in any breeding program. First an increase in heterogeneity will increase the resistance against abiotic and biotic stresses. Second allelic variations could be used to develop new combinations (van Esbroeck and Bowman, 1998). The various breeding tools like introduction of exotic germplasm, hybridization, mutation and polyploidy breeding can be used to increase the variability and estimation of this variability in the germplasm will enable the plant breeders to choose the parental lines those can generate diverse segregating population for selection of superior progenies (Saravanan et al., 2006; Esmail et al., 2008). The natural trend among the consumers and environment conscious people created a demand of textile products manufactured from naturally pigmented cottons (Veerland and James, 1987; Stankovic Elesini and Pavko Cuden, 2002), avoiding dyes for reducing environmental pollution (Veerland and James, 1987). The demand of coloured cotton is increasing but the cultivation of coloured cotton is not accelerated accordingly because of short fibre length, low fibre strength and undesirable micronaire value (Singh et al., 1993; Narayanan et al., 1996;

Dutt et al., 2004). Therefore, for the development of elite coloured cotton genotypes having good quality and high yield potential, it is thought that genetically diverse parent can generate superior progeny. The development of elite cotton cultivar by crossing distant parental lines has been reported (Bhatt, 1970; Punitha et al., 2004; Akter, 2009). The precise information on nature and degree of genetic diversity depends upon the techniques used for its estimation, like plant characterization based on agronomical, morphological and physiological traits (Ndour, 1998; Bajracharya et al., 2006). Multivariate analysis based on Mahalonobis's D^2 statistics (MDS), principal component analysis (PCA) and principal coordinate analysis (PCoA) are commonly used to assess the magnitude of genetic diversity among the germplasm (Thompson et al., 1998; Brown-Guedira et al., 2000). Among these biometrical techniques the main advantage of principal component analysis (PCA) is that each genotype can be assigned to only one group and it also reflects the importance of largest contributor to the total variation at each axis of differentiation (Sharma, 1998). Genetic diversity for morphological traits has been estimated using principal component analysis, which lead to identification of phenotypic variability in cotton (Sarvanan et al., 2006; Esmail et al., 2008; Li et al., 2008). In addition to

diversity analysis, the genotype-by-traits (GT) biplot analysis has been used to study the nature of association among the traits, evaluation of genotypes for multiple traits and identification of those genotypes which are superior in certain traits. These genotypes could be the parental lines for a breeding program or for commercial cultivation (Yan and Rajcan, 2002). However, in the presence of synthetic dyes, inferior fibre quality and low yield potential, the characterization of coloured cotton genotypes is not well documented. The objective of current study is to evaluate the genetic diversity of available coloured cotton germplasm, identify the ideal genotypes for future breeding program and to explore the relationship among multiple morphological traits.

Results

Analysis of variance for various plant traits

The analysis of variance indicated that genotypes belonging to *Gossypium hirsutum* L. and *Gossypium arboreum* L. had highly significant variation for all the traits. The data revealed non-significant interaction between genotypes and years. Therefore, the data for both years were pooled for secondary statistical analysis. The coefficient of variability percentage (CV%) for all traits varied from 0.00% to 15.60% (Table 1). The lowest CV % values were observed for fibre colour and seed volume 0.00% and 3.20% respectively. However, the highest CV% values were found for no of seed per boll (11.00%), seed density (11.00%) and lint per seed (15.60%).

Classification of genotypes

The assessment of cotton genotypes on PC1 and PC2 indicated the structure of population. Score plot depicted that principle component analysis divided the 20 cotton genotypes in four clusters. Two genotypes occupied distinct position, cute-98 was far from the origin while BWP 8 was near to the origin of biplot. Cluster I consisted of four white cotton genotypes. However, cluster II and III comprised of all tetraploid pigmented fibred cotton genotypes. Only two diploid cotton genotypes ARB-1 and ARB-2 having brown and white fibres respectively belong to the cluster IV (Fig 2).

Genotypes-by-traits analysis

The evaluation and identification of best genotypes for multiple morphological and quality traits was done using biplot analysis. The cotton genotypes generated a biplot with Cute-98, PB-899, CIM-496, Khaki AARI, BWP-4, BWP-5 and ABR-2 at the vertex of polygon (Fig 3). Among these vertex genotypes two genotypes i.e. PB-899 and CIM-496 were found near the trait vectors of lint per seed, fibre uniformity, fibre strength and fibre length, a brown cotton genotypes (BWP-8) was near to the origin of biplot. The biplot analysis using the data of only coloured genotypes had Khaki-AARI, Khaki American-A, Khakhi-900, BWP-1, ABR-1 and BWP-6 at the vertex of polygon and two genotypes i.e. Khaki American-A, Khakhi-900 were plotted near trait vectors of boll weight, seed weight per boll and fibre strength. However, biplot for only white cotton genotypes showed three genotypes (CIM=496, Cute-98 and ABR-2) at the vertex and two genotypes i.e. MNH-786 and PB-899 were found near the origin of biplot.

Relationship among morphological traits

The line between marked point of any trait and origin of a biplot is termed as traits vector and cosine angle between trait vectors determine interrelationship among the traits. The traits are positively correlated if the angle among their vectors is $< 90^o$ and negatively correlated if angle among trait vectors are $>90^o$. While trait vectors that are approximately at right angle behave independently (Yan et al., 2007). Biplot analysis for cumulative data of all collected cotton genotypes revealed significant positive associations among fibre length, fibre strength, fibre uniformity, lint per seed and ginning out turn. Similar kind of association was observed among seed weight per boll, hundred seed weight, seed density, seed volume and boll weight. The correlation between fibre colour and fibre quality traits was significantly negative (Fig 3). To plan an effective breeding strategy two more biplot analyses were done using data of all coloured and all white cotton genotypes separately (Fig 4 and 5). Biplot for the coloured genotypes revealed that fibre length, fibre strength and fibre uniformity had close positive association. While ginning out turn and fibre colour had negative association with fibre length, fibre strength, fibre uniformity and seed physical traits (Fig 4). Another biplot generated for white cotton genotypes depicted positive association among all traits except number of seeds per boll and fibre fineness (Fig 5).

Discussion

The cotton plants with coloured fibre have been grown since ancient. But the existing naturally pigmented fibre genotypes are lower in yield, poor in fibre quality and monotonous in colour. All these factors collectively posed a challenge to colour cotton breeding and innovation (Yatsu et al., 1983; Kohel 1985; Narayanan et al., 1995). Therefore, it is imperative to characterize the existing coloured cotton genotypes to provide the guidance to modern breeding and innovation of high yielding coloured cotton genotypes (Sun et al., 2009). Readily available coloured and white cotton genotypes were collected from different research organizations of Pakistan. These genotypes might have relatively higher frequency of favourable genes that could lead to the development of elite coloured cotton genotypes (Allard, 1996). Presence of significant degree of variations in all genotypes for all physical seed traits and fibre quality parameter depicted ample scope for characterization of coloured cotton genotypes (Killi et al., 2005; Ashokkumar and Ravikesavan 2011; Malik et al., 2011). The CV% is an effective statistical tool for the comparison of studied traits. In this study, the traits with lower CV% might be more repeatable than those with higher CV% value and these traits with lower CV% value could be reliable markers for the success of breeding program (Aghaee et al., 2010). Knowledge of extent of genetics divergence with in germplasm collection is of prime importance for the effective utilization, conservation and success of breeding program (Matus and Hayes, 2002). It also facilitates in the selection of divers parental line, which can generate new recombinants with desirable traits. The Principal Components Analysis is a powerful tool to choose parental lines for successful hybridization program (Akter et al., 2009). Distribution of 20 cotton genotypes irrespective of their center of origin in four clusters revealed that cluster analysis failed to indicate any relationship between genetic divergence and geographical origin (Punitha and Raveendran, 2004; Akter, 2009). Further, geographical distribution of genotypes is not the only factor that is responsible for genetic diversity. Genetic divergence

Table 1. Mean squares for analysis of variance of seed physical and quality traits in coloured cotton.

| SOV	DF	BW	SWB	HSW	SV	SD (10^{-4})	NSB	SCS (10^{-4})	LS (10^{-4})	GOT	FL	FS	FF	FU	FE	FC
Genotype	19	0.82**	0.50**	7.54**	16.22**	26.08**	46.62**	20.83**	7.90**	88.86**	9.35**	35.21**	6.71**	41.32**	3.19**	7.83**
Year	1	0.17^{NS}	0.07^{NS}	0.77^{NS}	2.08^{NS}	24.14**	130.14**	9.21**	1.60^{NS}	1.25^{NS}	7.62^{NS}	2.61^{NS}	0.05^{NS}	0.62^{NS}	1.06*	0.00^{NS}
Geno × Yr	19	0.06^{NS}	0.04^{NS}	0.31^{NS}	1.22^{NS}	2.64^{NS}	11.45^{NS}	1.49^{NS}	0.30^{NS}	3.91^{NS}	0.63^{NS}	0.05^{NS}	0.08^{NS}	4.25^{NS}	0.19^{NS}	0.00^{NS}
Error	78	0.07	0.04	24.54	3.46	2.42	8.55	0.89	0.30	8.70	2.53	1.78	0.10	8.11	0.24	0.00
CV (%)		8.8	9.5	7.0	3.2	11.0	11.0	8.1	15.6	9.9	6.7	7.2	6.4	6.3	9.8	0.00

BW=boll weight (g), NSB=number of seed per boll, SWB=seed weight per boll (mg), HSW=hundred seed weight (mg), SV= seed volume, SCS=seed cotton per seed (mg), LS=lint per seed (mg), GOT= ginning outturn (%), FL= fibre length (mm), FS=fibre strength (g/tex), FF=fibre fineness (micronaire), FU=fibre uniformity (%), FE=fibre elongation, Geno=Genotypes, Yr=Years, d.f=degree of freedom, SOV=source of variability, DF=degree of freedom, CV=coefficient of variability, *(P≤0.05), **(p≤0.01).
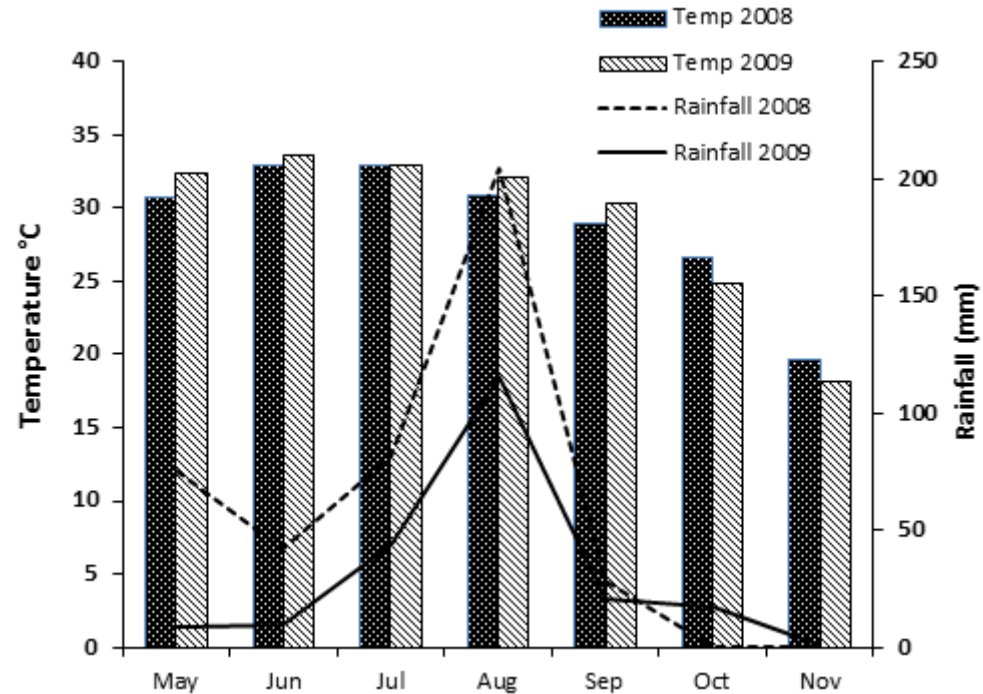

Fig 1. Average temperature and rainfall during cotton growing season for 2008-09 and 2009-10.
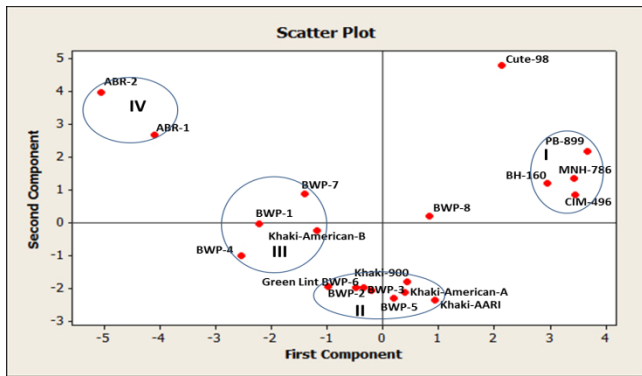
Fig 2. Principal Component analysis scatter plot depicting the genetic diversity based on morphological data of 20 cotton genotypes.
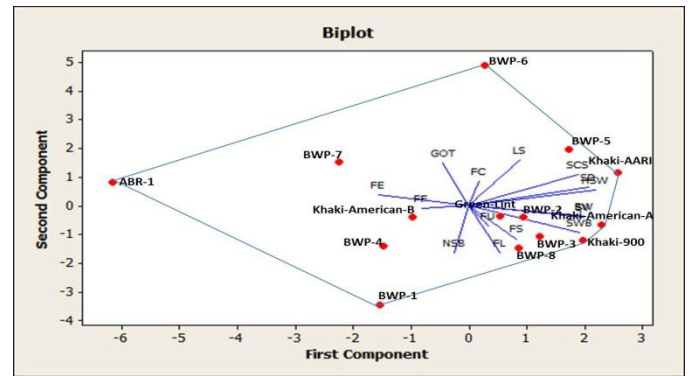
Fig 4. Biplot for genotypes-by-trait and correlation analysis among various morphological traits using data of only coloured cotton genotypes.
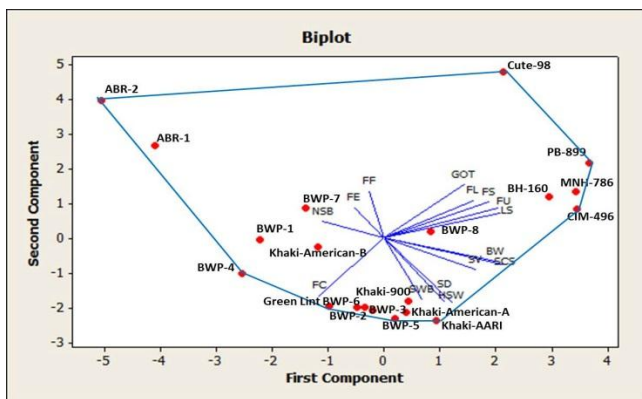
Fig 3. Biplot for genotypes-by-trait and correlation analysis among various morphological traits using cumulative data of all cotton genotypes
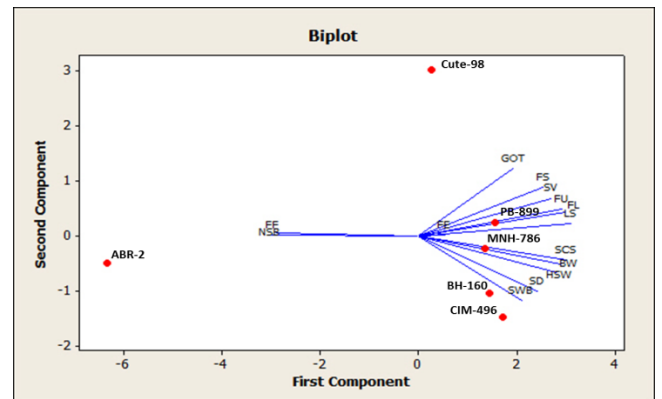
Fig 5. Biplot for genotypes-by-trait and correlation analysis among various morphological traits using data of only white cotton genotypes .

may be due to the result of numerous other factors like genetic drift, natural, artificial selection, environmental variation and exchange of breeding materials. Therefore, selection of parental lines for future cotton program should be based on genetics rather than geographical diversity (Thiyagu, 2011). All genotypes in cluster-I had white cotton fibre with good quality and seed traits. While coloured cotton genotypes falling in cluster II, III and IV were poor for seed and quality parameters (Punitha and Raveendran, 2004). It appeared that distribution of cotton genotypes to the various clusters was due to the performance of cotton genotypes and this interspecific and intraspecific diversity can further be exploited to get information about the genetic architecture of cotton plants (Ashokkumar and Ravikesavan, 2011). Biplot compares the genotypes on the basis of multiple traits and interrelationship among the traits (Yan and Rajcan, 2002). The vertex genotypes in the biplots have the longest distance from the origin of biplot and they can be best or poor in some or all of the traits (Yan et al., 2007). Perpendicular line between two vertex genotypes can be used for the comparison between neighboring genotypes (Yan and Kang, 2003). The biplot analysis for morphological traits of the cotton genotypes showed that two cotton genotypes at vertex i.e. PB-899 and CIM-496 were good for fibre length, fibre strength, fibre uniformity and lint per seed, and comparison between these two genotypes depicted that PB-899 had better fibre quality traits than CIM-496. The presence of genotypes (PB-899) near the origin in another biplot for white cotton

genotypes confirmed that PB-899 had potential for good fibre yield and quality parameter (Aghaee et al., 2010). Therefore PB-899 could be used for the development of a better cultivar (Badu-Apraku and Akinwale, 2011). However, biplot analysis for coloured cotton genotypes showed that two brown cotton genotypes i.e. Khaki American-A and Khaki-900 could serve as source for the development of elite coloured cotton genotypes and recurrent selection could be an appropriate breeding strategy to bring further improvement in these genotypes. Correlation coefficients are useful if indirect selection of secondary traits is to be used for the improvement of primary trait of interest (Hussain et al., 2010). The highly positive correlations suggested that all these traits provided the similar information about the variations among the genotypes and they all were tending to discriminate the genotypes in similar fashion (Aghaee et al., 2010). It is suggested that considerable efforts, time and resources could be saved without sacrificing the precious information if indirect selection strategy was adopted for the improvement of traits of interest (Hussain et al., 2010). Biplot for cumulative data of all genotypes showed significantly positive associations among fibre length, fibre strength, fibre uniformity, lint per seed and ginning out turn indicated that it was not difficult to improve the fibre quality of cotton genotypes (Pan et al., 2010). In contrary, biplot for coloured cotton genotypes depicted negative association of ginning out turn with fibre length, fibre strength, fibre uniformity and seed physical traits, suggesting that it would

be difficult to improve the fibre yield and quality simultaneously (Killi et al., 2005; Hussain et al., 2010). Such type of negative associations can be broken by introducing new alleles from wider genetic base or reforming the non-allelic interactions through several generations of intercrossing to develop populations (Meredith & Bridge, 1971; Hinze et al., 2011). The results of all biplots showed that fibre colour had strong negative association with fibre quality traits. This kind of association raised a question, how we can improve the fibre quality of coloured cotton so that it could be comparable with white fibred cotton?. Cotton breeders and geneticists have been studying this complex negative association and found that genes for lint pigmentation have pleiotropic effects (Richmond; 1943; Murthy 2001). Considering the complexity of this negative association it is challenging to single out any breeding method for the development of good quality coloured cotton genotypes. The breeding strategy aimed at disrupting gene pool followed by a planned selection scheme coupled with testing of colour intensity and fibre quality in a very scientific manner, are supposed to yield good results (Dutt et al., 2004). Three different biplots showed different kind of associations among the multiple morphological and quality traits. It is concluded that biplot generated from cumulative data of all the genotypes could not be compared with the biplots analysis for data of coloured and white cotton genotypes separately. The reason being that the performance of coloured genotypes was poor than white cotton genotypes and biplot describes the relationships among multiple traits only on the basis of overall pattern of data (Yan and Rajcan, 2002).

Materials and methods

Experimental site

The field experiments were conducted during the two consecutive years (2008-09 and 2009-10) at experimental farm located at University of Agriculture, Faisalabad (Latitude = 31°-26' N, Longitude = 73°- 06' E, Altitude = 184.4 m). Average temperature during the cotton growing season for years 2008-09 and 2009-10 are shown in Fig.1 (http://www.uaf.edu.pk/faculties/agri/depts/crop_physiology/agri_met_cell/met_bulletin.html).

Plant materials

A set of 20 cotton genotypes with brown, green and white lint colour belonging to *Gossypium hirsutum* L. and *Gossypium arboreum* L. were collected from different research organization of Pakistan (Supplementary Table S1). During 2007-08, the collected germplasm was planted in earthen pots and selfing was done for one generation to ensure purity of the plant material using Glasshouse facility at Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad.

Field experiments

Seeds of 20 cotton genotypes were sown on 20th May, 2008 and 10th May, 2009. The experimental design was randomized complete block design having three replications. The plant to plant and row to row distance was 30 cm and 75 cm respectively. The experimental plot of both years received the same cultural practices. Plants were fertilized with 143 kg/ha (N), 115 kg/ha (P) and 125 kg/ha (K). Nitrogen was applied in three equal doses i.e. at sowing, at first irrigation

and at the maximum flowering stage. Plant protection measures were also taken to provide insect control as required.

Sampling and data recording

Each genotype was represented by 10 plants in each replication. At maturity sample of 20 random bolls from middle, upper and lower plant parts were hand-picked. The dried and cleaned seed cotton samples were ginned using single roller ginning machine and lint obtained was expressed as ginning out turn (%). Boll weight was calculated using the following formula.
Boll weight (g) = Seedcotton weight per sample/number of bolls in a sample

Physical seed traits

Number of seeds per boll, 100-seed weight (g), seeds weight per boll (g), seed volume (ml), seed density (g ml^{-1}), seed cotton per seed (g) and lint per seed (g) were calculated following Coyle and Smith, (1997) and Rahman et al., (2005).

Fibre quality traits

Staple length (mm), fiber fineness (micronaire), fiber strength (g/tax), fibre uniformity (ratio) and fibre elongation (%) were taken from HVI (High Volume Instrument). The lint colour was scored against different colour intensities i.e. white=1, light brown/lite green=2, medium brown/medium green=3, dark brown/dark green=4.

Statistical analysis

The data were subjected to analysis of variance (Steel *et al.*, 1997) using Gen Stat statistical software. Principal Component Analysis (Ogunbayo et al., 2005) was performed to generate scatter plot for diversity and biplots for correlation and genotype-by-trait analysis using the Minitab-16 statistical package.

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