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# Genetic diversity for seed cotton yield parameters, protein and oil contents among various Bt. cotton cultivars

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## Abstract

Availability of sufficient genetic diversity is prerequisite for selection of economically important traits. Forty upland cotton genotypes were evaluated for genetic variability with respect to seed cotton yield attributes, fiber quality traits and protein and oil content in the seed through PCA and cluster analysis. Considerable genetic differences were estimated among the genotypes for most of the characters including bolls per plant, seed cotton yield, lint percentage, oil content, protein content and fiber uniformity while non-significant results were recorded for boll weight, fiber length, fiber strength and fiber fineness. Phenotypic variances were a little higher than genotypic variances for all the traits except bolls per plant and seed cotton yield for which environmental variance was higher indicating more influence of environmental factors. Heritability in broad sense was high for all the traits except bolls per plant and seed cotton yield. Genetic advance was the highest for seed cotton yield (31.50) followed by bolls per plant (13.77), fiber uniformity (9.26) and crude protein (8.97). Correlation analysis revealed the highest significant correlation between seed cotton yield and bolls per plant (0.858) followed by seed cotton yield and fiber fineness (0.469). Oil and protein contents were negatively correlated. Principal component analysis (PCA) revealed four PCs exhibiting Eigen value more than 1and contributing 66.1% in total variability. Dendrogram showed that the clusters I and II were the most diverse clusters indicating that their members had great genetic diversity for the characters under study. Genotypes belonging to distant clusters may be used for exploiting the maximum genetic diversity.

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**Introduction**

Cotton (*Gossypium hirsutum* L.) is the world's most important fiber crop and Pakistan ranks 4th among cotton producing countries in world (USDA, FAS, 2014). In Pakistan, cotton contributes 1.4% to GDP and 6.7% to value added in agriculture (Govt. of Pakistan, 2014-15). It provides livelihood to over 60 million people directly and indirectly and accounts for approximately 78% of Pakistan's export earnings (Govt. of Pakistan, 2014-15). There is fluctuation in cotton yield of Pakistan and its actual obtained in the field is low than the exact genetic potential (Esmail *et al.*, 2008).

Cotton seed contain a considerable amount of oil ranging from 16% to 24% (Khan *et al.*, 2010) and it is also second major source of domestic edible oil production(Govt. of Pakistan, 2014-15). Not only a rich source of edible oil, cottonseed has a considerable amount of protein ranging from 14% to 20% (Qayyum *et al.*, 2009).

During the year 2014-15 total requirement of edible oil was 3.069 million tonnes while local production from all the sources was 0.567 million tonnes (Govt. of Pakistan, 2014-15). To fill the gap between consumption and production, 2.502 million tonnes oil was imported by paying Rs.241.936 billion rupees (Govt. of Pakistan, 2014-15). Besides its major product i.e. lint, cotton is also an important source of edible oil (Ali *et al.*, 2016), though it is ignored as an oilseed crop.

In variety development programs fiber yield is given more importance than its oil yield. If we want to reduce the edible oil imports in the country we need to develop such varieties having both high fiber and oil yield (Munawar *et al.*, 2013).

Factors responsible for low cotton production include high temperature at flowering stage (Rahman *et al.*, 2006), cotton leaf curl disease i.e. CLCuD (Shakeel *et al.*, 2016), sucking insect pest (ref) and lack of resistant/tolerant varieties (ref) in major cotton growing areas (Panni *et al.*, 2012).

In Pakistan, there is a lack of yield sustainability. Sustainability in yield in future will depend on new cotton varieties with higher potential having tolerance against several biotic and abiotic factors (Ahmad *et al.*, 2012). The main objective of any breeding program is to improve the yield by using different techniques (Patial *et al.*, 2011). Cotton is often cross pollinated crop in which large amount of genetic variation is observed for many important traits. Breeders are using only fraction of the available germplasm for cultivar breeding, which mitigates the genetic variability. It has been speculated that reduction in yield and fiber characteristics is a result of a narrow genetic base in cotton germplasm (Bowman *et al.*, 2001).

The cluster analysis verifies sufficient diversity for any group of genotypes and this diversity is important in selecting useful genotypes. Principal components analysis is used in maintaining and utilizing genetic resource in which we divided the total variance into its components.

It is a powerful tool to achieve parental lines for any successful breeding programme (Saeed *et al.*, 2014). Knowledge of correlation between different traits is necessary in plant breeding. If two traits are positively correlated, then one trait can be improved indirectly by improving the other trait. In cotton, yield and quality are almost equally important for a successful breeding programme to improve both these parameters (Hussain *et al.*, 2010).

Successful breeding programme depend on good knowledge about genetic diversity, which exists in a crop germplasm (Esmail *et al.*, 2008). Levels and patterns of genetic variation vary greatly within and among species and populations. Genetic improvement of cotton has led to the evolution of large number of cotton varieties with improved yield and fiber quality by using genetic variation among cotton germplasm (Haider *et al.*, 2012) but still yield is less than many cotton growing countries. The main objective of proposed research work is to study genetic variation among upland cotton Btgermplasm for oil contents, crude protein and fiber quality traits.

## Materials and methods

Bt cotton germplasm consisting of 40 genotypes was planted in the research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, on 14thApril, 2015. Before sowing, seed of selected genotypes was treated with sulphuric acid to remove the fuzz, washed under tape water and air dried. The seed of cotton germplasm was sown in randomized complete block design with two replications. Plant to plant and row to row distances was maintained 30cm and 75cm respectively. Genotypes used in the study are listed in table 1.

At proper time, data on plant variables like bolls per plant, boll weight, seed cotton yield, lint percentage, oil percentage, protein percentage, fiber length, fiber uniformity, fiber strength and fiber fineness were recorded from five tagged guarded plants from each experimental unit.

*Crude protein (%)*

The cotton seeds samples were run on velp distillation apparatus and crude protein percentage were determined by using formula.

N% = (ml of acid × 0.0014 × 100/ Wt. of sample

Crude protein (dry weight basis) = N% × 6.25

Crude protein (on fresh basis) = Crude protein (dry weight basis) × D.M/100.

*Oil contents (%)*

The oil contents in cotton seed samples were determined using soxhlet apparatus. Oil percentage was calculated using formula.

W3 = Weight of filter paper (W1) + Weight of seeds (W2)

Oil contents = (Difference/Weight of seed) × 100.

*Fiber quality traits*

A high volume instrument (HVI-900 SA) was used to measure the fiber quality traits. This computerized instrument provides a comprehensive profile of raw fiber. It measures the most important fiber characteristics such as fiber length, fiber uniformity, fiber strength and fiber fineness according to the international trading standards.

The collected data for different yield and fiber quality traits were subjected to analysis of variance technique (steel *et al.*, 1997) through statistical software *STATISTICS 8.1*. The data were analysed by PCA (Principal component analysis) and cluster analysis described by Pearson and Neyman (1928) to study the genetic diversity among the Bt cotton germplasm through computer software *MINITAB 18*.

## Results

Basic analysis of variance revealed highly significant (P≥0.01) differences among genotypes for bolls per plant, seed cotton yield, lint percentage, oil percentage, crude protein and fiber uniformity while non-significant results were recorded for boll weight, fiber length, fiber strength and fiber fineness (Table 1).

**Table 1.** Analysis of variance (mean squares) for various traits in *G. hirsutum*.

| SOV | d.f. | Bolls/plant | Boll weight | Seed cotton yield | Lint percentage | Oil percentage | Crude protein | Fiber length | Fiber uniformity | Fiber strength | Fiber fineness |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replication | 1 | 166.234 | 5.243 | 15.990 | 0.173 | 2.264 | 0.113 | 0.084 | 1.860 | 0.512 | 0.0005 |
| Treatments | 39 | 79.688** | 0.410NS | 970.300** | 9.424** | 12.218** | 40.500** | 2.568 | 42.803** | 4.616 | 0.394 |
| Error | 39 | 10.613 | 0.737 | 220.380 | 0.504 | 0.305 | 0.702 | 0.118 | 0.596 | 0.326 | 0.005 |

Phenotypic variances were a little higher than genotypic variances for all the traits except bolls per plant and seed cotton yield for which environmental variance was higher indicating more influence of environmental factors (Table 2).

Heritability in broad sense was high for all the traits except bolls per plant and seed cotton yield. Genetic advance was the highest for seed cotton yield (31.50) followed by bolls per plant (13.77), fiber uniformity (9.26) and crude protein (8.97).

**Table 2.** Variability parameters and heritability for various traits in *G. hirsutum*.

| Components of variation | Bolls/ plant | Boll weight | Seed cotton yield | Lint percentage | Oil percentage | Crude protein | Fiber length | Fiber uniformity | Fiber strength | Fiber fineness |
|---|---|---|---|---|---|---|---|---|---|---|
| Genotypic variance (GV) | 34.53 | 3.14 | 374.95 | 4.46 | 5.95 | 19.89 | 1.22 | 21.10 | 2.14 | 0.19 |
| Phenotypic variance (PV) | 45.15 | 3.27 | 595.34 | 4.96 | 6.26 | 20.60 | 1.34 | 21.69 | 2.47 | 0.20 |
| Environmental variance (EV) | 10.61 | 2.01 | 220.38 | 0.50 | 0.30 | 0.70 | 0.12 | 0.60 | 0.33 | 0.01 |
| Genotypic coefficient of variance (GCV) | 143.33 | 8.09 | 488.02 | 11.80 | 42.01 | 110.43 | 4.25 | 48.25 | 7.24 | 4.34 |
| Phenotypic coefficient of variance (PCV) | 187.42 | 9.37 | 774.87 | 13.13 | 44.20 | 114.38 | 4.66 | 49.59 | 8.34 | 4.45 |
| Environmental coefficient of variance (ECV) | 44.04 | 3.84 | 286.84 | 1.33 | 2.12 | 3.89 | 0.41 | 1.36 | 1.10 | 0.11 |
| Heritability (broad sense) $h^2_{(BS)}$ | 0.76 | 0.84 | 0.62 | 0.89 | 0.95 | 0.96 | 0.91 | 0.97 | 0.86 | 0.97 |
| Genetic advance | 13.77 | 1.11 | 31.50 | 4.10 | 4.87 | 8.97 | 2.17 | 9.26 | 2.79 | 0.75 |
| Genetic advance as percentage of mean (GA%) | 57.16 | 3.59 | 40.99 | 10.84 | 34.39 | 49.80 | 7.52 | 21.17 | 9.42 | 16.77 |

Correlation analysis revealed the highest significant correlation between seed cotton yield and bolls per plant (0.858) followed by seed cotton yield and fiber fineness (0.469). Boll weight was positively associated with lint percentage, protein percentage and seed cotton yield. Fiber fineness exhibited direct association with lint percentage and seed cotton yield. While oil percentage and protein percentage were negatively correlated (Table 3).

Principal component analysis (PCA) revealed eleven PCs out of which first four PCs exhibited more than 1 Eigen value.

**Table 3.** Correlation coefficients of various traits in *G. hirsutum*.

| | Bolls/ plant | Boll weight | Lint percentage | Oil percentage | Protein percentage | Fiber length | Fiber uniformity | Fiber strength | Fiber fineness |
|---|---|---|---|---|---|---|---|---|---|
| Boll weight | -0.191 | | | | | | | | |
| Lint percentage | 0.017 | 0.352* | | | | | | | |
| Oil percentage | 0.129 | 0.068 | 0.202 | | | | | | |
| Protein percentage | -0.107 | 0.353* | -0.021 | -0.331* | | | | | |
| Fiber length | 0.158 | -0.232 | -0.023 | 0.297 | -0.350* | | | | |
| Fiber uniformity | -0.083 | -0.088 | 0.065 | 0.101 | 0.112 | -0.075 | | | |
| Fiber strength | 0.003 | -0.248 | -0.181 | 0.079 | -0.205 | 0.176 | 0.209 | | |
| Fiber fineness | 0.469** | 0.047 | 0.429** | 0.278 | -0.049 | -0.09 | 0.089 | -0.066 | |
| Seed cotton yield | 0.858** | 0.312* | 0.159 | 0.198 | 0.027 | 0.099 | -0.14 | -0.131 | 0.439** |

These four PCs contributed 66.1% in total variability amongst the upland cotton genotypes. The remaining 33.9% of the total variability towards total variation was contributed by remaining components (Table 4). The highest contribution was made by PC1 (22.4%) followed by PC II (19.6%). Bolls per plant, seed cotton yield and fiber fineness were the important traits contributing towards variability in PC1.It showed positive effect towards boll weight, lint percentage oil percentage and fiber length while negative effect for protein percentage, fiber uniformity and fiber strength.

In PC2, boll weight, protein percentage and fiber length were the major contributing traits for variability. It also showed positive factor loadings for seed cotton yield, lint percentage, and fiber fineness while negative factor loadings for bolls per plant, oil percentage, fiber length, fiber uniformity and fiber strength.

The third principal component explained 13.1% of the total variation with bolls per plant as the major variability contributing trait. This PC had positive loading for seed cotton yield while negative effect towards oil percentage, boll weight and fiber uniformity.

The fourth PC contribution toward total variability was 10.9%. It elucidated by the variation among genotypes for fiber length and boll weight. The PC-4 exhibited negative factor loadings for fiber strength, fiber fineness and protein percentage (Table 4).

**Table 4.** Principal component analysis for forty genotypes of *G. hirsutum* with respect to various characters.

| Variables | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Eigenvalue | 2.4669 | 2.1534 | 1.4459 | 1.2017 |
| Total variance | 22.4 | 19.6 | 13.1 | 10.9 |
| Cumulative eigen value | 0.224 | 0.420 | 0.551 | 0.661 |
| Bolls/plant | 0.510 | -0.054 | 0.437 | -0.181 |
| Boll weight | 0.106 | 0.453 | -0.295 | 0.242 |
| Seed cotton yield | 0.547 | 0.139 | 0.275 | -0.002 |
| Lint percentage | 0.276 | 0.191 | -0.517 | 0.072 |
| Oil percentage | 0.304 | -0.255 | -0.386 | 0.107 |
| Protein percentage | -0.124 | 0.490 | 0.077 | -0.244 |
| Fiber length | 0.132 | -0.416 | 0.056 | 0.282 |
| Fiber uniformity | -0.051 | -0.059 | -0.322 | -0.707 |
| Fiber strength | -0.075 | -0.359 | 0.017 | -0.410 |
| Fiber fineness | 0.467 | 0.063 | -0.176 | -0.267 |

*Scree Plot*

Scree plot explained the percentage variance associated with each principal component obtained by drawing a graph between eigen values and principal component numbers (Figure 1). PC1 showed 22.4% variability with eigen values 2.50.

By examining scree plot it was evident that first four PCs have considerable variability while from PC5 to PC11 variability reduced. From graph it was cleared that maximum variation was present in first PC. So selection of genotypes from this PC will be useful.

**Table 5.** Cluster membership of genotypes.

| Cluster No. | No. of genotypes | Genotypes |
|---|---|---|
| Cluster 1 | 17 | Lalazar, FH 177, MNH 886, AA 703, FH 170, FH 114, FH 113, IR 3, NS 131,SB 149, MHN 586,CRS 456, FH 172, FH 4243, KZ 181, NS 121, S-3 |
| Cluster 2 | 15 | CIM 599,VH 282, AA 803, MNH 888,CIM 602, CRS 2007, VH 295, VH 283,VH 329,KZ 189, MG 6, FH 154,FH 187,FH 169, IR 901 |
| Cluster 3 | 2 | CIM 591, AS 01 |
| Cluster 4 | 6 | VH 259, IR 3701,C 26, FH 118, NIAB 820,FH 941 |

*Biplot*

A principal component biplot showed that variables were super imposed on the plot as vectors. Distance of each variable with respect to PC1 and PC2 showed the contribution of this variable in the variation of germplasm.

The biplot figure depicted that as seed cotton yield, bolls per plant and fiber fineness shares were maximum towards variability in germplasm (Figure 2).

*Cluster Analysis*

Forty cotton genotypes were grouped into 4 clusters based on various traits (Table 5). Cluster analysis showed that cluster 1 comprised of 17 genotypes, cluster 2 contained 15, cluster 3 had 2 while cluster 4 comprised of 6 genotypes. Genotypes in cluster 1 showed significant values for bolls per plant, fiber fineness, fiber length, fiber uniformity and seed cotton yield.

The members of 2nd cluster represent the highest values for oil percentage, fiber length and protein percentage.

The members of 3rd cluster were characterized by highest values of lint percentage, oil percentage, fiber strength and fiber uniformity. Cluster IV showed highest values for bolls per plant, boll weight, seed cotton yield and fiber length. Description of traits in various clusters is shown in Table 6.

**Table 6.** Description of traits with respect to variability percentage in various clusters.

| Variables | Cluster I | Cluster II | Cluster III | Cluster IV |
|---|---|---|---|---|
| Bolls/plant | 34.67 | 15.67 | 10.42 | 33.08 |
| Boll weight | 2.39 | 3.29 | 3.25 | 3.71 |
| Seed cotton yield | 82.67 | 51.58 | 21.13 | 122.91 |
| Lint percentage | 36.01 | 37.10 | 40.81 | 36.98 |
| Oil percentage | 11.72 | 14.11 | 12.21 | 11.28 |
| Protein percentage | 18.50 | 19.50 | 17.50 | 18.50 |
| Fiber length | 28.65 | 29.45 | 26.50 | 29.55 |
| Fiber uniformity | 44.35 | 30.85 | 49.00 | 42.75 |
| Fiber strength | 30.05 | 26.70 | 32.65 | 29.75 |
| Fiber fineness | 4.65 | 3.55 | 4.25 | 4.55 |

After constructing dendrogram that displayed distance between the clusters, it was found that cluster I and cluster 2 are the most diverse clusters indicating that their members had great genetic diversity for the characters under study (Figure 3). Cluster III and cluster IV were the least diverse for the selected characters. It indicated that both the clusters had very small genetic diversity.

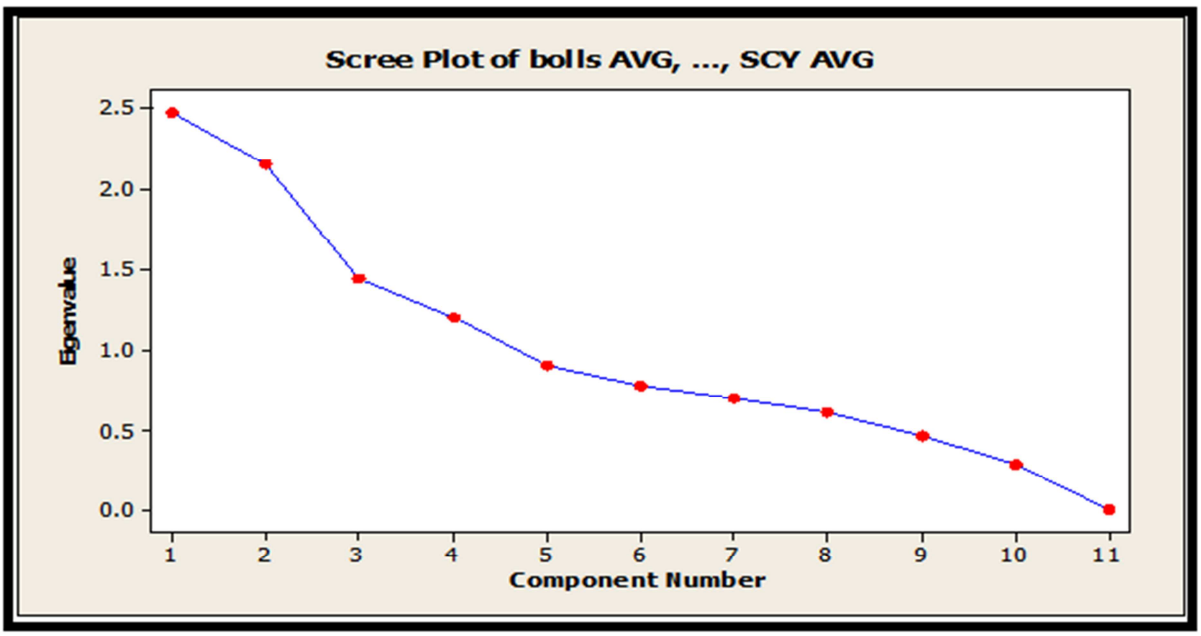


**Fig. 1.** Scree plot of principal component analysis between Eigen values and principal components.

**Discussion**

Plant genetic resources are the important components to provide raw material for producing new varieties of crops (Hoisington *et al.*, 1999). Knowledge of genetic diversity is a prerequisite of a successful breeding program (Ullah *et al.* 2012; Ali *et al.*, 2016). Precise calculation of the levels and patterns of genetic divergence can be irreplaceable in crop breeding for the following diverse applications (Ahmad *et al.*, 2012) analysis of genetic variability in cultivars (Ali *et al.*, 2016) finding diverse parental combinations to create segregating progenies with high genetic variability for further selection (Ali *et al.*, 2008) and introgressing appropriate genes from diverse germplasm into the available genetic base (Iqbal *et al.*, 1997; Rana and Bhat, 2005; Rasheed *et al.*, 2009; Khan *et al.*, 2010). Analysis of genetic diversity in germplasm collections can simplify reliable classification of accessions and identifications of subsets of core accessions with conceivable utility for specific breeding purposes (Campbell and Jones, 2005; Ali *et al.*, 2008; Ahmad *et al.*, 2012).

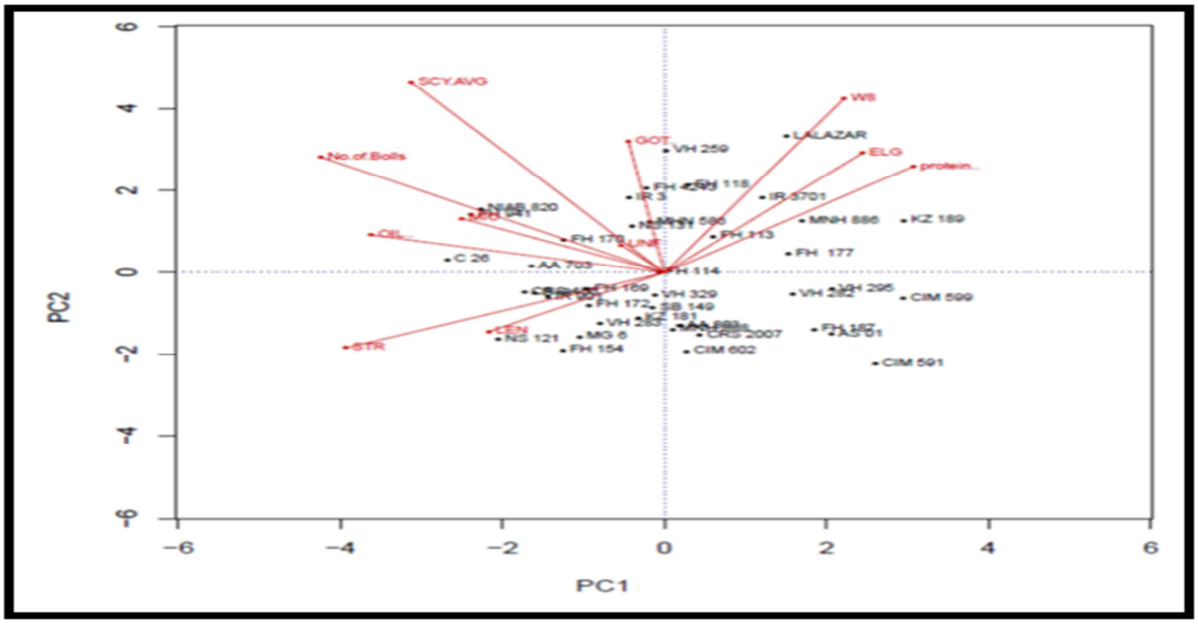


**Fig. 2.** Biplot between PC-1 and PC-2 showing contribution of various traits in variability among different genotypes.

The forty genotypes used in current study were clearly divided into various clusters indicating considerable genetic diversity among genotypes for various characters of economic importance. Out of eleven first four PCs exhibited more than 1 eigen value. All these four PCs contributed 66.1% in total variability amongst the upland cotton genotypes which were assessed for genetic diversity for fiber quality traits, protein and oil contents. With cluster analysis forty cotton genotypes were grouped into 4 clusters based on various traits. Among all four clusters the genotypes in cluster 1 showed significant values for more characters like bolls average, micronaire, staple length, fiber uniformity and seed cotton yield average, so the genotypes in this cluster may be used to exploit variation for the mentioned traits. A number of researchers used cluster analysis to assess genetic diversity and to select parental genotypes for breeding programs to improve various traits (Candida *et al.*, 2006; Khan *et al.*, 2010; Iqbal *et al.*, 2013).

Correlation analysis revealed significant positive correlation of number of boll with seed cotton yield and fiber fineness. Boll weight showed positive association with lint percentage, protein percentage and seed cotton yield on the other hand it revealed negative correlation with staple length, fiber strength and fiber uniformity.

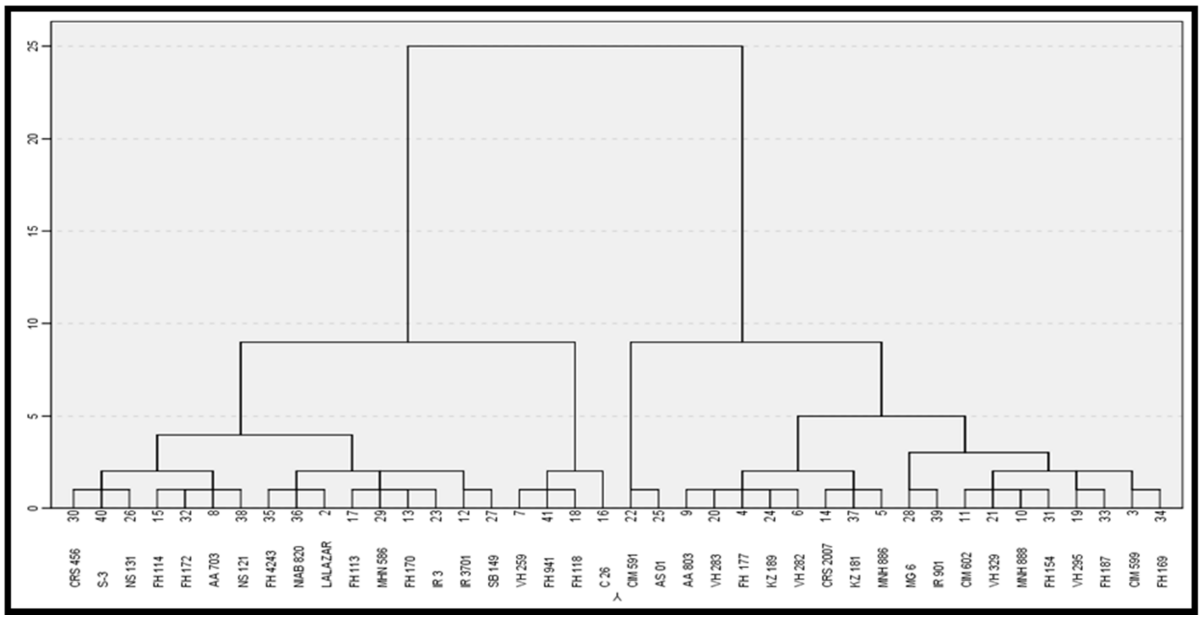


**Fig. 3.** Dendrogram using ward's method.

The negative correlation among yield and quality attributes has been reported previously by a number of researchers including Rana and Bhat (2005); Khan *et al.* (2007); Ahmad *et al.* (2012); Iqbal *et al.* (2013) and Ali *et al.* (2016). The oil percentage was negatively correlated with protein percentage and fiber elongation. Negative correlation of oil content with fiber and yield related traits were also reported by Khan *et al.* (2007); Khan *et al.* (2010); Qayyum *et al.* (2010) and many other researchers. Number of bolls, crude protein oil percentage and fiber quality traits showed high heritability. Fiber fineness, fiber elongation boll weight they showed low genetic advance. Seed cotton yield, number of bolls per plant and fiber uniformity showed high genetic advance. It is recommended that the germplasm have variation for all traits and showed high broad sense heritability, which concluded that it can be used in further breeding program for the improvement of yield and fiber related traits.

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