Research Article

Diversification Assessment in Cotton for Lint Quality, Disease Response and Economic Attributes by Multivariate Analysis

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Abstract | Genetic erosion is a major pitfall in man directed evolution faced by cotton crop in Pakistan during recent years. Present study was carried out at Cotton Research Station, Vehari during 2019-20. The main purpose was to explore the genetic diversity in the strains under study. Principal component analysis, along with agglomerative hierarchical clustering tools were employed. Twenty-four recently bred upland cotton strains bearing diversified origin were configured in triplicate following Randomized Complete Block Design (RCBD). The data for novel attributes were recorded. Analysis of variance results indicated that a significant level of variation was existing among the strains for disease index, plant population and micronaire value at (p<0.05), while means for all other study traits were found highly significant at (p<0.01). Descriptive statistics illustrated presence of sufficient range in the studied traits. Out of 11 principal components (PC), first 5 PC indicated Eigen value >1 and contributed 75.826% towards cumulative variability. Yield related traits plus lint quality attributes depicted positive loading behavior, while disease index (-0.215) and plant population (-0.054) showed negative loading attitude in PC-1. Similarly, boll counts plant^{-1} (0.778) and fiber length (0.480) indicated strong positive loading trend, whereas fiber fineness (-0.742) and ginning out turn (-0.709) showed negative loading attitude towards PC-2. The correlation analysis indicated presence of significant (0.632) association at (p<0.01) between fiber fineness and ginning out turn. Whereas disease index showed negative association with all studied traits except monopodial branches. All strains were categorized in two clusters. The strains included in clusters 1 inclusive of check cultivar CIM-602 were distinguished by 44% higher within class variance than strains in cluster 2 respectively. The cotton strains S-1918 and S-1923 were found most diverse and can be utilized in future gene pyramiding schemes to breed cotton cultivars with broad genetic base.

Introduction

Cotton is a unique crop due to its yarn production for textile industry. This aspect makes it a major cash crop in a true sense. It is backbone of our nation-al economy by adding 0.81% to GDP and roughly 4% value addition in agriculture sector (Anon, 2019). Cotton is ranked as the 6th largest source of edible oil globally which is obtained from its seeds as by product (Xu *et al.*, 2016). Its genus Gossypium, consists of 5

allotetraploid and 44 diploid species as primary gene pool. Among all species, only 4 are cultivated globally. Pakistan, India, U.S.A and China are the prominent cotton producing countries contributing two third of the crop area worldwide (Dahab *et al.*, 2013). Central Asian countries like Uzbekistan and Turkmenistan are present at 8th and 10th positions in the world ranking for cotton production respectively, while Tajikistan and Egypt are placed below top 10th ranking positions (Shuli *et al.*, 2018). Textile sector demands huge quantity of superior quality lint to promote national exports and earning foreign currency reserves. Bakhtavar *et al.* (2015) suggested that development of cotton varieties bearing improved morphological and fiber traits is ultimate solution to combat the bottlenecks in successful production of this crop.

Multivariate analysis is a powerful statistical tool which enabled researchers to explore the multidimensional association among studied variables (Grahic *et al.*, 2013). This approach on cotton genotypes enabled scientists to classify existing gene pool into typical clusters based on lint quality and yield related traits (Shakeel *et al.*, 2015). Variation behavior observed in genotypes during principal component analysis leads scientists to choose parental genotypes for initiating fruitful crop improvement schemes (Isong *et al.*, 2017). Hierarchical clustering procedure followed after principal component analysis (PCA) enhanced chances to select subgroup of clusters at the highest dissimilarity level (Rizwan *et al.*, 2021). In any plant breeding program, genetic diversity plays key role for selection of desirable genotypes (Riaz *et al.*, 2019). Saeed *et al.* (2014) indicated the classification of imported cotton germplasm into five distinct clusters. Seed cotton yield is a complicated character, highly influenced by metrological conditions (Abbas *et al.*, 2013). For improving such complex polygenic trait, many yield related attributes such as plant stature, number of monopodial branches, sympodial branches count and fruit retention plant^-1 are accountable (Anil and Abhay, 2017). Munir *et al.* (2020) found similarities in scatter behavior of interspecific cotton hybrids with their parents. For quantification of genetic divergence, researchers have extensively employed the PCA procedure which assists in isolation of suitable parental genotypes for further breeding programs (Rehman *et al.*, 2015). Sarwar *et al.* (2020) reported development of disease resistant cotton genotypes having better yield along with premium lint quality traits in a similar study.

Continuous man made selective breeding efforts in cotton crop resulted genetic erosion and loss of diversity. This process has ultimately led to high yielding cultivars bearing narrow genetic base. Such cultivars with narrow genetic base are highly vulnerable to different biotic and abiotic stress factors. Pakistan is ranked globally at fifth position with respect to facing climate change hazards in the form of uneven rains, elevated temperature in summer and biotic stress in the form of insect pests (Global climate risk index report, 2018). The presence of genetic divergence is vital for successful breeding program to combat unexpected stress on crop plants posed by climate changing conditions as indicated by Jarwar *et al.* (2019). Trial location in this study is unique and hot spot for cotton leaf curl virus. Recently bred twenty-four cotton strains bearing diversified origin were included in the study never reported earlier. It was hypothesized that crossing between diversified parents will outcome the strains with better adaptability and yield performance.

The main objective of present research was to explore the magnitude of diversification in upland cotton strains under the study. These selected strains may be employed by plant breeders as a potential tool for choosing parental material in future crossing programs.

Materials and Methods

Description of site
The present experiment was carried out at Cotton Research Station, Vehari, Pakistan (located at 72° 37″E longitude and 30° 25″N latitude, 175m above sea level). The climate of Vehari district is semi-arid and sub-tropical type. The soil texture was loamy with PH value 8.1 and retained 0.9 % organic matter contents with history of wheat-cotton cropping pattern under irrigated conditions. The available phosphorus and potash were 7 and 136 ppm respectively. Moreover, 36 % water saturation ability made that soil well suited for raising cotton crop. The soil analysis was carried out following the method of Homer and Pratt (1961) at soil and water testing laboratory, Vehari.

Experiment layout and planting material
Twenty-four strains of upland cotton bred by the method of pedigree selection after genetic pyramiding having the diversified origin (Table 1) including check cultivar (CIM-602) were sown in randomized complete block design with three blocks on 23th May, 2019.

Table 1: *Detail of studied 24 cotton strains.*

S. No.	Code	Parentage	S. No.	Code	Parentage
1	S-1901	CIM-632 X VH-402	13	S-19013	VH-447 X NS-181
2	S-1902	QSR-189 X VH-305	14	S-19014	VH-450 X China-1
3	S-1903	Thakar-808 X VH-321	15	CIM-602	Check cultivar
4	S-1904	LRA-5166 X RH-668	16	S-19016	Tipu-9 X BS-18
5	S-1905	ASP-59 X SLH-19	17	S-19017	LS-191 X BS-18
6	S-1906	LS-191 X BS-18	18	S-19018	NIAB-545 X VH-189
7	S-1907	NIAB-545 X NIAB-112	19	S-19019	NIAB-444 X VH-363
8	S-1908	NIAB-444 X VH-305	20	S-19020	US-3090 X RH-662
9	S-1909	MNH-786 X RH-662	21	S-19021	N-414 X FH-490
10	S-19010	N-414 X VH-305	22	S-19022	VH-402 X NIAB-78
11	S-19011	VH-402 X CH-18	23	S-19023	QSR-189 X US-3090
12	S-19012	N-414 X VH-345	24	S-19024	VH-440 X MNH-886

Each plot consisted of 7.5 m long 4 cotton rows. The seeding was done on 75 cm wide raised beds by maintaining 20-25 cm spacing within hills. First irrigation was applied immediately after sowing and the subsequent irrigation on five days later to ensure optimum seed germination. All the remaining irrigations were applied at the uniform interval of 14 days till the crop maturity. Thinning was done at the four leave stage and left one healthy plant per hill. The recommended fertilizer dose NPK@ 80:35:30 kg ha^{-1} was applied to the trial (Rizwan *et al.*, 2021). All the phosphorus and potassium were applied at the time of land preparation. Nitrogen was applied in three equal split doses namely at sowing, at flowering and boll formation stages. Pest populations were kept below the economic injury level by spraying recommended pesticides. Picking was done on 28th November, 2019 after opening of more than 90% bolls determined by counting.

Recording of Data
Ten randomly selected healthy plants were tagged from each plot for recording the data. Plant height was measured in cm with wooden scale starting from base to top of the stem. Number of monopodial branches and mature bolls were recorded by counting each tagged plant and then average of ten plants was calculated. Average boll weight in grams was obtained by picking all open bolls from each tagged plant, weighing seed cotton and dividing by sum of picked bolls. Number of plants were counted in each entry after picking for plant population ha^{-1}. On the picking day seed cotton yield of each plot was obtained and converted into kg ha^{-1}. After sun drying and cleaning the seed cotton samples were taken and

ginned with electric single roller ginning machine and calculated Ginning Out Turn % as per following formula.

G.O.T % = (Weight of lint / weight of seed cotton) × 100

Fiber analysis
The lint samples were drawn and evaluated for lint quality parameters after cleaning and sun drying by High Volume Instrument model USTER 1000 using methodology previously reported by Sasser (1981). This equipment provided data of fiber attributes as, fiber length (mm), fiber strength (g tex^{-1}) and micronaire value which indicated lint fineness.

Cotton Leaf Curl Virus Disease index
This trait was measured in % age based on leaf symptoms. Disease response was calculated according to the procedure reported by Sarwar *et al*. (2020).

Biometric Analysis
Average data of the studied traits was subjected to Analysis of Variance (ANOVA) (Steel *et al.* 1997). Principal component analysis (PCA) was calculated according to the procedure given by Jackson (1991) Agglomerative hierarchical clustering (AHC) with wards method was performed according to procedure given by Anderberg (1993). Correlation analysis, biplot and the dendogram based on euclidean distance were generated by XLSTAT software.

Results and Discussion

The ANOVA results revealed that the studied cotton strains had significant variation for traits like CLCuD

Table 2: *Analysis of variance (ANOVA) and descriptive statistics results in 24 cotton strains used in the study.*

		Mean squares values										
Source	DF	SCY	BC	BW	DI	MB	PH	PP	GOT	FL	FS	MK
Blocks	2	218057	11.542	0.061	44.097	0.117	319.389	1360	0.844	0.942	1.402	**0.273**
Strains	23	422820[A]	83.109[A]	0.192[A]	97.086[B]	0.703[A]	470.811[A]	3050[B]	15.006[A]	3.119[A]	20.604[A]	0.478[B]
Error	46	51716	1.455	0.803	15.329	0.563	105.374	1047	0.221	0.049	0.280	0.021

Descriptive statistics

Traits	Minimum	Maximum	Mean ± S.E	Std. Deviation	C.V %
Seed cotton yield kg ha^{-1}	534	2452	1280±84.63	414.598	18.63
Average bolls plant^{-1}	19	39	28.458±1.095	5.365	4.2
Average boll weight in (g)	2.4	3.6	2.992±0.084	0.412	9.53
CLCuV disease index %	35	70	57.917±1.901	9.315	18.93
Monopodia plant^{-1}	0	4.6	0.908±0.201	0.985	16.86
Average plant height (cm)	93	164	126.058±3.979	19.492	8.4
Plant population ha^{-1}	23250	38750	32452±796	3900	11.2
Ginning out turn %	36.5	46.6	40.8±0.479	2.349	1.16
Staple length (mm)	23.9	28.34	26.048±0.225	1.102	0.85
Fiber strength (g tex^{-1})	19.1	33.5	26.088±0.604	2.957	2.01
Micronaire value	3.89	5.8	4.656±0.082	0.082	3.08

[A]*Significant (p<0.01)* [B] *significant at (p<0.05)*
SCY: *seed cotton yield in kg ha^{-1}*; **BC:** *Number of bolls plant^{-1}*; **BW:** *Average boll weight (g)*; **DI:** *Disease index %*; **MB:** *Number of monopodia plant^{-1}*; **PH:** *Average plant height (cm)*; **PP:** *Plant population ha^{-1}*; **GOT:** *Ginning out turn %*; **FL:** *Fiber length (mm)*; **FS:** *Fiber strength (g tex^{-1})*; **MK:** *Fiber micronaire value.*

disease index, plant population ha^{-1} and micronaire value indicating fiber fineness at (p<0.05), while all other studied traits displayed highly significant variation at (p<0.01) as presented in Table 2. These results provided sufficient grounds for further analysis.

The results of descriptive statistics indicated that ample variation range is present in studied cotton strains. Seed cotton yield ranged from 534 kg ha^{-1} to 2452 kg ha^{-1} with 18.63% coefficient of variability (CV). Minimum (0.082) standard deviation from means was recorded in micronaire value, while maximum (3900) for plant population ha^{-1}. The CV value was (0.85%) for fiber length and (18.93%) for CLCuV disease index (Table 2).

Correlation analysis illustrated the presence of few significant association between novel traits (Table 3). Significant correlation among key traits is a good aspect for success of any breeding program, as it enhances chances for isolation of genotypes bearing desirable attributes concurrently (Ali *et al.*, 2009). Seed cotton yield indicated positive (r = 0.271) correlation with boll weight similarly plant height depicted positive (r = 0.366) correlation to boll counts plant^{-1} at (P < 0.05). These results confirmed the outcomes given

by Salahuddin *et al.* (2010), who discovered positive connection between seed cotton yield and boll weight in upland cotton. The GOT showed highly positive (r = 0.632) links with micronaire value indicating fiber fineness. By increasing GOT lint quantity is increased hence, ultimately quality of lint is deteriorated and lint became coarser possessing higher micronaire value. These results are confirmatory to the earlier findings of Khan *et al.* (2017). Similarly, monopodial branches depicted positive (r = 0.406) association with plant height at (p<0.01). In general CLCuV disease index indicated negative correlation with all study parameters except for monopodial branches. These results partially contradicted to earlier findings of Saeed *et al.* (2014) in which CLCuV indicated negative bonding with monopodial branches while studying imported cotton genotypes. The reason behind this contradiction was dominance of virus strain (Burewala) at trial location. The strains studied in this trial were domestically bred and well adapted to this viral disease.

Principal Component Analysis
It is an excellent data mining tool employed to isolate the potential parental combinations for initiation of a successful breeding scheme (Nazir *et al.*, 2013). The prime function of this tool is to allocate the entire

Table 3: *Pearson correlation coefficients for key traits in 24 cotton strains.*

Variable	SCY	BC	BW	DI	MB	PH	PP	GOT	FL	FS	MK
SCY	1										
BC	0.251	1									
BW	0.271 [B]	0.112	1								
DI	-0.012	0.098	-0.299	1							
MB	0.366	0.360	-0.090	0.263	1						
PH	0.045	0.366 [B]	-0.029	-0.186	0.406 [A]	1					
PP	0.113	-0.145	-0.196	-0.099	-0.160	-0.008	1				
GOT	0.189	-0.327	0.030	-0.176	0.141	0.239	0.019	1			
FL	0.010	0.358	0.058	-0.118	0.087	0.380	0.095	-0.190	1		
FS	0.379	0.062	0.013	-0.179	0.232	0.204	-0.043	0.193	0.246	1	
MK	0.210	-0.341	-0.190	-0.196	0.048	0.208	0.011	0.632 [A]	-0.145	0.253	1

[A] Significant at (p<0.01) [B] significant at (p<0.05)

Abbreviations: **SCY:** *seed cotton yield in kg ha⁻¹ ;* **BC:** *Number of bolls plant⁻¹ ;* **BW:** *Average boll weight (g) ;* **DI:** *Disease index %;* **MB:** Number of monopodia plant⁻¹ ;* **PH:** *Average plant height (cm) ;* **PP:** *Plant population ha⁻¹ ;* **GOT:** *Ginning out turn %;* **FL:** *Fiber length (mm) ;* **FS:** *Fiber strength (g tex⁻¹) ;* **MK:** *Fiber micronaire value.*

variance into the several factors, which is applicable for selection of parental combinations to plan to a valuable breeding scheme (Akter *et al.*, 2009). The results of eleven calculated PC, s depicted maximum cumulative variability packed in order of PC-1 > PC-2 > PC-3 and so on (Table 4).

According to the Eigen value >1 rule as proposed by Kaiser (1960), first five PC, s were selected containing 75.83% of the total variability. Remaining six PC, s represented 24.17 % of the residual variation. The broad range of variation was observed with respect to the factor plains PC-1 and PC-2 viz., 21.33% and 19.38% respectively (Table 4). Jarwar *et al.* (2019) also found wider variation with respect to PC-1and PC-2 factor plain while study on chines origin cultivars of upland cotton. Higher magnitude of variability squeezed at this factor plains is important and gave clue for the importance of PC-1 and PC-2 for traits under the study.
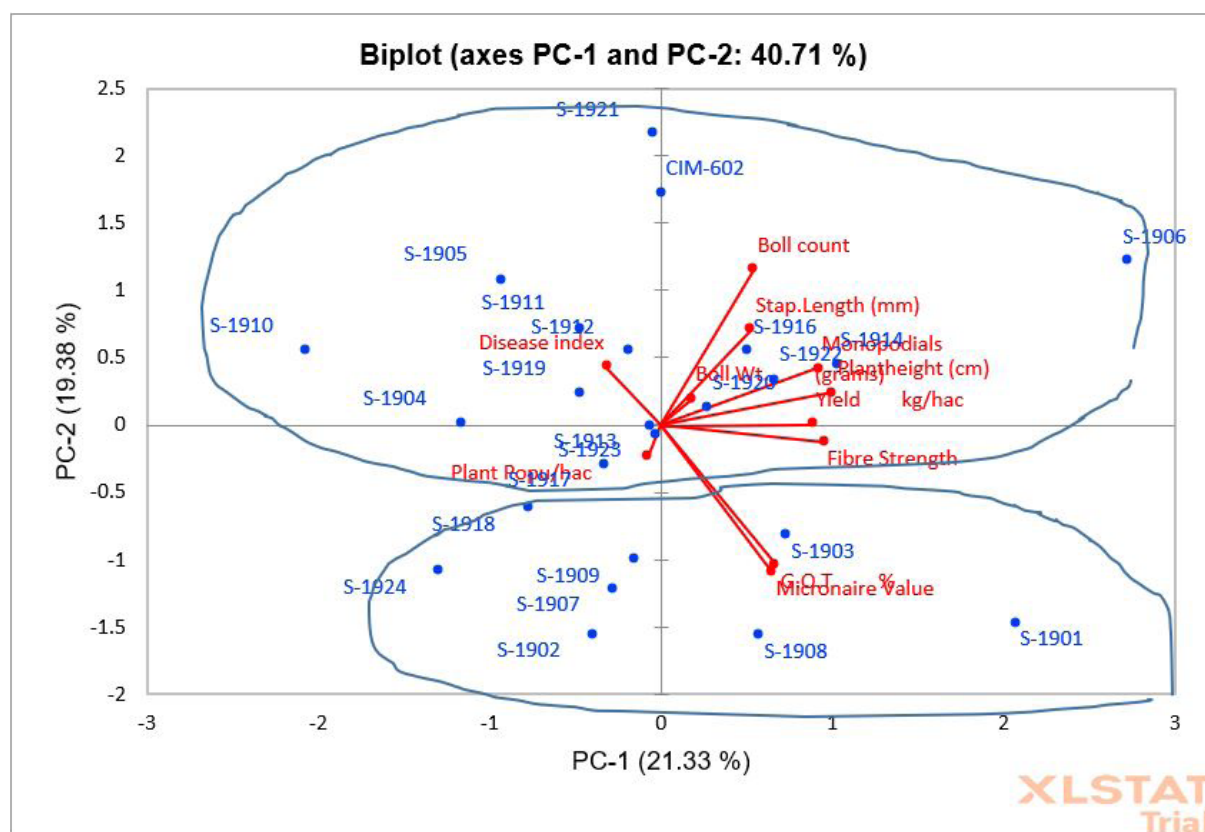
Each PC is derived from the component factors contributing towards that particular PC. In the present study, following traits showed positive loading trend with respect to magnitude in the order of plant height > fiber strength > monopodial branches plant⁻¹ > seed cotton yield > GOT % age, while slightly negative loading pattern towards attributes in the following order of CLCuV disease index %age > plant population ha⁻¹ in PC-1 (Table 4). Similar factors loading pattern for seed cotton yield, boll counts plant⁻¹, boll weight, GOT % age, fiber length and fiber strength

towards PC-1 in cotton was reported earlier by Shah *et al.* (2018). PC-1 is vital and reliable for the expression of disparity among traits studied in cotton. (Malik *et al.*, 2011). In PC-2 boll counts plant⁻¹ showed strong positive loading trend followed by fiber length, while micronaire value (fiber fineness) indicated highly negative loading attitude followed by GOT % age respectively. These findings confirmed the component loading behavior of plant height, monopodial plant⁻¹, fiber length and miconaire value indicating fiber fineness towards PC-2 in cotton as reported by Sarwar *et al.* (2020). Hence, current results in comparison to early findings by Nazir *et al.* (2013) depicted importance of PC-1 and PC-2 for information related to key traits in upland cotton.

One of the informative graphical representations of multivariate dataset is biplot graph which is linked to a relevant data matrix (Sarwar *et al.*, 2020). Since calculated PC, s are unrelated with each other, PC-1 and PC-2 are selected to draw biplots on opposite axis due to packing of maximum amount of variability in them (Table 4). Other researchers like Sarwar *et al.* (2020) explained results on similar factor plain in the study on upland cotton genotypes. The presence of cotton strains in all biplot graph area indicated occurrence of ample variation for component traits at selected factor plain. The distance of vectors with respect to origin of biplot indicated input magnitude of variables towards total divergence among genotypes studied. The biplot showed contribution of traits in the following order, micronaire value > GOT

Table 4: *Results of all calculated PC, s to study attributes in 24 cotton strains.*

	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6	PC-7	PC-8	PC-9	PC-10	PC-11
Eigen value	2.346	2.132	1.467	1.304	1.092	0.828	0.545	0.474	0.348	0.285	0.180
Variability %	21.33	19.38	13.333	11.854	9.927	7.523	4.954	4.306	3.162	2.588	1.641
Cumulative %	21.33	40.713	54.046	65.9	75.83	83.35	88.3	92.61	95.77	98.36	100

Component matrix/ loading factors

Traits under study	PC-1	PC-2	PC-3	PC-4	PC-5
Seed cotton yield kg ha^{-1}	0.597	0.001	0.004	0.480	0.552
Boll counts plant^{-1}	0.366	0.778	0.012	0.041	-0.023
Average Boll weight in (g)	0.121	0.131	-0.636	0.617	-0.095
CLCuV disease index %	-0.215	0.289	0.778	0.121	0.139
Monopodial plant^{-1}	0.620	0.285	0.531	0.136	-0.032
Plant height (cm)	0.682	0.163	-0.013	-0.420	-0.361
Plant population ha^{-1}	-0.054	-0.164	-0.134	-0.490	0.739
Ginning out turn %	0.458	-0.709	0.091	0.057	-0.166
Fiber length (mm)	0.360	0.480	-0.342	-0.477	0.023
Fiber strength (g tex^{-1})	0.641	-0.090	-0.109	0.022	0.210
Micronaire value	0.439	-0.742	0.140	-0.097	-0.094

Figure 1: *Biplot indicating scatter pattern of cotton strains along with contribution of various traits in total divergence (PC-1 x PC-2).*

% age > boll counts plant^{-1}, whereas minimum divergence was loaded by plant population followed by boll weight (Figure 1). The strain (S-1901) bearing 5.8 miconaire value as highest, the strain (S-1906) giving highest (2452 kg ha^{-1}) seed cotton yield and the strain (S-1910) distinguished as most dwarf in stature bearing (93 cm) plant height depicted unique behav-ior and found present on periphery of the plot. The distinct scatter behavior of these 3 strains confirmed the loading behavior of respective traits in PC-1 for which trait these strains are famed (Table 2). It also illustrated presence of two groups among 24 cotton strains as proved in cluster analysis.

Table 5: *Clustering behavior of 24 advance cotton strains on the basis of euclidean distance.*

Cluster number	Number/ central objects	Average euclidean distance to centroids	Within class variance	Strains
1	16 (S-1923)	2961.3	15169099	S-1904, S-1905, S-1906, S-1910, S-1911, S-1912 S-1913, S-1914, CIM-602(Check Variety) S-1916, S-1917, S-1919, S-1920, S-1921, S-1922 S-1923,
2	8 (S-1918)	2712.276	10495217	S-1901, S-1902, S-1903, S-1907, S-1908, S-1909 S-1918, S-1924

Cluster Analysis

Agglomerative hierarchical clustering (AHC) technique with ward's method was used in this study based on euclidean distance. The results indicated presence of two clusters in 24 cotton strains. Contrary to these results Munir *et al.* (2020) reported presence of four clusters in cotton during diversity analysis in interspecific hybrids. This contradiction was due to presence of greater range of variability in cotton hybrids obtained by crossing two species as compared to strains studied here. First cluster contains 16 strains inclusive of check cultivar (Table 5) and second cluster contains 8 strains respectively.
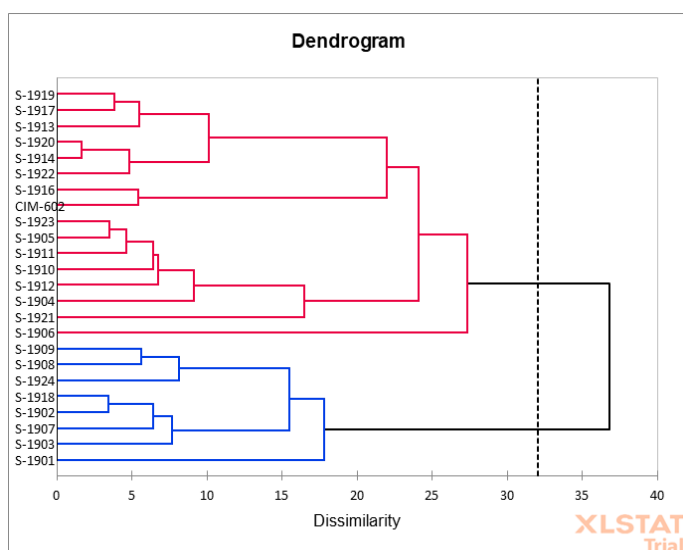

Figure 2: *Dendrogram constructed with wards method based on euclidean distance in 24 cotton strains.*

The dendogram was plotted on scores of dissimilarities among cotton strains. The dotted line represents automatic truncation, leading to two groups (Figure 2). S-19018 and S-19023 strains were found central objects in their respective class indicating highest euclidean distance from the main centroid, so these are most divergent strains with respect to the selected factor plain. Any strain did not depict obvious separation from clustering behavior as already indicated by Sarwar *et al.* (2020). It means all study strains were fallen in mentioned two clusters and no strain displayed deviation to the prevailing trend.

Conclusion and Recommendations

PC-1 and PC-2 proved novel for seed cotton yield and lint quality parameters in current study. Two clusters were clearly identified among 24 cotton strains during PCA based cluster analysis. S-19018 and S-19023 were found the most divergent strains with highest euclidean distance between them. These two strains can be crossed to obtain genotypes bearing high seed cotton yield and superior fiber traits. Such upcoming genotypes will help to boost cotton production in the country.

Acknowledgements

Novelty statement

Multivariate analysis is a modern technique used for examining the relationship of multiple variables simultaneously. XLSTAT software was applied in present research endeavor, rarely exploited by researchers for data analysis and graphics. Narrow genetic base is an ultimate consequence of man directed evolution faced by cotton cultivars in Pakistan, which resulted decline in cotton production in the country. Changing climate is an additional factor contributing towards cotton decline. Upcoming genotypes as a result of crossing between divergent strains found in this study will be a solution to above mentioned pitfalls.

Author's Contribution

Muhammad Jamil: Created the idea, reviewed the literature and wrote final draft of this paper.
Kamran Javed: Performed trial in the field and recorded data.

Imran Akhtar: Analyzed data and prepared figures and tables.

Conflict of interest
The authors have declared no conflict of interest.

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