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# Comparative Study of Morpho-Physiological Diversity in Interspecific Wheat (Triticum aestivum L.)

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**Novelty statement** Wheat is considered as king of all cereals and consumed in most of world as staple crop. So estimation of morpho-physiological diversity and their correlation especially for yield and traits related to yield is very crucial in our rainfed areas where wheat might faces various biotic and abiotic stresses. For current study most sophisticated biometrical techniques were used to accomplish this study.

**Abstract**
Genetic diversity in wheat is crucial to meet the diversified goals of plant breeding mainly breeding for increasing yield, desirable quality and disease. The aim of research was to quantify the total amount of variability existing in the genetic material for morpho-physiological traits. The proposed study was conducted in the experimental area and laboratory of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Poonch Rawalakot. Sixty three wheat accessions of three species (*aestivum, durum,* and *sphearococum*) including one check variety BARS-2009 were used. Diversity was found significant among different accessions by means of cluster analysis and individual contribution of each trait by means of principal component analysis. Most diversity was noted for traits like plant height, number of tillers per plant, specific flag leaf weight, specific flag leaf area and peduncle length etc. Taken as whole it was revealed that the accessions 13011, 13005, 12976, 12983, 19028, 12982, Land race -LR-30, LR-42, 12978, LR-36, LR-3, 13010, 13015, LR-33, 12979, LR-7, 12996, 12984, 13002, 13004, 13006, LR-13, LR-38, 12977, 12999, 13003, LR-43, 13014, LR-16 and 12980 were outliers and traced to be superior as they contributed maximum divergence for morpho-physiological traits. Ample diversity was found in all three species accessions, which could be exploited in future studies for wheat improvement.
**Keywords:** Accession, Diversity, Outliers, Significant, Wheat.

## 1. INTRODUCTION

Wheat is considered the universal cereal originated from south west and East Asia. It is believed that wheat is originated in Turkey 10000 years ago. Iran, Jordan and Iraq are also considered as the center of origin along with Turkey. Wheat is occupying 17% of crop acreage world over, feeding approximately 40% of total world population with providing 20% of the total food calories in human nutrition (Gupta et al., 2005).

Wheat is the most important cereal crop of many world countries such as in Pakistan it is cultivated on the largest acreages. It contributes 14.4 percent to the value added in agriculture and 3.0 percent to the GDP. The production of wheat was 25.97 million tons during 2014-15. Pakistan keeps 8$^{th}$ position in wheat production in the world and 3$^{rd}$ position in Asia. (FAOSTAT, 2014-15).

In past three decades, increased agricultural productivity was obtained chiefly due to the cultivation of high-yielding cultivars and also due to increased and better use of fertilizers. By the introduction of semi-dwarf wheat cultivars, productivity of wheat has been increased in all the major cropping systems representing the diverse and varying agro-ecological conditions (Anon., 2012-13).

Wheat is grown under different agro-climatic such as arid and semi-arid regions and in both regions it faces different kinds of biotic and abiotic stresses at different growth and development stages. Among abiotic stresses drought, heat and nutrient deficiency are the mainly important stresses that limit wheat crop production (Ghulam et al., 2011).

In recent few years increasing wheat production under abiotic stress conditions has become essential since wheat production in areas with optimal growing conditions does not meet the requirements of the increasing population (Slafer and Peltonen-Sainio, 2001; Soufizadeh et al., 2006).

Wheat gained special interest in respect to morphological and physiological characters affecting drought tolerance because drought is the one of major abiotic stress of wheat, characters affecting drought tolerance including leaf (area, expansion, shape, orientation, pubescence, waxiness, senescence, cuticular resistance), stomata (number, size, aperture), root (length, dry weight, density), relative water content, evapo-transpiration efficiency, water use efficiency, abscisic acid levels, heat shock proteins, cell membrane stability and carbon isotope discrimination (Anil et al., 2017).

Morphological characters are usually used to measure genetic variability. They offer a simple technique of measuring genomic variation while defining genotype performance under normal cultivation locations. (Fufa *et* al., 2005). Traits related to drought resistance, such as small plant size, reduced leaf size and early maturity lead to reduced total seasonal evapo-transpiration (Dencic et al., 2000).

 

To overcome these environmental constraints genetic diversity is needed because the presence of genetic diversity and variability in wheat crop play a vital role to fulfill world food requirements (Sial et al., 2005). For effective evaluation and utilization of germplasm, measure of extent of available genetic diversity is of utmost importance (Zubair et al., 2007). Crop genetic diversity is important at both global and local or domestic levels. At the global level, plant genetic resources are valued for their role in the genetic 'improvement' of all cultivated species to meet the challenges posed due to population growth, by changing levels of the demand, and changing supply conditions, due to climatic changes and attendant changes in pathogen and pest risks. Description of genetic variability between various genotypes and estimation of the genetic association are essential and crucial for breeders, because artificial crosses between dissimilar or divergent parents permit the huge segregation and also the grouping of various favorable alleles or genes (Bered et al., 2002).

Plant consistency that can be resulted by the use of modern plant breeding techniques can produce plants that are more efficient by means of different goals including better tolerance under stress; however more research must be performed to point out the most optimized methods that can be used for the efficient plants production. This is of significance for the production of food for the world increasing population (Fu and Somers, 2009). Genetic divergence analysis estimates the extent of diversity existed among selected genotypes (Mondal, 2003).Genetic diversity and variability exists both for quantitative and qualitative traits in wheat and has great importance for breeder( Muhammad et al., 2016).

The prime objective of any breeding program is grain yield, which is a complex trait in wheat and greatly influenced by various environmental fluctuations and genetic factors (Dixet & Dubey, 1984). Direct selection for yield could be misleading (Marouf & Naghavi, 2016). Breeders believe that successful selections rely on the information on the genetic diversity and relationship of morpho-agronomic traits with grain yield or using indirect selection that may bring about genetic upgradation of complex traits like yield (Rayees et al., 2017).

The production of wheat in terms of its yield expressed by grain weight, number of spikes per plant and number of grains per spike (Ompal et al., 2017). One of the most important yield contributing traits is 1000-grain weight and has been an important selection criteria of higher yielding plants (Roder et al., 2008).

Investigation of genetic divergence in wheat germplasm is prerequisite for high yielding cultivars development and in order to maintain the desirable level of genetic variation in future wheat breeding. Higher heritability and genetic variation for the trait under selection are necessary to have response to selection. Various statistical measures are available for the assessment of diversity and among these multivariate techniques is greatly used in plant breeding and genetics for the study of genetic divergence in the different breeding materials. (Kahrizi et al., 2010).

In plant breeding program, several characters are simultaneously considered that make it feasible to approximate the genetic diversity by using multivariate techniques. Principal component analysis (PCA) reflects the importance of the largest contributor to the total variation at each axis of differentiation (Stehno et al., 2006). Similarly cluster analysis is a efficient method for determining the family relationships (Mellingers, 1972).

The main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only (Mohammadi, 2002). These methods are used in breeding programs to study the centre of origin, genetic diversity identification, tracing the pathway to evolution of crops, parental selection, and to study interaction between the environment that are currently available or prevail (Hair et al., 2006).

## 2. MATERIAL AND METHODS

**Sample Description:**

To assess the morpho-physiological diversity, total sixty three wheat accessions were used. Seed of accessions was acquired from Department of Plant Breeding and Genetics, PMAS-University of Arid Agriculture, Rawalpindi-Pakistan. Crop was grown during 2013-2014. Research was conducted in the experimental field and laboratory of Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Poonch, Rawalakot. Sixty two accessions of different wheat species including *durum*, *aestivum* and *sphearococum* with one check variety BARS-2009 (total 63) were included in study. Detail of accession names were listed in table 1. There were sown in the experimental field of PBMG, University of the Poonch Rawalakot, Azad Kashmir during 2013-14. All accessions were sown in plots (five plots, each plot have sixteen entry rows). In field seeds of all accessions were sown in well prepared soil having 30cm row to row distance. Fertilizer having nitrogen was applied twice. Replications were not practiced. All cultural practiced were implemented uniformly.

**Traits to be Studied:**

### 2.1 Morphological Traits

#### 2.1.1. Days to 50% heading

For each line days to heading was recorded in days from the date of sowing till 50% spikes head emerged.

 

**2.1.2 Days to maturity**
Days to maturity was noted at the period of the 90% turning of spikes of plants into yellow colour.
**2.1.3. Plant height (cm)**
By means of the meter rod plant height was measured in centimeters at maturity from randomly selected plant of each line from bottom of the plant to the tip of the tallest tiller (mother tiller) excluding awns.
**2.1.4. Number of tillers plant$^{-1}$**
Number of the fertile tillers plant$^{-1}$ of all selected plants of every line was counted at maturity stage.
**2.1.5. Spike length (cm)**
Spike length of the mother shoot was measured in centimeters from base of the spike to the tip excluding awns at the time of maturity from the ten randomly selected plants of each line.
**2.1.6. Number of spikelets spike$^{-1}$**
Number of spikelets spike$^{-1}$ was counted from mother shoot spike at the maturity but excluding non fertile spikelets of all selected plants of each line and their mean values were calculated.
**2.1.7. Peduncle length (cm)**
Mother shoot of each selected plant was measured from upper most node to the base of spike.
**2.1.8. 1000-grain weight (g)**
After threshing 1000-grains of each entry were counted and weight was taken with electric balance in gram.
**2.1.9. Grain yield (g)**
Total produce of ten selected plants from each line was weighed on an electronic balance and then average grain yield per plant was taken in grams .

**2.2.PHYSIOLOGICAL TRAITS**
**2.2.1. Residual transpiration (g $H_2o$/min/cm$^2$/$10^5$)**
Residual transpiration was measured according to Clarke et al. (1991). It was calculated as follows:

$$RT = (W_1 – W_2)/ (LA.180 \text{ min})$$

**2.2.2 Relative water contents (%)**
Relative water contents (RWC) was calculated by means of the following formula.

$$RWC (\%) = (FW – DW) / (TW – DW) \times 100$$

After excision, the leaves were immediately taken to laboratory and fresh weight (FW) and leaf area (LA) was taken. TW i.e. the full turgid weight of leaves was determined after 24 hours at 4ºC in darkness by rehydrating them in test tubes containing distilled water, DW i-e dry weight was recorded after oven drying at the 80ºC for 48 hours .
**2.2.3. Osmotic adjustment (g)**
The osmotic adjustment (OA) was calculated by following formula:

$$OA = TW – FW$$

**2.2.4. Flag leaf area (cm$^2$)**
This parameter was measured by means of leaf area meter from randomly selected five plants from each line.
**2.2.5. Specific flag leaf area**
Specific flag leaf area (SFLA) was measured as:

$$SFLA = FLA / DW$$

**2.2.6. Flag leaf weight (g)**
By using electronic balance flag leaf weight was recorded in grams.
**2.2.7. Specific flag leaf weight**
Specific flag leaf weight (SFLW) was calculated as:

$$SFLW= DW/ FLA$$

**Statistical Analysis**
Data was analyzed by means of the of principal component analysis by help of computer software PAST (Hammer et al., 2001). The cluster analysis (Sneath and Sokal 1973) was done by employing computer software 'Statistica' (www.statsoft.com) on the basis of standard distance of k-means and in each cluster the accessions were then analyzed for the basic statistics.

## 3. RESULTS
Three species of wheat were utilized to generate physiological and morphological data, which was subsequently analyzed and used to access performance as well as diversity.

### 3.1 PRINCIPAL COMPONENT ANALYSIS
Principal component analysis is presented for the morpho-physiological traits among different accessions of wheat (Table 2). Joliffe cut-off was 0.7 hence number of the extracted components were six. Maximum eigen value is showed by PC1 (6.48) and PC 6 showed minimum eigen value (0.84). All the six components

 

contributed the 88.57% of variability. Maximum variance was contributed by PC 1 (40.53%) that was followed by PC 2 (16.46 %), PC 3 (11.35%), PC 4 (8.75%), PC 5 (6.21%) and PC 6 (5.27%).

**Scree plot for 16 morpho-physiological traits**

Scree plot enlighten the percentage of variance linked with each principal component acquired by drawing the graph between eigen value and PC number. For quantitative traits Scree plot of 16 principal components was drawn, which showed that the most of the contribution to total variance is accomplished by first six principal components for morpho-physiological traits. scree plot it was noted that line which indicating the contribution of PC's to the variance was going in steep mode up to about PC 6 and then it started to straighten, which shows that first 6 components were main contributor to total variance (Figure.1) Among these first six PC's, the PC 1 and PC 2 contributed maximum about (56%). It also justified the results of table 2. that was based on principal component for morpho-physiological traits.

**Loadings for 16 morpho-physiological traits in wheat accessions**

**Loading for factor 1**

For factor 1 positive load was noted maximum for morphological trait days to maturity (0.891). Minimum positive load was seen for grain yield (0.096). For factor 1 the maximum negative load was for specific flag leaf weight (-0.614). Number of tillers per plant had minimum negative (-0.445) load for factor 1. Factor 1 revealed that SFLW was highly significant but negatively correlated with DTM and DTH (Figure 2). For this factor maximum positive load was contributed by days to maturity so it can be called as the effective factor for**" maturity"**.

**Loadings for factor 2**

Loadings for factor 2 expressed that maximum positive load was contributed by grain yield (0.891). Positive load was minimum for NS (0.101). Maximum negative load was noted for residual transpiration of (-0.530). Negative load was minimum for trait like DTH (-0.254). Current factor declared that RT was highly significant and negatively associated with RWC and grain yield (Figure 3). As grain yield added maximum positive load for factor 2 of (0.891) so factor 2 is effective factor for **"grain yield".**

**Loadings for factor 3**

Osmotic adjustment showed the maximum positive load of (0.638) for this factor. Minimum positive load was recorded for PL (0.077). Negative load was noted maximum for relative water content (-0.897) for factor 3. For factor 3 the least negative load was added by NS of (-0.032). (Figure 4). It was also exposed that RWC was negatively associated with RT and O.A was positively correlated with 1000- g wt and grain yield. As it was noted that maximum positive load for factor 3 contributed by osmotic adjustment (0.638) so we can called the factor 3 the effective factor of **"osmotic adjustment"**

**Loadings for factor 4**

For this factor highest positive load was observed for trait specific flag leaf weight (0.684) and this was followed by the residual transpiration (0.348). Specific flag leaf area showed the maximum negative load (-0.728) for factor 4. Minimum negative load was present in peduncle length (-0.016). This factor demonstrated an important result that SFLA was negatively highly significant with SFLW (Figure 5). Factor 4 is effective factor for **"specific flag leaf weight"** because positive load was noted maximum for this trait SFLW.

**Loadings for factor 5**

For this factor, 1000-g wt showed the maximum positive load (0.298). SFLW contributed minimum for positive load of (0.043) followed by the DTM (0.071). Moreover figure suggested that maximum negative load was showed by osmotic adjustment (-0.630) for factor 5. Minimum negative load was contributed by RWC (-0.005). This factor also exhibited correlation between various traits (Figure 6). As 1000-g wt was main donor for maximum positive load followed by the PL and grain yield and most of these were grain yield allied parameters so this factor can be the effective factor of **"1000-g wt** and **peduncle"** as well.

**Loadings for factor 6**

For factor 6 the trait 1000-grain weight showed the utmost positive load (0.569). Positive load was minimum for peduncle length (0.012) followed by NT. Highest negative load was observed for number of spikelets per spike (-0.484). Whereas relative water content showed the minimum negative load (-0.031) for this factor 6 under study (Figure 7). It revealed two important interrelations such as spike length and number of spikeltes were negatively associated with 1000-g wt. It can be called as the effective facor for **"grain or yield"** linked traits.

**Biplot for morpho-physiological (quantitative) traits in different accessions of wheat**

Principal component scatter plot illustrated that accessions that were close together are being alike when related on 16 variables. Scatter diagram also verified the result of PC loadings by confirming the positive and negative loads added by particular factors. Biplot orientation for sixteen morphological characters exposed that PC1 and PC2 added utmost for genetic variance demonstrating accessions location depending on traits load. Traits like SFLW, NT, Grain yield, plant height, Peduncle length, SFLA, and NS showed the maximum variation because these traits were found far away from the point of origin. Whereas OA, RWC, SL, DTH, DTM were close to the origin and fall near to each other (Figure 9). These biplot results were also justified by the factor loadings of

 

PCA. Diversity among accessions was also showed by dividing the scatter plot into 4 groups. Land races and *durum* accessions occupy different position on scatter plot. Land races were closer to *sphearococum* and check variety BARS-2009 but far away from the *durum*. Group 1 was comprised of LR-3, 20, 34, 16, 26, 43, 27, 30, 15, 33, 38, 36, 42, 37, 19029 and BARS-2009. Diverse accessions of group 1 included LR-33, LR-37, LR-30, LR-36, LR-44 and BARS-2009. Whereas LR-42, LR-3, LR-38 and 19029 were less diverse as they were closer to origin. Group 2 included the LR-35, LR-11, LR-6, LR-7, LR-12, LR-5, LR-13, LR-11, LR-10, *durum* 12978, 13016 and *sphearococum* 19027, 19028. In group 2 the LR-35, LR-6 and 12978 were closer to the origin but LR-10 and 19027, LR-7 and LR-12 were more divergent. Group 3 included 12979, 12977, 13009, 13004, 13010, 12982, 13001, 13005, 12983, 12981, and 13002. The accessions 13009, 12983, 13004, 12977 showed maximum diversity in this group and 13002, 12981, 13004 were not diverse as they were near to the origin. As for as group 4 was concerned it included the 12992, 12999, 13003, 13006, 12998, 13000, 12989, 13007, 12980, 13013, 12986, 12985, 12984, 13012, 12976, 13008, 12996, 12988, 13014, 12993, 13015. Maximum diversity for group 4 showed by 12983, 13009, 13015, 12993, 13005, 12988 and 13014 while the 13003, 12999, 13007, 12989 were less diverse among these. Over all it was observed that 12999, 13003, 13007, 12989, 12977, 13009, 12983, 13005, 19027, LR-7, LR-12, LR-10, LR-37, LR-33, LR-15, LR-30, LR-36 and check variety showed maximum diversity for 16 morpho-physiological (quantitative) traits because these were far away from the point of origin in scatter diagram hence considered as outliers.

### 3.2. CLUSTER ANALYSIS

#### A. Quantitative Traits

#### Hierarchical Clustering

The morpho-physiological traits of 63 genotypes were classified in the four main clusters. There were four key clusters namely as I, II, III and IV. Cluster I included the 12978, 19029, LR-7, BARS-2009, LR-6, 19027, LR-10, LR-11, LR-12, LR-3, LR-5, 19028, LR-13, LR-35. Cluster II was comprised of 12985, 13008, 12984, 13002, 12989, 13012, 13007, 12992, 13003, 13016, 13015, 12999, 12976, 13014, 12993, 12996 and 12988. Whereas LR-37, LR-20, LR-44, LR-30, LR-15, LR-34 were the members of cluster III and cluster IV comprised of the following accessions LR-27, LR-33, LR-42, LR-26, LR-16, LR-36, LR-38, LR-43, LR-12979, 13001, 12983, 13009, 13010, LR-41, 13004, 13011, 12982, 12977, 12981, 12980, 12986, 13005, 13013, 13000, 13006. The Figure 10, showed the four main clusters at the linkage distance of (Ramji et al., 2017). Clusters were named as cluster I, II, III and IV. Cluster I was split into two sub clusters ‘a’ and ‘b’. The sub cluster ‘a’ of main cluster I comprised of LR-7, 19029, 12978, BARS-2009, LR-6. In this sub group LR-7, 12978, 19029 were present on maximum genetic distance so these were confirmed as outliers. Sub cluster ‘b’ of the main cluster I comprised of accessions namely as LR-11, LR-12, LR-10, LR-3, 19028, LR-13, 19027, LR-35 and LR-5, this sub cluster was larger one than sub cluster ‘a’. In this sub cluster 19027, LR-10, LR-3, LR-5, 19028 were outliers. Cluster II was divided into two sub clusters ‘e’ and ‘i'. Sub cluster ‘e’ contained further two sub-sub cluster ‘e1’ and ‘e2’. The sub-sub group ‘e1’ composed of following 12985, 13008, 12984, 13002, due to similarities in mean values for morpho-physiological traits, in this small sub cluster 12984 was most diversed accession. The sub cluster ‘e’ of cluster II was also categorized into another sub-sub cluster e2 that included the 12992, 13012, 12989, 13007, and 13003, only 13007 showed most of variation so it was an outlier in this small cluster. Whereas sub cluster ‘i’ of main cluster II was again distributed into two new sub sub groups ‘ia’ and ‘ib’. Sub-sub cluster’ ia’ of sub cluster ‘i ‘was comprised of following accessions on the basis of similarity and diversity such as durum 13015 and 13016. They shared the same cluster because of similarities in their means for quantitative traits. No such outlier was identified in this cluster; it was small group that only contained two members. Sub-sub cluster ‘ib’ of sub cluster ‘i’ of cluster II consisted of 12996, 12999, 12993, 12976, 13014, and 12988. In this sub group 12999, 12993 were declared as the outliers because they showed the diversity. The cluster III was the smallest cluster among all four clusters of morphological traits. This cluster was only composed of landraces such as LR-20, LR-37, LR-30, LR-44, LR-34, and LR-15 due to similarities. No *durum* and *sphearoccocum* accession existed in this main cluster. Outliers for this cluster were LR-30, LR-37. As far as cluster IV was concerned, it was divided into the two sub group’s i-e the sub cluster ‘v1’ and ‘v2’. The sub cluster v1 included the LR-27, LR-33, LR-42, LR-26, LR-16, LR-36, LR-38, and LR-43. While LR-16, LR-26, and LR-42 were outliers in this sub cluster due to showing the variation. Sub cluster ‘v2’ of cluster IV was further divided into two new sub-sub clusters the ‘c1’ and ‘c2’. The sub-sub cluster ‘c1’ was consisted the 13004, LR-41, 12977, 12981, 12982, 13011. In this sub sub cluster LR-41 and 2981 contributed the most towards divergence. On the other hand ‘c2’ contained the wheat accessions named as 13006, 12980, 13005, 12986, 13013, and 13000. These accessions shared same sub cluster due to similarities in mean values for metric traits.

#### Non-hierarchical clustering (K-mean clustering)

#### Analysis of variance (ANOVA)

Analysis of variance between and within 63 accessions for 16 metric traits. All traits like relative water content, peduncle length, number of tillers, spike length, residual transpiration, plant height, days to maturity, days to

 

50 % heading, 1000 grains weight, flag leaf weight, flag leaf area, specific flag leaf weight and area, number of spikelet's and grain yield showed highly significant results(Table 3). Non significant results were not found. While plot of means values that showed the most variable traits such as relative water contents, specific flag leaf area, specific flag leaf weight, days to heading, plant height, peduncle length and number of tillers according to non-hierarchical clustering (Figure 11).

## 4. DISCUSSION

Objective of using the PCA was to reduce and summarized the data but with no loss of information. It also helped in greater understanding of data and most importantly helped in reduction of dimensionalities by extracting minimum number of components that accounted for most of variation. PCA studies were comprised of Eigen values, factor loadings, scree plot and scatter or biplot. PCs were extracted from the Eigen values and it can be also extracted from correlation matrix of original variables. Eigen value actually computed the amount of variation explained by each PC. Joliffe cut was actually the value used in PCA which determined the number of significant PCs. Factor loadings were truly correlation coefficients between original variables and PC scores. Biplot exposed the interrelation ship between observations and variables in multivariate data. Sixteen quantitative (morpho-physiological) parameters were analyzed namely as residual transpiration, relative water content, flag leaf area, specific flag leaf area, flag leaf weight, specific flag weight, osmotic adjustment, days to heading, days to maturity, plant height, spike length, peduncle length, number of spikelet's per spike, number of tillers, 1000-grain weight and grain yield. Joliffe cut was 0.7 which included the first six factors (PCs). It was estimated that first six factors contributed the most to total variance. Also noted that PC 1 and PC 2 added maximum i.e. more than 50 % for variance. Factor loadings of six PCs showed the positive and negative loading of individual traits towards respective PCs. In PC 1 positive load was noted maximum for days to, for PC 2 positive load was highest for grain yield. Maximum positive load was observed for osmotic adjustment for PC 3, where as in PC 4 the trait SFLW showed the highest positive load, as for as for PC 5 and PC 6, the maximum positive load was added by 1000-grain weight. Scree plot diagram also demonstrated that variance was going in steep manner to about PC 6 but after PC6 it was running as straight line. The projections of accessions on first two PCs was use full to recognize the diverse sets of parents for better and enhanced transgressive segregation. In addition biplot studies also justified the results of scree plot and factor loadings. Biplot showed that most of the divergence was noted for number of tillers, grain yield, SFLA, SFLW, PL, plant height and NS. Accessions like 12999, LR-7, 13007, 12977, 12987, LR-36, LR-12, LR-3, LR-33 and BARS-2009 contributed well for diversity in scatter plot whereas LR-6, LR-37, 13005, 13013, 12986, 13015 and 13016 added most in biplot for diversity so these were declared as outliers in PCA.

Whereas aim of cluster analysis was to increase the homogeneity of individual or members within the cluster and also maximize the heterogeneity between clusters, measures the extent of diversification. Through cluster analysis we concluded that the accessions grouped mutually in one cluster were less divergent than those which were positioned in a different cluster. Sixty three accessions were used for both types of study. Both hierarchical and Non- hierarchical cluster analysis was done. Cluster analysis estimated diversity that was based on Euclidean distance using Ward's method for morpho-physiological traits. Non-hierarchical clustering showed most diverse metric traits among four clusters. According to plot of means, all clusters members were divergent for following traits such as SFLA, SFLW, DTH, RWC, PL, NT and PH. For quantitative traits the tree diagram was divided into four main clusters named as cluster I, II, III and IV. Cluster I was comprised of BARS-2009, LR-10, LR-35, 12978, LR-7, LR-6, 19029, 19027, LR-11, 19028, LR-12, LR-3, LR-5, LR-13. Cluster II included the 12985, 13012, 13015, 12984, 13002, 12989, 12992, 13003, 13014, 13016, 12999, 12976, 13007, 12993, 13008, 12996 and 12988. Cluster III had the, LR-44, LR-34, LR-30, LR-20, LR-15, LR-37 and cluster IV comprised of the accessions named as LR-27, LR-42, LR-38, LR-16, LR-36, LR-43, LR-12979, 12983, 13009, 13010, LR-41, 13004, 13011, 12977, 12981, 12980, 13001, 12986, 13013, 13005, LR-26, 13000, 12982, LR-33, 13006. Outliers for quantitative traits were LR-41, 12982, LR-42, LR-16, LR-26, LR-36, 12983, LR-30, LR-37, 12999, 12993, 12984, 13007, 19027, LR-10, 19028, LR-5, LR-3, LR-7, 12978, 19029 because they showed the variation for quantitative traits of study. Scatter plot of PCA also complimented these results of cluster analysis because LR-5, 19027, LR-10, LR-37, LR-30, 12999, LR-7, 13007, 13015, 13016, 12983, 13005 and BARS-2009 were most divergent accessions in PCA. These accessions are recommended for future studies.

## CONCLUSION

Variability is the basis of selection for wheat and the plant characters that illustrate direct and or indirect influence on yield and yield contributing traits could be potential source that needs to be focused for crop improvement. Morphological and physiological studies collectively all are of equal importance that can give us the reliable picture of genetic diversity. In current study one of most significant and sophisticated biometrical techniques for estimation and description of diversity present in different accessions of wheat were used. The accessions were checked for certain morphological as well as physiological parameters. Diversity was assessed

 

by cluster analysis and PCA. PCA and cluster analysis used to characterize and evaluate the genetic diversity. PCA exposed that first six factors contributed maximum to the total variability and PC 1 and PC 2 added more than 50% in total variance. Most diversity in was found for traits like plant height, number of tillers, SFLW, SFLA, peduncle length and grain yield. Cluster analysis was comprised of hierarchical and non-hierarchical clustering in which RWC, SFLA, SFLW, DTH, PH and NT were most variable traits, while Outliers for these metric traits were LR-41, LR-42, LR-26, 12983, LR-30, LR-37, 12984, 19027, 19029, 12982, LR-36. Nutshell physiological and morphological study in means of diversity is equally essential in wheat crop being a staple food and study of these traits have provided us the primary base about the genetic variation present in accessions that can be used as the breeding material for improvement of wheat. Hybridization between the diversed accessions choose from this research study will be very much use full for devising additional breeding strategies.

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**Authors' Contribution**
Main project was of Huma Tariq and Dr. Shahid Iqbal Awan. All other authors of this paper contributed equally, all helped in its discussion and involved in overall planning of work.

**Abbreviations**
LR= Land race, cm= Centimeters, g= Grams, RT= Residual transpiration, RWC= Relative water content, OA=Osmotic adjustment, PCA= Principal component analysis, DF= Degree of freedom, Prob=Probability, NT= Number of tillers, NS= Number of spikelet's, SFLA= Specific flag leaf area, SFLW= Specific flag leaf weight, DTH= Days to heading, PH= Plant height, PL= Peduncle length,

**LITERATURE CITED**

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**Figure 1. Scree plot for morpho-physiological traits in wheat accessions**

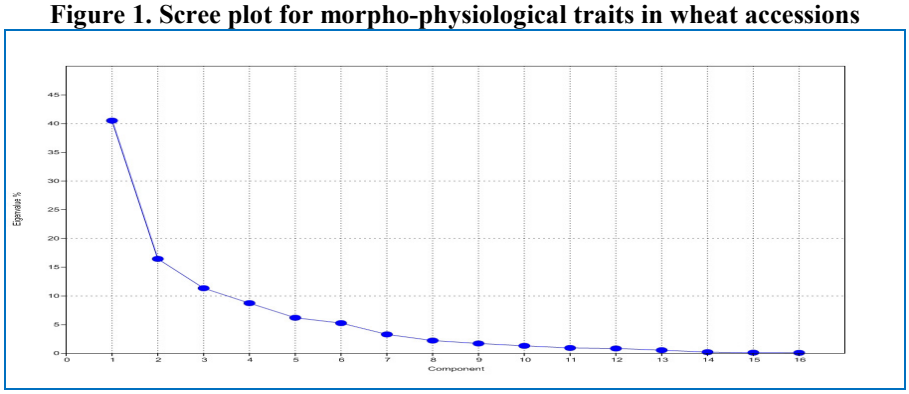


**Figure 2. Loadings for factor 1**

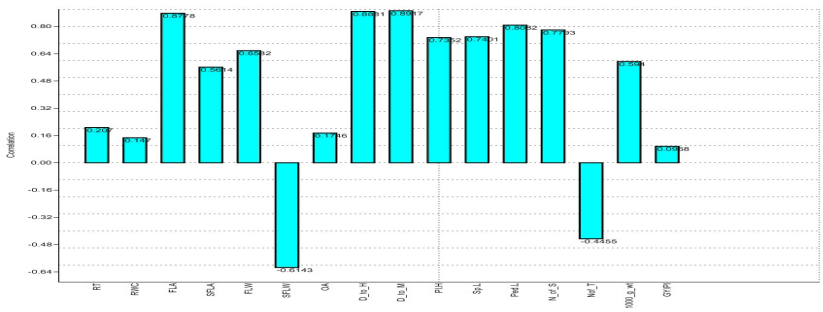

 

**Figure 3. Loadings for factor 2**

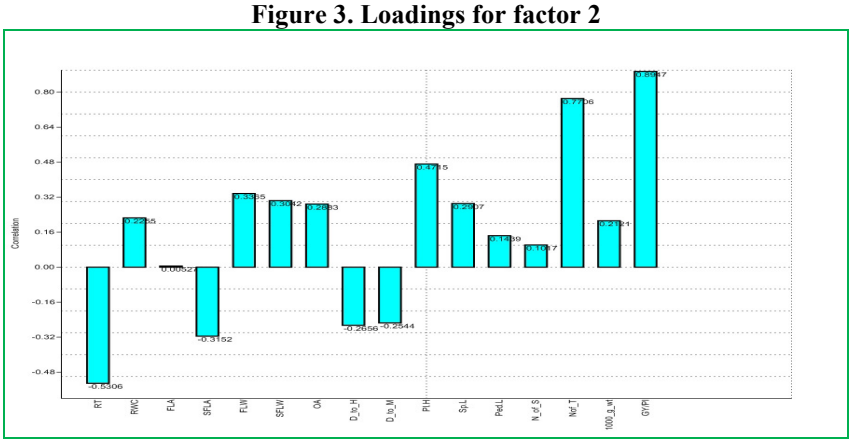


**Figure 4 showing Loadings for factor 3**


**Figure 5 depicting Loadings for factor 4**

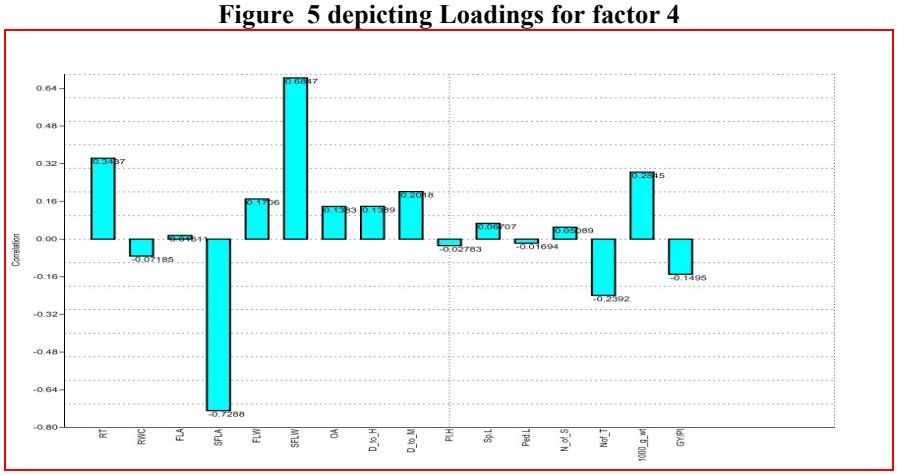

 

**Figure 6 presenting Loadings for factor 5**

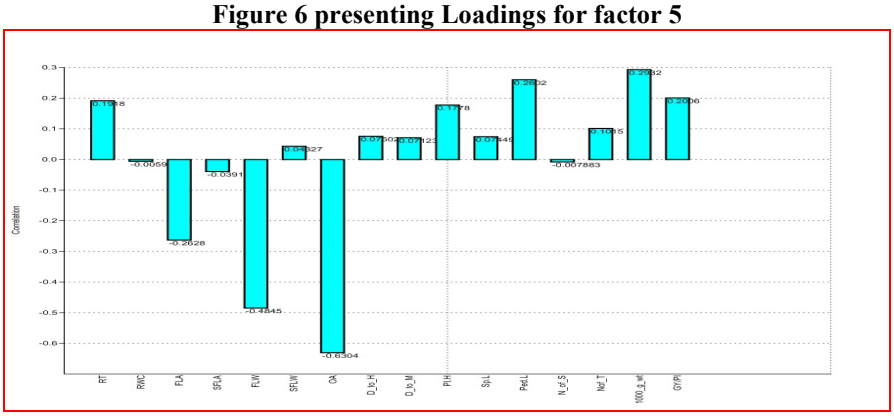


**Figure 7 showing Loadings for factor 6**

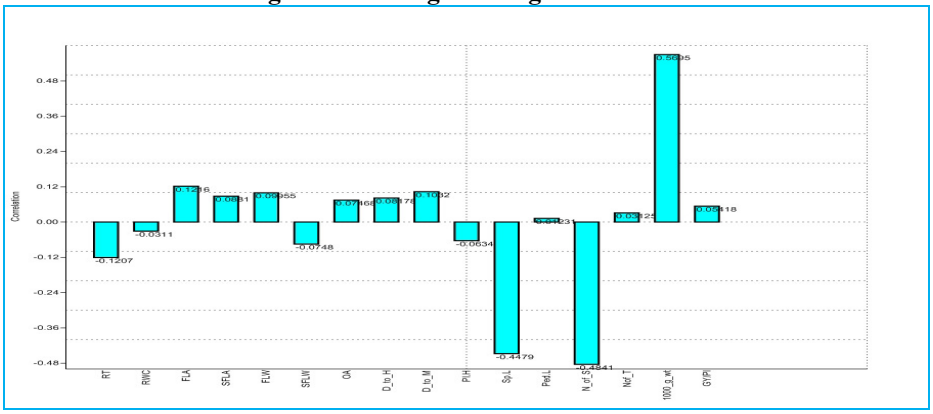


**Figure 8. Showing accessions distribution for PC1 and PC2**

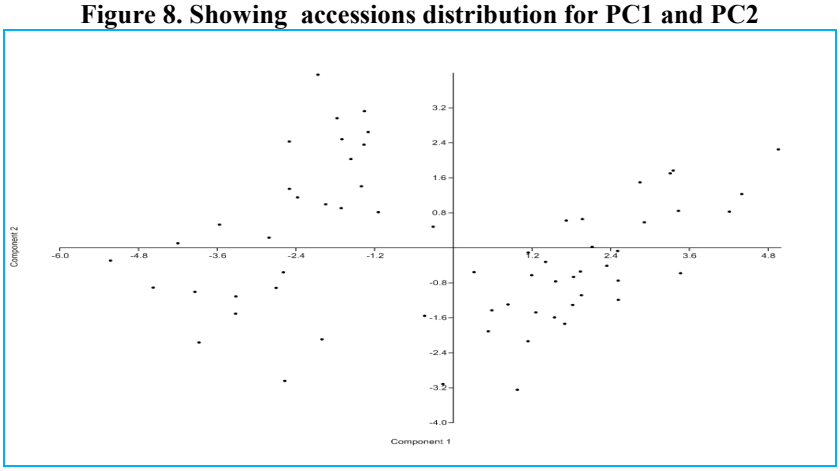

 

**Figure 9. Biplot for 16 quantitative traits of wheat accessions**

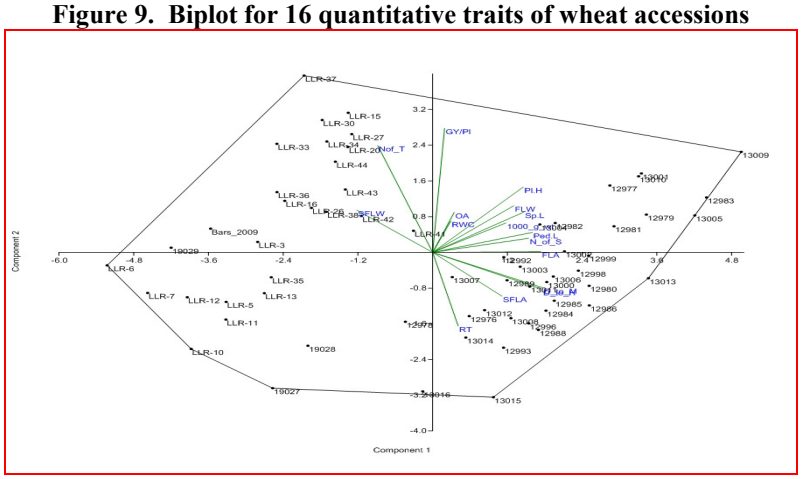


**Figure 10 showing Dendrogram based on average linkage distance in 63 accessions**

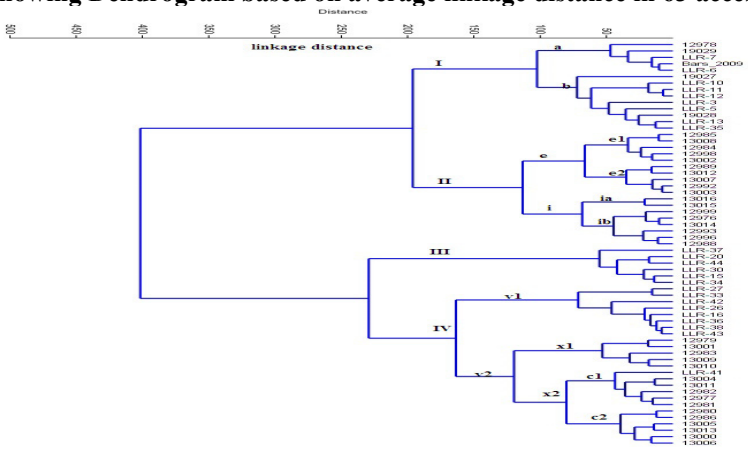


**Figure 11 consisting plot of mean values for 16 morphological traits among all accessions**

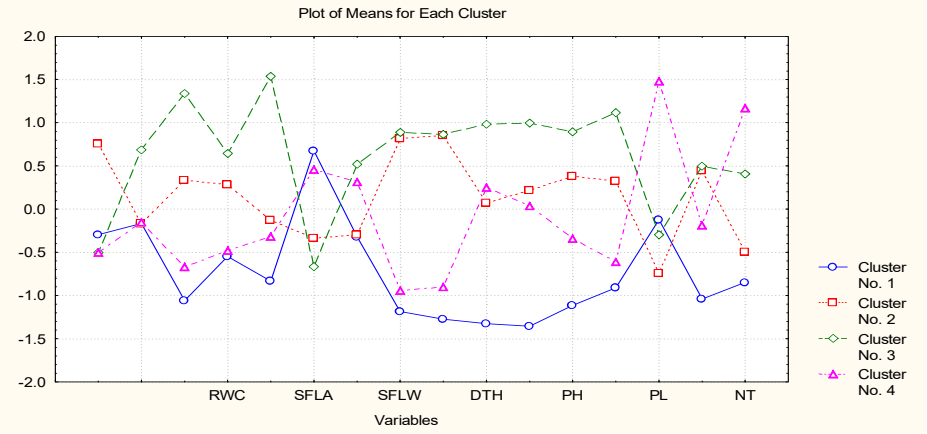

 

**Table 1. List of wheat accessions of different species**

| Sr. No | Acc. No | Former Acc. | Genus | Species | C/D-Org. Name |
|---|---|---|---|---|---|
| 1. | 012976 | PAK0016525 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 2. | 012977 | PAK0016526 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 3. | 012978 | PAK0016527 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 4. | 012979 | PAK0016528 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 5. | 012980 | PAK0016529 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 6. | 012981 | PAK0016530 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 7. | 012982 | PAK0016531 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 8. | 012983 | PAK0016532 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 9. | 012984 | PAK0016533 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 10. | 012985 | PAK0016534 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 11. | 012986 | PAK0016535 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 12. | 012988 | PAK0016537 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 13. | 012989 | PAK0016538 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 14. | 012992 | PAK0016541 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 15. | 012993 | PAK0016542 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 16. | 012996 | PAK0016545 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 17. | 012998 | PAK0016547 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 18. | 012999 | PAK0016548 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 19. | 013000 | PAK0016549 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 20. | 013001 | PAK0016550 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 21. | 013002 | PAK0016551 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 22. | 013003 | PAK0016552 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 23. | 013004 | PAK0016553 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 24. | 013005 | PAK0016554 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 25. | 013006 | PAK0016555 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 26. | 013007 | PAK0016556 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 27. | 013008 | PAK0016557 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 28. | 013009 | PAK0016558 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 29. | 013010 | PAK0016559 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 30. | 013011 | PAK0016560 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 31. | 013012 | PAK0016561 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 32. | 013013 | PAK0016562 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 33. | 013014 | PAK0016563 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 34. | 013015 | PAK0016564 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 35. | 013016 | PAK0016565 | *TRITICUM* | *Durum* | ICARDA-SYRIA |
| 36. | 019027 | PAK0018144 | *TRITICUM* | *Sphaerococcum* | CRP-WHEAT/NARC |
| 37. | 019028 | PAK0018145 | *TRITICUM* | *Sphaerococcum* | CRP-WHEAT/NARC |
| 38. | 019029 | PAK0018146 | *TRITICUM* | *Sphaerococcum* | CRP-WHEAT/NARC |
| 39. | BARS-2009 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 40. | LLR-3 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 41. | LLR-5 | -------- | *TRITICUM* | *Sphaerococcum* | PAKISTAN |
| 42. | LR-6 | -------- | *TRITICUM* | *aestivum* | PAKISTAN |
| 43. | LR-7 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 44. | LR-10 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 45. | LR-11 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 46. | LR-12 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 47. | LR-13 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 48. | LR-15 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 49. | LLR-16 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 50. | LR-20 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 51. | LR-26 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 52. | LR-27 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 53. | LR-30 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 54. | LR-33 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 55. | LR-34 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 56. | LR-35 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 57. | LR-36 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 58. | LR-37 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 59. | LR-38 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 60 | LR-41 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |

 

| Sr. No | Acc. No | Former Acc. | Genus | Species | C/D-Org. Name |
|---|---|---|---|---|---|
| 61. | LR-42 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 62. | LR-43 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |
| 63. | LR-44 | -------- | *TRITICUM* | *Aestivum* | PAKISTAN |

**Table 2. Principal component analysis for metric traits in 63 wheat accessions**

| PC | Eigen value | Variance% | Cumulative variance% |
|---|---|---|---|
| **1** | 6.48 | 40.53 | 40.53 |
| **2** | 2.63 | 16.46 | 56.99 |
| **3** | 1.82 | 11.35 | 68.34 |
| **4** | 1.40 | 8.75 | 77.09 |
| **5** | 0.99 | 6.21 | 83.3 |
| **6** | 0.84 | 5.27 | 88.57 |

**Joliffe cut-off 0. 7**

**Table 3 indicating analysis of variance between and within 16 quantitative traits**

| | Between | | Within | | | Signi |
|---|---|---|---|---|---|---|
| | SS | Df | SS | Df | F | Prob |
| **RT** | 21.11 | 3 | 40.89 | 59 | 10.15** | 0.000 |
| **RWC** | 7.05 | 3 | 54.95 | 59 | 2.52** | 0.066 |
| **FLA** | 45.40 | 3 | 16.60 | 59 | 53.79** | 0.000 |
| **SFLA** | 14.18 | 3 | 47.82 | 59 | 5.83** | 0.001 |
| **FLW** | 39.30 | 3 | 22.70 | 59 | 34.05** | 0.000 |
| **SFLW** | 16.96 | 3 | 45.04 | 59 | 7.41** | 0.000 |
| **OA** | 8.11 | 3 | 53.89 | 59 | 2.96** | 0.039 |
| **D TH** | 56.44 | 3 | 5.56 | 59 | 199.57** | 0.000 |
| **D M** | 58.95 | 3 | 3.05 | 59 | 380.33** | 0.000 |
| **P.H** | 35.57 | 3 | 26.43 | 59 | 26.47** | 0.000 |
| **S.L** | 36.90 | 3 | 25.10 | 59 | 28.91** | 0.000 |
| **P.L** | 30.81 | 3 | 31.19 | 59 | 19.42** | 0.000 |
| **N S** | 33.77 | 3 | 28.23 | 59 | 23.53** | 0.000 |
| **N .T** | 46.83 | 3 | 15.17 | 59 | 60.73** | 0.000 |
| **1000g wt** | 22.11 | 3 | 39.89 | 59 | 10.90** | 0.000 |
| **G.Y** | 37.50 | 3 | 24.50 | 59 | 30.11** | 0.000 |