STUDY OF GENETIC VARIABILITY IN CHICKPEA GERMPLASM AND ITS CONTRIBUTION TOWARDS GENETIC ADVANCEMENT

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Abstract Genetic variability is a pre-requisite to develop novel varieties in crop plants and to strengthen crop breeding programs at Research institutes. For this purpose, the genetic variability of nineteen chickpea genotypes was evaluated at Pulses Research Institute Faisalabad, Ayub Agricultural Research Institute during the Rabi season 2020-2021. The data were analyzed using D2 statistics, measured coefficient of variation, range, and standard deviation of various morphological traits of genotypes expressed significant value of variability. Seven principal components (PC) were extracted from the data by principal component analysis. Eigenvalues of the first two components were recorded >1 pointing out that these components have a major share in genetic variability. Data also expressed that no. of pods per plant (0.47) and root length (0.48) and plant height (0.48) and root length have the highest positive contribution of PC1 and PC2 respectively. In cluster - IV genotypes with higher yield potential were grouped, therefore the members of cluster- IV (D19025, D-19029, D-19036, and Bittle-2016) possessing higher grain yield along with sufficient amount of genetic diversity that can be incorporated into the genetic improvement program of chickpea.

Keywords: Chickpea; D2 Statistics; Principal component analysis; Cluster analysis; Eigen values; Grain yield

Introduction Chickpea is a self-pollinated, diploid species that has paramount importance in pulses. It is the third most important grain in the world being rich in crude protein (15-22%), carbohydrates (50-58%), minerals (Calcium, Iron, and Magnesium), Vitamins (B1, B2, B3, B6, B9) and different important nutrients (Mahmood et al., 2021; Rashid et al., 2021). Based on seed morphology there are two main chickpea groups i.e. Desi and Kabuli, desi has angular brown or black microsperma with a rough coat while the other type Kabuli has round white or creamy macro sperma with a smooth coat. Chickpea desi type is cultivated in larger areas as compared to that of chickpea Kabuli type i.e. about 80-85% acreage of cultivated chickpea in the world is under the desi chickpea type (Kumar et al., 2021). Desi-type chickpea is used for the production of split Dal and Basin while Kabuli is used for salads, soups, and hummus (Aliu et al., 2016). Being a leguminous crop with and symbiotic nature of chickpea, it helps to boost soil fertility by fixation of nitrogen in the soil and it can fix up to 140 kg/ha nitrogen with the aid of nodules which is favorable for plant growth (Bulti and Haji, 2019). Pakistan is in 3rd position having its share in overall chickpea production (5.7%); India is in 1st position with (66.3%) share and Australia on 2nd with (6.2%) contribution. Currently, it is grown on approximately 13.7 million hectares worldwide, with an average annual production of 12.8 million tonnes.

The major supply of pulses depends upon the production of chickpea and mungbean; it is cultivated in an area of about 2.2 million ha in Pakistan. The chickpea sector has the potential to uplift the country’s agricultural growth and development. The production potential of Pakistan for chickpea is much lower than that of the production of leading countries (FAO Stat, 2019). High-yielding cultivars have paramount importance in increasing chickpea production in Pakistan, but the yield potential of these cultivars is much lower in Pakistan as compared to cultivars of other countries, that’s why the average production of Pakistan is lower than leading chickpea-producing countries.

Genetic variability plays a key role in developing varieties and for the successful crop breeding program. Parents for the breeding program are selected based on diversity present for different traits in genotypes and correct estimation of genetic variability helps to identify the parents for the breeding program (Tshaye et al., 2020; Rafiq et al., 2020). Each genotype is different from other genotypes based on genetic divergence that helps to plan a suitable breeding program. Incorporation of desirable genes in high-yielding cultivars from wild genotypes is easy after having true information about genetic diversity (Pavan et al., 2017). Therefore, this study was designed to assess genetic variability in chickpea genotypes. Principal component analysis (PCA) and cluster analysis have been used by many scientists (Mahmood et al., 2021; Farshadfar, 2018; Zubair et al., 2017) for the estimation of genetic variability and identification of most diverse genotypes and traits contributed towards genetic diversity. D² Statistics proposed by Mahalanobis in 1936 was used by scientists for the estimation of genetic divergence/variability (Mahmood et al., 2021; Rafiq et al., 2020). The major purpose of this study was to identify the genotypes with desirable genetic makeup after generating information about the contribution of different yield-related traits towards genetic variability.

**Material and Methods**
The study was conducted during crop season 2020-21 at Pulses Research Institute, Faisalabad, for the finding of best-performing genotypes through assessment of genetic variability.

**Genetic Material**
Nineteen (19) genotypes D-19019, D-19020, D-19021, D-19022, D-19023, D-19024, D-19025, D-19026, D-19027, D-19028, D-19029, D-19030, D-19031, D-19032, D-19033, D-19034, D-19035, D-19036 and CH-2016 along with check cultivar BITTLE-2016 were sown during the last week of October under RCB Design in three replications.

**Planting Geometry and Agronomic Practices**
Four rows (each of 4m in length) of nineteen genotypes by maintaining 15 cm plant-to-plant and 30 cm row-to-row space were sown in the fields of Pulses Research Institute, Faisalabad. Sowing was done under ideal soil moisture conditions. During the complete crop season no irrigation was applied, the crop was totally under natural conditions. Weeds were eradicated with the help of manual hoeing as per the requirement of the crop.

**Data Collection**
Data for the following traits were recorded from randomly selected plants of each plot:
1. Plant height (cm)
2. No. of pods/plant
3. Days to maturity
4. No. of Primary branches
5. No. of Secondary branches
6. Root length (cm)
7. Grain Yield (kg/ha)

**Statistical Analysis**
D2 Statistics was used for the statistical analysis by following the outlines of Mahalanobis (Mahalanobis, 1936). STAR (Statistical Tools for Agriculture Research) 2.0.1 was utilized for Principal component analysis and cluster analysis.

**Results**
**D2 statistics**
D2 statistics was utilized to measure the mean values, coefficient of variations, and range of morphological characters of genotypes. Results showed that studied traits have significant value for variability. The traits like grain yield showed a coefficient of variability (28.5%), No. of pods per plant (23.76%) and plant height (15.25%) (Rashid et al., 2021). It exhibited that a lot of diversity was present between the genotypes in the performance of these attributes. (Table 1)

**Table (1). Mean performance of different attributes of chickpea Genotypes**

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Range</th>
<th>Mean (μ)</th>
<th>Standard Deviation (σ)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Height (cm)</td>
<td>35 – 60</td>
<td>42</td>
<td>7.12</td>
<td>15.25</td>
</tr>
<tr>
<td>No of pods/plant</td>
<td>35 – 82</td>
<td>58.2</td>
<td>12.76</td>
<td>23.76</td>
</tr>
<tr>
<td>Days to maturity</td>
<td>152 – 165</td>
<td>159.9</td>
<td>4.15</td>
<td>10.2</td>
</tr>
<tr>
<td>Primary branches</td>
<td>3.2 – 5.2</td>
<td>4.3</td>
<td>0.66</td>
<td>2.4</td>
</tr>
<tr>
<td>Secondary branches</td>
<td>4.0 – 10.0</td>
<td>6.65</td>
<td>1.46</td>
<td>3.45</td>
</tr>
<tr>
<td>Root length</td>
<td>10.15 – 18.5</td>
<td>13.31</td>
<td>2.83</td>
<td>6.48</td>
</tr>
<tr>
<td>Yield (kg/ha)</td>
<td>1495 – 2015</td>
<td>1717</td>
<td>161.2</td>
<td>28.5</td>
</tr>
</tbody>
</table>

**Principal component Analysis**
Principal component analysis is a simple non-parametric method for extracting relevant information from data. The principal component is a statistical procedure that employs an orthogonal transformation to convert a set of observations of...
possible correlated variables into a set of values of linear uncorrelated variables called principal components (PCs). The objective of the principal component analysis is to identify the minimum number of components that can explain maximum variability out of total variability and also rank germplasm based on PC scores. Seven principal components (PCs) were classified from data by using principal component analysis (PCA). Eigenvalues greater than 1 showed in PCs indicate that these principal components are the main contributors to genetic variability. In the present studies, main contributor to variation is PC 1 and PC2. PC1 has an Eigen value of 3.56, whereas PC2 has 1.46 (Table 2).

### Table 2. Eigen values of Principal Components and Variance PCA

<table>
<thead>
<tr>
<th>PC</th>
<th>Eigen values</th>
<th>Percentage of variance (%)</th>
<th>Cumulative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>3.56</td>
<td>50.79</td>
<td>50.79</td>
</tr>
<tr>
<td>PC2</td>
<td>1.46</td>
<td>20.83</td>
<td>71.62</td>
</tr>
<tr>
<td>PC3</td>
<td>0.93</td>
<td>13.24</td>
<td>84.86</td>
</tr>
<tr>
<td>PC4</td>
<td>0.52</td>
<td>7.46</td>
<td>92.32</td>
</tr>
<tr>
<td>PC5</td>
<td>0.26</td>
<td>3.73</td>
<td>96.05</td>
</tr>
<tr>
<td>PC6</td>
<td>0.19</td>
<td>2.77</td>
<td>98.82</td>
</tr>
<tr>
<td>PC7</td>
<td>0.08</td>
<td>1.18</td>
<td>100</td>
</tr>
</tbody>
</table>

The percentage of variance showed that PC1 contributed the largest amount of share (50.79%) of total variation. After PC1 main share was of PC2 (20.83%) followed by PC3 (13.24%) and PC4 (7.456%). The minimum contribution to variation was in PC7 (1.18%) (Table 2). These results indicate that for the selection of desirable chickpea genotypes based on diversity, variables that construct PC1 and PC 2 may be considered as these two components have a major contribution in genetic variability. The Eigenvalues showed that in the principal component (PC1), root length manifested a maximum value of 0.4884 followed by No. of pods per plant of 0.4787 and then followed by grain yield of 0.4699. The minimum positive value shown by plant height was 0.2581. Similarly, in PC2 the highest value of Eigenvalue was showed in plant height of 0.4875 followed by root length of 0.4601 then followed by grain yield of 0.4092 (Table 3). It indicates that plant height and root length were major contributors to the construction of genetic variability of PC2. Therefore, root length, No. of pods per plant, plant height should be considered for the selection of parents for introgression of high-yielding genes in cultivars of low yield. Eigenvalues of PC1 are also presented in graphical form Scree plot was constructed (Fig 1).

### Table 3. Principal component analysis of various chickpea traits (Eigen Value)

<table>
<thead>
<tr>
<th>Variables</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
<th>PC6</th>
<th>PC7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Height (cm)</td>
<td>0.2581</td>
<td>0.4875</td>
<td>0.5391</td>
<td>0.4366</td>
<td>0.3043</td>
<td>0.3491</td>
<td>0.0091</td>
</tr>
<tr>
<td>No of pods/plant</td>
<td>0.4787</td>
<td>0.0536</td>
<td>0.2491</td>
<td>0.0881</td>
<td>0.0211</td>
<td>0.7457</td>
<td>0.3764</td>
</tr>
<tr>
<td>Days to maturity</td>
<td>-0.1033</td>
<td>-0.1031</td>
<td>0.7436</td>
<td>0.3833</td>
<td>0.0206</td>
<td>0.0875</td>
<td>-0.1684</td>
</tr>
<tr>
<td>Primary branches</td>
<td>-0.0958</td>
<td>-0.7006</td>
<td>0.1337</td>
<td>0.6544</td>
<td>0.1445</td>
<td>0.1712</td>
<td>-0.0609</td>
</tr>
<tr>
<td>Secondary branches</td>
<td>-0.4785</td>
<td>-0.0096</td>
<td>0.0319</td>
<td>0.0761</td>
<td>-0.7994</td>
<td>-0.2043</td>
<td>0.2888</td>
</tr>
<tr>
<td>Root length</td>
<td>0.4884</td>
<td>0.4601</td>
<td>0.2181</td>
<td>-0.1887</td>
<td>0.4709</td>
<td>0.1282</td>
<td>0.6634</td>
</tr>
<tr>
<td>Yield (kg/ha)</td>
<td>0.4699</td>
<td>0.4092</td>
<td>0.1662</td>
<td>-0.4303</td>
<td>0.1579</td>
<td>-0.4763</td>
<td>-0.5502</td>
</tr>
</tbody>
</table>

**Fig 1: Scree plot of Eigen values**

Results manifested those characteristics like No. of pods per plant, Root length, and Plant height should be considered for the selection of parents for introgression of high-yielding genes in cultivars (Sharifi et al., 2018).

### Cluster Analysis

Genotypes were grouped into four clusters based on similarities, dissimilarities, and performance of traits by cluster analysis. Data from Cluster analysis showed that a sufficient amount of genetic variability is present between genotypes and traits. Mean data and range data of all clusters showed that No. of pods (74-82), primary branches (4-5) Secondary branches (8-10), Root length (14.5-18.5) and grain yield (1700-2015) were found in cluster-IV (Table 4). Maximum number of genotypes were in cluster-III (7) followed by Cluster-II (5) and then Cluster-I & 1V (Table 4).

### Table 4. Range values of chickpea traits in different clusters

<table>
<thead>
<tr>
<th>Clusters</th>
<th>PH</th>
<th>NPP</th>
<th>DM</th>
<th>PB</th>
<th>SB</th>
<th>RL</th>
<th>YLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50-60</td>
<td>45-64</td>
<td>160-164</td>
<td>3-4</td>
<td>5-8</td>
<td>10.2-13.4</td>
<td>1558-1675</td>
</tr>
</tbody>
</table>

Maximum days to maturity of 160-165 and primary branches (4-5) per plant were reported in cluster II whereas a maximum plant height of 50-60 cm was found in cluster I. Five out of seven traits showed a higher range in cluster-IV than that of others, so genotypes in cluster-IV (D-19025, D-19029, D-19036, and Bittle-16) must be exploited for the breeding program (Table 5). Maximum yield was recorded in cluster-IV followed by cluster-II, cluster-III, and cluster-I respectively. Higher yield genotypes; D-19025, D-19029, D-19036, and Bittle-16 were found in cluster IV. So, different chickpea genotypes were selected as parents based on traits like No. of pods per plant, primary branches per plant; secondary branches, root length, and grain yield.

Table .5 Clusters Memberships of different chickpea genotypes

<table>
<thead>
<tr>
<th>Clusters</th>
<th>No of members</th>
<th>Membership</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster-I</td>
<td>4</td>
<td>D-19019, D-19026, D-19033 and D-19035</td>
</tr>
<tr>
<td>Cluster-II</td>
<td>5</td>
<td>D-19020, D-19021, D-19022, D-19034 and CH-2016</td>
</tr>
<tr>
<td>Cluster-III</td>
<td>7</td>
<td>D-19023, D-19024, D-19027, D-19028, D-19030, D-19031 and D-19032</td>
</tr>
<tr>
<td>Cluster-IV</td>
<td>4</td>
<td>D-19025, D-19029, D-19036 and BITTLE-2016</td>
</tr>
</tbody>
</table>

Discussion
The success of any crop improvement program is largely dependent on the genetic divergence of existing germplasm. Genetic Variability has prime importance in breeding programs of all crops, it provides a broader genetic base to breeders and more chances for better selection. Therefore, this study was designed to evaluate genetic variability among chickpea genotypes to improve its breeding program. D2 statistical analysis showed significant differences among chickpea genotypes for grain yield as reported in Table 1. Rashid et al., 2021 and Ningwal et al., 2023 also reported genetic variability among chickpea genotypes and its importance for breeders. Principal component analysis classified the data into seven principal components. In the present studies, the main contributors to variation are PC 1 and PC2. PC1 has an Eigenvalue value of 3.56, whereas PC2 has 1.46 (Table 2) Similar, results were also reported by Rafique et al., 2020 in which a major contribution was found by two principal components (PC1 and PC2). The percentage of variance showed that PC1 contributed the largest amount of share (50.79%) of total variation. In PC2, plant height, and root length were major contributors to genetic variability. Therefore, root
length, No. of pods per plant, plant height should be considered for the selection of parents for introgression of high yield genes in cultivars of low yield (Mohammad et al., 2021; Zubair et al., 2017). Data from Cluster analysis showed that a sufficient amount of genetic variability is present between genotypes and traits. Maximum yield was recorded in cluster IV followed by cluster II, cluster-III, and cluster-I respectively. Higher yield genotypes; D-19025, D-19029, D-19036, and Bittle-16 were found in cluster IV. So, different chickpea genotypes were selected as parents based on traits like No. of pods per plant, primary branches per plant; secondary branches, root length, and grain yield. These parents may be utilized for crossing programs (Kumar et al., 2019; Pavan et al., 2015).

CONCLUSION

Chickpea genotypes showed maximum variability for different traits and they were statistically different from each other. Cluster analysis showed that members of cluster-IV (D19025, D-19029, D-19036, and Bittle-2016) possess higher grain yield along with a sufficient amount of genetic diversity that can be incorporated into the genetic improvement program of chickpea.

Novelty Statement

Genetic variability is crucial in the evolution of new crop varieties under changing environments for sufficient crop production. For this purpose, chickpea genotypes were evaluated using different statistical analyses and parents were selected for further breeding programs.

References


Johnston PL, RN Sharma and HC Nanda (2015). Genetic diversity and association analysis for yield traits chickpea (Cicer arietinum L.) under rice based cropping system. The Bioscan 10, 879-884.


**Statements and Declarations**

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**Author contributions**
All authors contributed equally.

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N/A

**Informed consent**
N/A

**Ethical Approval**
Current study is approved from concerned ethical review committee

**Competing interests**
The authors have no competing interests.

**Data availability statement**
All data has been given in manuscript.

**Submission declaration and verification**
The work is not been published previously, and it is not under consideration for publication elsewhere.

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