Research Article

Quantitative Studies in Upland Cotton (*Gossypium hirsutum* L.) using Multivariate Techniques

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Abstract | Sustainable production of cotton be contingent upon development of genotypes having better yield, tolerance with respect to abiotic and biotic stresses and enhanced fiber quality. Forty five elite lines of cotton were used to evaluate the genetic variability for fifteen parameters viz., plant height (cms), days taken in 50% flowering, monopodia plant^{-1}, sympodia plant^{-1}, boll weight (g), No. of bolls plant^{-1}, ginning out turn (%), lint index(g), seed index(g), 2.5 percent span length(mm), bundle strength(g/tex), micronaire (µg/in), fibre elongation (%), uniformity ratio and yield plant^{-1}(g). Using Mahalanobis D^2 analysis, these parameters were assembled into seven clusters. Among these clusters, cluster I and VII were largest each having nine and eight genotypes respectively, followed by cluster VI having seven genotypes. According to the illustrations by using hierarchical cluster analysis, total genotypes were grouped in seven clusters with 11 genotypes in cluster VI after that cluster II comprising 9 genotypes. The random distribution among genotypes showed that no parallelism exists amongst genetic and geographical diversity. First seven components in principal component analysis (PCA) having eigenvalue more than 1, showed 91.131 % the cumulative variance, while PC-1 alone showed 32.47 % variance. Hierarchical cluster analysis and PCA provided an opportunity to identify subgroups of clusters at different stages, so that every single subgroup may be analyzed critically and it will be helpful for incorporation of desirable characters in future breeding programmes.

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Introduction

Cotton (*Gossypium hirsutum* L.), generally grown for its fiber in more than eighty countries of the world, is considered chief cash earning commodity and backbone of Pakistan's economy by contributing 0.8% to GDP and 4.1% to value added in agriculture (Anonymous, 2019). The cotton industry needs higher quantity and quality of raw cotton due to revolution in textile technology. Therefore, it is need of the time to develop high yielding cotton genotypes with superior fiber quality. The development of genotypes having genetically superior qualitative and quantitative traits is inevitable to combat with biotic and abiotic stresses (Bakhtavar *et al.*, 2015). Exploitation of genetic diversity is very useful to identify desirable genotypes in existing germplasm for cotton improvement (Asha *et al.*, 2013). The genetic diversity is assumed as

major prerequisite for breeding program to overcome unexpected effects on crop plants due to frequent changes in climatic conditions (Rathinavel, 2017; Jarwar *et al*., 2019). The selection of genotypes having wider genetic diversity for numerous yield and fiber quality parameters is vital for future strategies of cotton breeding (Shabbir *et al*., 2016).

Multivariate analysis is being used as major tool to explore genetic divergence in genotypes (Jarwar *et al*., 2019). Normally, hierarchical cluster analysis, Mahalanobis D^2 statistics and principal component analysis are used to explore genetic divergence in multivariate studies. Cluster analysis is important because it is helpful for in-depth analysis by splitting the clusters into sub clusters. The PCA is multivariate tool which extracts most valuable facts from data array into principal components (Sharma, 2006). While partitioning the total variation, PCA is very appropriate statistical tool which is useful to obtain suitable parents for effective breeding strategies (Akter *et al*., 2009; Nazir *et al*., 2013). Multivariate analysis approaches on cotton genotypes were accomplished by scientists, which enabled them to categorize the existing germplasm into distinctive clusters based upon fibre quality and yield traits (Shakeel *et al*., 2015). The experiment aimed to investigate genetic divergence and association amongst forty-five cotton genotypes and categorize them into different classes using multivariate techniques. So, that this information can be utilized in further breeding studies for heterosis and ultimately resulting in improvement of yield and quality parameters by selecting highly divergent parents.

Materials and Methods

Plant materials and location properties

Forty-five cotton strains (Table 1), were studied during Kharif 2019-20 at Cotton Research Station, Faisalabad, Punjab, Pakistan which is situated at altitude of 184 m at 31° 21′ 52′ N 72° 59′ 40′ E having mean annual rainfall 43.2 mm, humidity 61.64 % and temperature 24.25 °C. The trial was sown on 1st May to evaluate the genetic variability of genotypes for morphological traits, fibre parameters and yield.

Management and design of trial

The genotypes were arranged in completely randomized block design having 3 replicates. The plot size of each entry was 4.54 m × 3.03 m, which comprised of four rows having plant x plant spacing 30 cms, while row x row spacing was 75 cms. Delinted seed of each entry was treated with fungicide and insecticide before sowing on beds. Gap filling was practiced after one week of sowing to ensure the plant population. Pre-emergence weedicide was applied before sowing and after germination manual weeding was practiced in the trial. Thinning was done at 25 days after sowing. Recommended dose of fertilizer was used viz., N: P: K @ 80:35:30 kg/ha respectively. Twelve irrigations were applied to the experiment during the season while plant protection measures were adopted as per requirement.

Traits measurement

Ten representative and undamaged plants from each plot were randomly marked for identification and data collection of parameters viz., plant height (cms), days to 50 % flowering, monopodia plant^{-1}, sympodia plant^{-1}, boll weight (g), No. of bolls plant^{-1}, ginning out turn (%), lint index (g), seed index (g), 2.5 percent span length (mm), bundle strength (g/tex), micronaire (µg/in), fibre elongation (%), uniformity ratio and yield plant^{-1}(g). Plant height (cms) was recorded with measuring rod from the base to the tip of the plant. Days to 50 % flowering was obtained by counting the days from sowing date to flower appearance on 50% plants. Monopodia plant^{-1} and sympodia plant^{-1} were calculated by counting the number of indirect and direct fruit bearing branches respectively. Boll weight (g) was calculated by picking 50 bolls from top, middle and base of each guarded plant and dividing the total weight by number of bolls. No. of bolls plant^{-1} was obtained by counting the total no. of bolls of guarded plants. Yield plant^{-1}(g) was obtained on plot basis. The seed of each entry was ginned with single roller ginning machine and lint gained from samples was weighed to calculate GOT % with the formula given below:

$$\text{GOT \%} = \frac{\text{Lint Weight}}{\text{Seed Cotton Weight}} \times 100$$

Fiber characteristics

At full maturation, the seed cotton was picked carefully and ginned after drying under sunshine. The fiber quality traits were assessed by Uster-1000 High Volume Instrumentation (HVI) (Sasser, 1981).

Statistical analysis

Tochers' method was used for Mahalanobis D^2

analysis as given by (Rao, 1952). Agglomerative heirarchial clustering method was worked out for cluster analysis following the method demonstrated by (Anderberg, 1993). Principle component analysis (PCA) was performed as given by (Jackson, 1991).

Table 1: *List of Genotypes included in the study.*

Sr. No.	Name of Genotype	Status	Source of Seed	Sr. No.	Name of Genotype	Status	Source of Seed
1	520/18	Cultivar	Cotton Research Station, Faisalabad	24	6030/18	Cultivar	Cotton Research Station, Faisalabad
2	521/18	Cultivar	Cotton Research Station, Faisalabad	25	6034/18	Cultivar	Cotton Research Station, Faisalabad
3	522/18	Cultivar	Cotton Research Station, Faisalabad	26	6037/18	Cultivar	Cotton Research Station, Faisalabad
4	523/18	Cultivar	Cotton Research Station, Faisalabad	27	6039/18	Cultivar	Cotton Research Station, Faisalabad
5	524/18	Cultivar	Cotton Research Station, Faisalabad	28	FH-496	Advanced Line	Cotton Research Station, Faisalabad
6	494/18	Cultivar	Cotton Research Station, Faisalabad	29	FH-497	Advanced Line	Cotton Research Station, Faisalabad
7	495/18	Cultivar	Cotton Research Station, Faisalabad	30	FH-500	Advanced Line	Cotton Research Station, Faisalabad
8	455/18	Cultivar	Cotton Research Station, Faisalabad	31	FH-505	Advanced Line	Cotton Research Station, Faisalabad
9	505/18	Cultivar	Cotton Research Station, Faisalabad	32	6006/15	Cultivar	Cotton Research Station, Faisalabad
10	456/18	Cultivar	Cotton Research Station, Faisalabad	33	FH-419	Advanced Line	Cotton Research Station, Faisalabad
11	6035/17	Cultivar	Cotton Research Station, Faisalabad	34	FH-504	Advanced Line	Cotton Research Station, Faisalabad
12	452/18	Cultivar	Cotton Research Station, Faisalabad	35	FH-492	Advanced Line	Cotton Research Station, Faisalabad
13	458/18	Cultivar	Cotton Research Station, Faisalabad	36	FH-495	Advanced Line	Cotton Research Station, Faisalabad
14	6028/17	Cultivar	Cotton Research Station, Faisalabad	37	FH-498	Advanced Line	Cotton Research Station, Faisalabad
15	6038/17	Cultivar	Cotton Research Station, Faisalabad	38	FH-453	Advanced Line	Cotton Research Station, Faisalabad
16	6061/17	Cultivar	Cotton Research Station, Faisalabad	39	FH-455	Advanced Line	Cotton Research Station, Faisalabad
17	6007/18	Cultivar	Cotton Research Station, Faisalabad	40	FH-503	Advanced Line	Cotton Research Station, Faisalabad
18	6008/18	Cultivar	Cotton Research Station, Faisalabad	41	FH-506	Advanced Line	Cotton Research Station, Faisalabad
19	6009/18	Cultivar	Cotton Research Station, Faisalabad	42	FH-507	Advanced Line	Cotton Research Station, Faisalabad
20	6011/18	Cultivar	Cotton Research Station, Faisalabad	43	FH-510	Advanced Line	Cotton Research Station, Faisalabad
21	6019/18	Cultivar	Cotton Research Station, Faisalabad	44	FH-511	Advanced Line	Cotton Research Station, Faisalabad
22	6021/18	Cultivar	Cotton Research Station, Faisalabad	45	FH-142*	Advanced Line	Cotton Research Station, Faisalabad
23	6023/18	Cultivar	Cotton Research Station, Faisalabad				

Table 2: *Analysis of variance for yield and related parameters in cotton (Gossypium hirsutum L.).*

Source of variation	d.f.	Plant height (cm)	Days to 50 % flowering	Monopodia per plant	Sympodia per plant	Boll weight (g)	No. of bolls per plant	Ginning out-turn (%)	Lint index (g)
Mean sum of squares									
Replications	2	232.1720	1.1062	0.0001	3.4680	0.1511	19.3016	1.8410	0.0316
Treatment	44	652.1102**	9.5438**	0.3251**	18.2460**	1.0961**	159.6583*	14.4749**	1.0714**
Error	88	129.3870	0.3261	0.0042	3.0474	0.0510	9.6298	2.3493	0.0431
Source of variation	**d.f.**	**Seed index (g)**	**2.5% span length (mm)**	**Bundle strength(g/tex)**	**Micronaire (µg/in)**	**Elonga-tion (%)**	**Uniformity ratio**	**Seed cotton yield plant^{-1} (g)**	
Mean sum of squares									
Replications	2	0.0742	0.1747	0.9734	0.0843	0.0424	5.5268	56.3466	
Treatment	44	3.4186**	16.7834**	10.5626**	1.0124**	0.1453**	22.2974**	1428.4240**	
Error	88	0.0708	0.9207	0.5106	0.0643	0.0276	2.5396	89.7302	

Results and Discussion

Highly significant differences were observed in analysis of variance among 45 genotypes of cotton for 15 quantitative parameters (Table 2). The genetic divergence depicted by 15 parameters was illustrated in Table 3 and Figure 1, which showed the contribution of each trait to the total genetic divergence. All the forty five cotton strains were congregated into seven clusters based upon D^2 statistics by using Tocher's method which depends upon the principle that the inter-cluster D^2 values must be higher than intra-cluster D^2 average values (Table 4). There was random distribution of forty five strains among seven clusters having maximum strains in cluster I (9 strains) while 8 strains in cluster VII and 7 strains in cluster VI. Six strains were present in cluster III and IV while 5 strains in cluster II and 4 strains in cluster V. Diagrammatic relationship among clusters was illustrated in Table 5, keeping in view the mean of intra and inter cluster D^2 values. Similar findings were observed by (Singh *et al.*, 2012; Singh and Dubey, 2011; Srinivasulu *et al.*, 2010; Eswara *et al.*, 2009; Gopinath *et al.*, 2009). Cluster-V showed maximum intra-cluster distance whereas it was minimum for cluster-VI and VII, which indicates that the strains in cluster-V was of diverse genetic makeup and these strains might belong to different genetic pool, whereas the inclinations were contrary for cluster VI and VII (Table 5, Figure 2). The strains of clusters IV and VI showed maximum inter-cluster distance which indicates existence of divergence in genetic makeup of strains among those clusters. Lowest inter-cluster distance was exhibited among strains in cluster-I and II, demonstrating the similarity among strains of this group regarding all parameters. The grouping array of the strains in clusters and inter-cluster distances showed that very less domestication was occurred and ultimately no parallelism amongst geographical distribution and genetic divergence of studied strains. Similar observations were described by (Singh *et al.*, 2012; Mohanty and Prusti, 2002; Mohanty, 2001; Lokhande *et al.*, 1987). While considering inter-cluster distances among the groups, best desirable segregants may be obtained by crossing the strains of clusters IV and VI after confirming their general combining ability. These outcomes are in line with the findings of (Asha *et al.*, 2013).

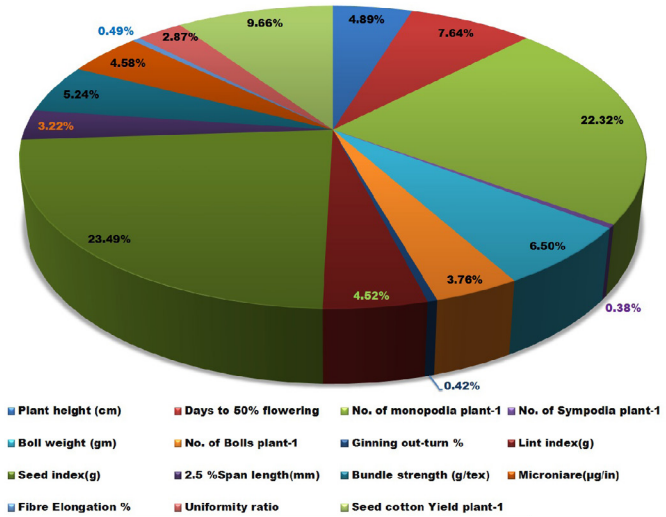

Figure 1: *Contribution of each character to the genetic divergence.*

According to hierarchial clustering (Ward's minimum variance) method, forty five strains were assembled into 7 clusters (Table 6). Cluster-VI was biggest comprising 11 strains after that cluster-II having 9 strains, cluster-V having 7 strains, cluster-I having 6 strains, cluster-IV and-VII (5 strains each) and cluster-III (2 strains). Average intra and inter-cluster Euclidean distance (D^2) was computed following

Table 3: *Contribution of parameters studied towards genetic variability in 45 strains of cotton (Gossypium hirsutum L.).*

Source	Times ranked first	Contribution %
Plant height (cm)	39	4.89
Days to 50% flowering	66	7.64
No. of monopodia plant^{-1}	165	22.32
No. of Sympodia plant^{-1}	3	0.38
Boll weight (gm)	44	6.5
No. of Bolls plant^{-1}	22	3.76
Ginning out-turn %	4	0.42
Lint index(g)	26	4.52
Seed index(g)	193	23.49
2.5 % Span length(mm)	31	3.22
Bundle strength (g/tex)	55	5.24
Microniare (µg/in)	45	4.58
Fibre Elongation %	4	0.49
Uniformity ratio	20	2.87
Seed cotton Yield plant^{-1}	63	9.66

Ward's minimum variance technique and was illustrated in Table 7. Cluster-II exhibited maximum intra-cluster Euclidean^2 distance having value of 259.54 after that clusters-I (181.65), III (151.93), IV (149.78), VII (115.33), VI (107.84) and V (0.00) demonstrating maximum variability contained by cluster-II related to other clusters. The range of inter-cluster Euclidean distance (D^2) was from 169.13 (among clusters VI and VII) to 769.72 (clusters VI and V). According to the study, the clusters VI and VII are highly divergent, consequently, the strains from these two clusters may further be exploited in future breeding strategies for heterosis studies. Similar conclusions were submitted by (Asha *et al.*, 2013; Lakshmi *et al.*, 2009; Srinivasulu *et al.*, 2010; Altaher and Singh, 2003).

Principal Component Analysis (PCA) revealed that Eigen values of first seven components was more

Table 4: *Distribution of 45 cotton strains in various clusters using Tocher's method.*

Cluster No.	Name of genotype(s)	No. of genotypes
I	FH-498, FH-453, 6038/17, 522/18, 6021/18, FH-507, 6035/17, FH-505, 6011/18	9
II	523/18, 6034/18, FH-496, FH-452, FH-142	5
III	6030/18, 524/18, 494/18, 6007/18, FH-510, 520/18	6
IV	FH-458, 6028/17, 6061/17, FH-511, 6023/18, FH-419	6
V	6008/18, FH-503, FH-504, FH-495	4
VI	6019/18, 521/18, 455/18, 505/18, 456/18, 6006/15, FH-492	7
VII	495/18, FH-455, 6009/18, 6037/18, 6039/18, FH-497, FH-500, FH-506	8

Table 5: *Average intra- (Diagonal values) and inter (Above diagonal values) cluster divergence D^2 and D^* values of among 7 clusters of 45 Cotton (Gossypium hirsutum L.) genotypes.*

Clusters	I	II	III	IV	V	VI	VII
I	25.21(5.02)	41.37(6.43)	76.41(8.74)	61.27(7.83)	82.84(9.10)	231.95(15.23)	173.59(13.18)
II		31.42(5.60)	59.65(7.72)	68.93(8.30)	81.33(9.02)	183.29(13.54)	128.36(11.33)
III			48.96(6.99)	79.86(8.94)	105.2(10.26)	123.81(11.13)	104.96(10.25)
IV				51.75(7.19)	140.68(11.86)	241.47(15.54)	155.65(12.48)
V					83.62(9.14)	192.58(13.88)	152.81(12.36)
VI						0.00(0.00)	88.61(9.41)
VII							0.00(0.00)

** Values in parentheses are D values.*

Table 6: *Distribution of 45 cotton strains in various clusters using Ward's minimum variance method.*

Cluster no	Name of genotype(s)	No. of genotypes
I	455/18, 6008/18, 456/18, FH-497, FH-500, FH-507	6
II	520/18, 495/18, 6028/17, 6038/17, 505/18, FH-495, FH-503, FH-506, FH-142	9
III	522/18, FH-455	2
IV	6061/17, 6007/18, FH-419, FH-453, FH-511	5
V	494/18, FH-505, 6035/17, 6021/18, 6034/18, FH-496, FH-492	7
VI	521/18, 523/18, 524/18, FH-452, 6009/18, 6011/18, 6023/18, 6030/18, 6037/18, 6006/15, FH-504	11
VII	FH-458, 6019/18, 6039/18, FH-498, FH-510	5

Table 7: *Average Eucledian² (intra and inter-cluster) values among clusters of 45 Cotton (Gossypium hirsutum L.) strains.*

Cluster No.	I	II	III	IV	V	VI	VII
I	181.65	407.91	275.83	272.82	621.95	277.34	256.86
II		259.54	293.51	459.16	712.65	556.16	553.7
III			151.93	279.3	631.29	230.42	254.59
IV				149.78	378.43	282.42	196.98
V					0	769.72	536.83
VI						107.84	169.13
VII							115.33

Table 8: *Factor loadings and Eigen values of principal components.*

	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7
Eigen value (Root)	3.246	2.874	2.024	1.628	1.367	1.0354	1.024
% Var. Exp.	32.472	17.654	11.107	9.421	8.223	6.015	6.239
Cum. Var. Exp.	32.472	50.126	61.233	70.654	78.877	84.892	91.131

than one and contributed 91.131 percent to the total variability (Table 8). Therefore, inference can be drawn that the most valuable information of data set was present in first seven principal components. Similar observations were previously described by (Kumari *et al.*, 2019; Shah *et al.*, 2018; Latif *et al.*, 2015; Kaleri *et al.*, 2015; Saeed *et al.*, 2014).

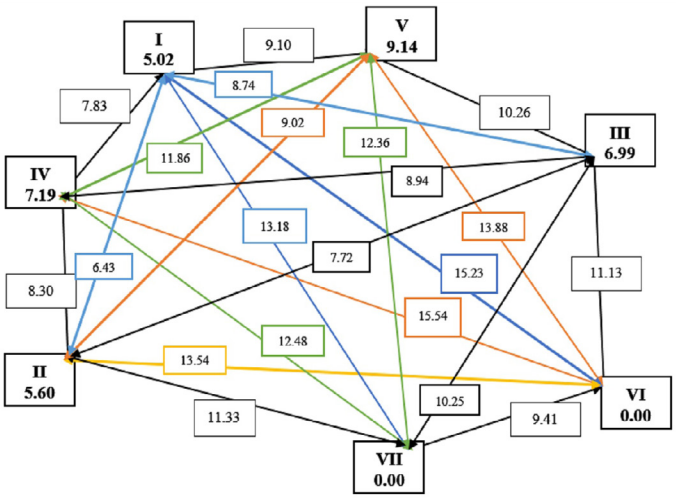

Figure 2: *Cluster diagram.*

Conclusions and Recommendations

The PCA and hierarchical cluster analysis confirmed findings of each other. Non-correspondence between geographic diversity and genetic divergence was confirmed by adopting these three methods of grouping. Hierarchical cluster analysis provided an opportunity to identify subgroups of clusters at different stages, so that every single subgroup may be analyzed critically and will be helpful for incorporation of desirable characters in future breeding programs.

Novelty Statement

Hierarchical cluster analysis and principal component analysis provided an opportunity to identify subgroups of clusters at different stages, so that every single subgroup may be analyzed critically and it will be helpful for incorporation of desirable characters in future breeding programmes.

Author's Contribution

Muhammad Rizwan: Performed the experiment and collected data.
Jehanzeb Farooq: Managed overall crop and gave technical input.
Muhammad Farooq: Helped in paper write up,
Muhammad Aqeel Sarwar: Performed the statistical analysis.
Farrukh Ilahi: Helped in data collection.
Abid Ali: Helped in data analysis.
Muhammad Asif: collected the literature.
Ghulam Sarwar: Supervised the study.

Conflict of interest

The authors have declared no conflict of interest.

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