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Research Article

Assesment Genetic Diversity of Kabuli Chickpea (*Cicer Arietinum* l.) Genotypes in Awabel District, Northwestern Ethiopia

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Abstract

The examination was conducted to point out genetic diversity of 36 Kabuli chickpea genotypes, including two varieties 6 x 6 in simple lattice design, during the main growing season 2019/2020-2020/2021 in Awabel district for two consecutive years. Thirty-six genotypes were grouped into six (6) distinct groups based on 13 (thirteen) traits. Intragroup distance was maximum in cluster IV (D2 = 438.6, indicating that the genotypes enclosed in this group were diverging as compared to others groups. In descending order maximum inter cluster distance was obtained among cluster VI and IV (D2 = 860. 9), cluster VI and V (D2 = 454.9), between groups II and VI and between groups VI and I. Genotypes that have a large intergroup distance can give a high heterotic response leading to better recombinants. The paramount six principal components elucidate approximately 78.52% of the total variation. Seed yield (0.439), number of seeds plant $^{-1}$ (0.459) and number of seeds pod^{-1} (0.368) were the main contributors of variation for (PC1). The current experimental finding shows the presence of a wide range of diversity among the genotypes tested, which is an essential role for further use in the breeding program. However, multi-locus testing among a wider conventional of contestant genotypes is needed to prove the stability across environment and advance good-performing varieties of the existing genotypic diversity.

Keywords

Chickpea, Cluster, D2 Statistics, Genotypes, PCA

1. Introduction

Chickpea (*Cicer arietinum* L.) is among the supreme significant annual food pulses and first domesticated grain legume cultivated in more than 57 countries under varied environmental conditions throughout the glob [19]. Chickpea is the one of an abundant legume grown in Ethiopia under wide ranging agro ecological conditions [2]. Chickpea is considered as a very nutritious and economical source of proteins, carbohydrates, fats, a good source of minerals and essential with enhanced levels of essential dietary nutrients for many people [5, 22, 29] and generating income for small households [11].

Nevertheless, seed production on a national scale in Ethiopia remains low, about 21800 kgha^{-1} [8] far below its genetic potential yield of while under best adoption it gave 35000 kgha^{-1} [11]. Because, the production of this crop is limited at the national level due to usage of unsuitable improved varie-

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ties, the employment of fundamentally unproductive agricultural varieties, and both biotic and abiotic factors [11]. Stable yields are the major goals of plant breeding programs. Hence, enhancement of crops needs conception and introduction of genetic diversity, combined with inbreeding and selection.

Assessment of breeding resources across various locations over years is crucial to determine wider adaptable and well-performed genotypes with favorable characters [9, 25]. The inception of chickpea breeding program has boarded on understanding the challenges of chickpea production and looking for genetic improvement basis to curb some of those key gaps [11]. Information regarding genetic diversity is vital to determine breeding materials for further breeding programs [7]. The diversity of genetic assets is the basis of any genetic advance of cultivated plants. The convenience of genetic divergence in a gene pool is a precondition for the realization of breeding program [1] to attain the anticipated genetic advancement via selection and/or hybridization. Estimating genetic diversity of Kabuli chickpea accessions helps to determine a very diverse germplasm that provides numerous opportunities for breeders to search for the desired characteristics to develop new and super-performing varieties. Evaluation of genetic assortment among genotypes is the breeder's duty [10].

Principal component analysis (PCA) highlights the prominence of the primarily involves the overall variance at each alignment for distinction [23]. PCA remained utilized to identify the characteristics that contributed more to the entire variance. Numerous reports had been noted by researchers for genetic diversity of chickpea [3, 12, 28, 13] and prove the presence of high genetic difference between tested genotypes. Despite, Ethiopia is well-thought out the second center of diversity for chickpea [16] genotypic diversity is invaluable; it is promptly conserved and effectively utilized in crop improvement programs. An evidence behind is up to date about 29 high-performing varieties have been released in both types since 1970s [11]. However, the majority of the developed varieties were also of the Desi type. Previous information's available on genetic variation is limited and there is lack of availability of genetic diversity to improved Kabuli chickpea varieties [21]. This indicates conducting such an original research specific to a site will have an important role on generating genetic information on diversity of genotypes based on morpho-agronomic traits for future progress via assortment and/or hybridization. The field experiment was aims to assess the level of genetic diversity among examined Kabuli chickpea genotypes and to define the important characters contributing more to the total variation.

2. Materials and Methods

2.1. Description of Experimental field

Genotypes were evaluated at experiment site of Awabel district, East Gojjam Zone, during 2019/2020-2020/2021 main cropping season. The site laid 10 °29' latitude N and 37 °44' longitude with an altitude of 2104 m.a.s.l. The average mean yearly rainfall was 1090 mm and its lowest and highest temperatures are 15 ℃ and 24 ℃. The soil was black vertisol with pH value 6.45 (ADAO).

2.2. Planting Material

Thirty four (34) Kabuli chickpea genotypes and two check varieties (Habru and Arerti) were used in the experimental study. Those experimental materials were collected from the Highland Pulse Research Program, Debre Zeit Agricultural Research Center (DZARC).

Table 1. *List of experimental materials examined.*

Code assigned	Genotype pedigree	Code assigned	Genotype pedigree	Code assigned	Genotype pedigree
G-01	FLIP-93-93C	G-13	Arerti	G-25	FLIP-12-343C
G-02	FLIP-12-53C	G-14	FLIP-12-60C	G-26	FLIP-12-311C
G-03	FLIP-12-110C	G-15	FLIP-12-57C	G-27	FLIP-12-176C
G-04	FLIP-12-37C	G-16	FLIP-12-86C	G-28	FLIP-12-40C
G-05	Habru	G-17	FLIP-12-342C	G-29	FLIP-12-01C
G-06	FLIP-12-198C	G-18	FLIP-12-263C	G-30	FLIP-12-331C
G-07	FLIP-12-107C	G-19	FLIP-82-150C	G-31	FLIP-12-265C
G-08	FLIP-12-287C	G-20	FLIP-88-85C	G-32	FLIP-12-55C
G-09	FLIP-12-06C	G-21	FLIP-12-108C	G-33	FLIP-12-197C
G-10	FLIP-12-18C	G-22	FLIP-12-322C	G-34	FLIP-12-210C

Code assigned	Genotype pedigree	Code assigned	Genotype pedigree	Code assigned	Genotype pedigree
G-11	FLIP-12-79C	G-23	FLIP-12-310C	G-35	FLIP-12-75C
G-12	FLIP-12-61C	G-24	FLIP-12-109C	G-36	FLIP-12-192

2.3. Experimental Layout, Procedures and Field Management

The research was carried out in simple lattice design 6 × 6 configurations for two consecutive years. Replication, blocks and plots was spaced 2 m, 1 m and 0.5 m, respectively. The total net area of the plots was 1.35 m^2. Every genotype was randomizing, planted with a space of 30 cm between rows and 10 cm between plants. All agronomic practices were implemented in harmony with the recommendations for chickpea production package [20].

2.4. Data Collected

Data were recorded from the middle entire rows, specifically in the three central rows, by arbitrarily selecting and tagging five individual plants by agreeing [15]. Thirteen morph-agronomic traits were collected on plant and plot base. The collected data were phonological: Days to 50% flowering (DF), days to 90% maturity (DM), pod-filling period (PFP).

Growth related data: plant height (PH) in cm, number of primary branch (NPB), number of secondary branches (NSB)) and yield and yield related data (100-seeds weight (HSW) in gm, biomass yield (BY) in $Kgha^{-1}$, seed yield (SY) in $kgha^{-1}$, harvest index (HI), number of pods $plant^{-1}$ (NPP) and number of seeds per pod (NSP)).

2.5. Data Analysis

2.5.1. Genetic Divergence Investigation

Genetic divergence analysis was calculated using multivariate analysis based on Mahalanobis's D2 statistic [17].

2.5.2. Estimation of Genetic Distance

Genotypes are grouped based on trait similarity and difference based on squared distance values (D2) using the multivariate statistical technique developed by Mahalanobis [17]. This technique allows different genotypes to be classified into different groups based on triads. Grouping of genotypes was done using Tocher's method as designated by [24]. Squared distances (D2) were calculated for each pair of genotype groupings using the following formula as defined by Mahalanobis:

$D^2_{ij} = (X_i - Xj)\, S^{-1}\, (X_i - X_j)$

Where, D^2_{ij} = the square distance between any two genotypes i and j, X_i and X_j = the vectors for the values for genotype i^{th} and j^{th} genotypes, and S^{-1} = the inverse of pooled variance covariance matrix.

2.5.3. Estimation of Intra and Inter-Cluster Distances

Average intra and inter cluster D2 values was estimated using the formula where ∑Di2 is the sum of distance between all possible combinations (n) of the genotypes included in a cluster [17].

Average intra and inter cluster D^2 values

Average intra cluster D^2, $D^2 = \sum Di^2 / n$

Where, $\sum Di^2$ is sum of distances between all possible combinations (n) is the population included in a cluster.

Average inter cluster D^2, $D^2 = \Sigma Di^2 / ni.nj$

Where, ni= number of population in cluster i, nj=number of population in cluster

2.6. Principal Component Analysis (PCA)

Principal component analysis was performed to recognize the traits that contribute ample to the total variation with association performed by the SAS computer software program [26]. Principal components eigenvalues greater than one were used for analysis according to [6]. Principal components were computed using SAS statistical software based on formulas formulated by [4].

The first PCA value (Y1) is given by the linear combination of the variables X1, X2...Xp Y1 = a11X1 +a12X2 +...+a1pXp

The next principal component is computed in the same way, Y2 = a21X1 +a22X2 +...+a2pXp this continues until a total of p principal components have been computed [3].

3. Results

3.1. Genetic Divergence Analysis

The scattering of tested chickpea genotypes categorized into six separate clusters (Table 2 and Figure 1) based on similarity and difference of traits.

3.1.1. Clustering Analysis of Genotypes

Large quantity of genotypes included in Cluster-II containing ten (10) genotypes accounts 27.78 % of the total genotypes. Cluster-I and cluster-III containing eight (8) genotypes each and cumulatively accounts 44.44 % of the total

genotypes evaluated. Cluster- IV included three genotypes and covers 8.33 % of total genotypes tested. Cluster-V contained four genotypes (11.11%) share of the total genotypes evaluated and cluster-VI comprises of a least number of two genotypes (5.56%). The overall results, indicates a extensive range of diversity amid the tested genotypes. The result showed genotypes existed under the same cluster as compared to others, more related in characters. Whereas genotypes grouped in different clusters related in some extent, but it is important for improvement of kabuli chickpea genotypes.

Table 2. *Scattering of 36 Kabuli Chickpea genotypes in six diverse clusters.*

No of cluster	Name of genotypes Pedigree	No of genotypes	Percent
I	FLIP-12-18C, FLIP-12-01C, FLIP-12-342C, Arerti, FLIP-12-343C, FLIP-12-263C, FLIP-12-86C, FLIP-12-197C and FLIP-12-322C	9	25
II	FLIP-12-331C, FLIP-12-210C, FLIP-12-109C, FLIP-12-265C, FLIP-88-85C, FLIP-12-310C, FLIP-12-79C, FLIP-12-60C, FLIP-12-311C and FLIP-12-55C	10	27.78
III	FLIP-12-110C, FLIP-12-192, FLIP-12-53C, FLIP-12-40C, FLIP-12-75C, FLIP-12-107C, FLIP-12-176C and FLIP-12-287C	8	22.22
IV	FLIP-12-06C, FLIP-12-61C and FLIP-82-150C	3	8.33
V	FLIP-12-37C, Habru, FLIP-12-198C and FLIP-12-108C	4	11.11
VI	FLIP-93-93C and FLIP-12-57C	2	5.56

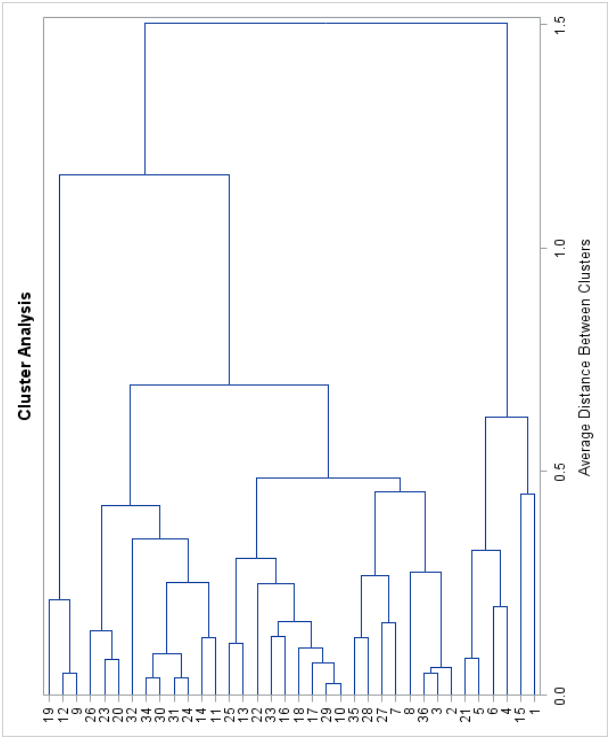

Figure 1. *Dendrogram displaying 36 Kabuli chickpea genotypes based 13 traits.*

3.1.2. Cluster Distance Analysis

Intra cluster distance was maximum in cluster-IV (D^2 = 438.6), the succeeding highest cluster distance was observed in I (D^2 = 67.08) and the lowest for cluster-VI (D^2= 19.2). The highest intra cluster distance of cluster- IV, indicates the genotypes comprised in this cluster were more divergent than

other clusters. The maximum inter-cluster distance were also observed between cluster VI and IV (D^2= 860.9), cluster VI and V (D^2= 454.9), between cluster-II and VI (D^2= 373.2) and between cluster I and VI (D^2=278.6) in descending order, this shows the existence of maximum genetic difference among the examined genotypes important for future chick-pea improvement. While, the minimum inter-group distance was observed between cluster I and II (D^2=22.1), indicates presence of narrow gap between these clusters as comparatively with other clusters.

Table 3. *Intra (crosswise) and inter (off-diagonal) square distance (D^2) values of 36 chickpea genotypes.*

Clusters	I	II	III	IV	V	VI
I	67.08	22.14	28.03	190.67	84.23	278.64
II		66.42	36.07	120.64	134.36	373.17
III			64.32	263.41	44.26	206.93
IV				438.6	454.89	860.95
V					19.2	76.81
VI						0

3.1.3. Mean of Clusters

The average mean value of the 13 traits in every cluster was explained in (Table 4). Among the cluster means, cluster six was considered through the maximum number of seeds per pod (1.26), highest number of seeds per plant (57.59), number of secondary branch (7.41) and the highest days to flowering (63.25), pod filling period (82), days to maturity (144.5), seed yield (4701.2 $kgha^{-1}$) and biomass yield (13301 $kgha^{-1}$). Genotypes in this cluster revealed the best performance for desirable agronomic traits above ground biomass yield, number of pods per plant, number of seeds per pod, and number of secondary branches. This might be the best situation for Kabuli chickpea improvement through selection and hybridization of desired traits. However, the cluster mean value of other cluster also not neglected, it is best performed and it could be selected in some important traits as shown in (Table 4). presented.

Table 4. *Mean values of 13 traits of the six clusters of 36 tested genotypes.*

Clusters	DF	PFP	DM	PH	NPB	NSB	NPP	NSP	NSPP	HSW	GY	BY	HI
CL1	57.92	71.69	128.36	47.57	3.24	5.92	48.16	1.20	40.76	30.94	3642.23	7754.33	0.49
CL2	59.67	75.45	133.98	47.22	4.74	7.31	51.33	1.11	30.30	30.18	2223.54	6294.50	0.39
CL3	53.53	76.09	128.56	48.10	4.08	6.78	47.09	1.12	34.43	35.91	2681.70	8823.75	0.32
CL4	49.75	73.25	121.75	52.00	3.37	6.57	50.77	1.03	22.40	35.50	2437.10	9704.00	0.26
CL5	51.81	72.13	123.19	47.39	3.67	7.02	43.33	1.26	40.34	33.19	4260.60	11299.50	0.40
CL6	63.25	82.00	144.50	50.45	4.24	7.41	57.59	1.26	37.35	28.13	4701.20	13301.00	0.37

3.2. Principal Component Analysis

The five principal components (PC) from PC1 to PC5 with eigenvalues greater than one contributed about 78.52 % of the total variation, with PC1, PC2, PC3, PC4 and PC5 in that order explaining 25.85 %, 19.71%, 12.87%, 11.03% and 9.06% of the gross variation among 36 Kabuli chickpea genotypes evaluated for 13 traits. PC1 was major contributors of variation, which were seed produce (0.439), number of seeds per plant (0.459) and number seeds per pod (0.368). Number of primary branches, number of secondary branches and number of pod per plant enlighten the highest variation on PC2. Uppermost contributors for explained variance in PC3 include pod-filling period, days to maturity and biomass yield. Variation in PC4 was mainly due to hundred seed weight, biomass yield and number of secondary branches. Plant height (0.53) and number of primary branches (0.36) contributed more in PCA5.

Table 5. *Total variance explained by the first five principal components (PC) for 13 traits in 36 chickpea genotypes.*

Traits	PC1	PC2	PC3	PC4	PC5
DF	-0.253	0.097	0.337	-0.528	0.046
PFP	-0.281	0.114	0.393	0.263	-0.353
DM	-0.314	0.088	0.561	-0.066	-0.182

Traits	PC1	PC2	PC3	PC4	PC5
PH	-0.012	-0.136	0.238	-0.109	0.785
NPB	0.109	0.547	0.000	0.052	0.237
NSB	0.051	0.497	0.029	0.275	-0.024
NPP	0.160	0.486	0.087	0.022	0.161
NSP	0.368	-0.131	0.149	0.024	-0.110
NSPP	0.459	0.090	0.023	0.025	-0.232
HSW	-0.088	-0.332	0.098	0.456	0.074
GY	0.439	-0.125	0.367	-0.071	-0.016
BY	0.307	-0.126	0.429	0.287	0.127
HI	0.279	-0.031	0.036	-0.510	-0.231
Eigenvalue	3.360	2.562	1.673	1.433	1.179
Percent	25.85	19.71	12.87	11.03	9.06
Cum Percent	25.85	45.56	58.43	69.45	78.52

Note: PC-principal component

4. Discussion

4.1. Divergence Analysis of Tested Genotypes

4.1.1. Range and Mean of Clusters Analysis of Tested Genotypes

The grouping of thirty-six genotypes of Kabuli chickpea into six different clusters based on square distance, demonstrating that the exhibit of wide range of diversity amongst the tried genotypes (Table 3). The result shows that the assessed Kabuli chickpea genetic resources represent a precious basis of genetic diversity that is predictable to be very convenient for existing and upcoming breeding programs. Achievements of genetic enhancement of any crop are highly dependent on the accessibility of genetic assets and the level of genetic diversity among key parameters [14]. Table 4 demonstrates the mean value of each groups of 13 characteristics. The cluster mean value of genotypes in Cluster (I) showed the best performance for desirable agronomic traits above ground biomass yield, number of pod per plant, number of seed per pod, number of primary branch, number of seed per plant and number of secondary branch. This might be the best situation for Kabuli chickpea improvement through selection and hybridization of desired traits. Fikre and Bekele [11], reported that the first level of priority for producers is productivity, resistance, adaptive traits, next concern is market and nutritional quality. In accordance with these results, genotypes grouped in Cluster-I rate consideration for their straight use as parents in hybridization programs to develop varieties that demanded by farmers in yield. The present results average cluster mean of 13 characters shown that none of the clusters contains a genotype with all the desired traits and therefore the recombinative selection between genotypes of different groups is necessary. In addition, to generate genetic diversity in various desired traits, several crosses made with these divergent parental lines can give good results. Correspondingly, Thakur et al. [27], Fikre, and Bekele [11] stated that parental selection should also consider the better gain of each group and each genotype within a group depending on the specific hybridization objective.

4.1.2. Intra and Inter Group Distances of Studied Genotypes

The results presented above in (Table 3), revealed that the highest group distance was that of cluster-IV, indicating that the genotypes included in this group were more divergent than the other groups. The genotypes which had large inter group distance between cluster VI and IV (D^2= 860.9) followed by cluster VI and V (D^2= 454.9) can be used for hybridization program and may give wide heteroticity response resulting in better recombinants. Whereas, genotypes which had the lowest inter group distance was observed between cluster I and II (D^2=22.1), representing existence of near closeness between these groups as comparatively with other clusters. Supreme genetic recombination and distinction in the next generation is predicted from crosses involving parents from the groups characterized by the maximum distance, crosses between the genotypes of clusters VI to IV, group VI to V are expected to be the best scenario for the development of more genetic recombination. Hence, selection of generation of desirable segregants with a broad genetic base seems to give promising results. Similar findings were noted by grouped 60 chickpea genotypes into five clusters and using 100 chickpea genotypes reported twelve clusters based on square distance values [18, 27] in chickpea genetic diversity study.

4.2. Principal Component Analysis

The consequences of the principal components analysis in (Table 5) displayed that the first five (5) principal components with eigenvalue exceeds one have been clarified 78.52 % of the total variation between genotypes. The major positive contributors of variation for (PC1) were seed yield (0.439), number of seeds per plant (0.459) and number seeds per pod (0.368). The first principal component contributed more (25.85%) for the total variation in figure compared to following components. The second principal component explained (19.71) of total variation and traits number of primary branches, number of secondary branches and number of pod per plant positively associated this principal component. The consequences designated that latent candidate traits for breeding materials could be derived from the genotypes of the first two principal components PC1 and PC2 contributing more to the total variation and suggested that many opportunities for genetic improvement through selection were more

numerous than the less common ones. In line with the present finding, Hailu [13] conveyed the first four PC explained about 74.3% of the total variation between 49 chickpea genotypes, indicated existence of genetic diversity. Zerfu et al. [30] also noted that four PCS from PC1 to PC4 contributed for 98.2% of the total variation. Overall, the results from this finding, indicates wide genetic diversity among the tested genotypes important for successful breeding program with respect to most studied traits.

5. Conclusions and Recommendations

In conclusion, wide genetic diversity were observed among genotypes between clusters, indicating that the crossing of genotypes in these groups could be the finest situation to create a moderately enhanced genetic recombination and create appropriate segregants. The first six principal components (PC) explain near 78.52% of the total variation among Kabuli chickpea genotypes. The current finding allowed to be observe a sufficient existence of diversity for furthermost of the characters of the evaluated genotypes, which should be used in the upcoming breeding of Kabuli chickpea. Nevertheless, this experimental research was carried out for two consecutive main cropping years only and at one site, which should be carried out in more breeding processes seeing more experimental sites to develop best-suited, maximum-yielding varieties and marketable produce for progress improvement for further Kabuli chickpea breeding.

Abbreviations

ADAO	Awabal District Agricultural Office
MOAAR	Ministry Agriculture and Animal Resource
CSA	Central Statistical Agency
SAS	Statistical Analysis Software
PCA	Principal Component Analysis

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Author Contributions

Gebremeskel Mequanint Mulu: Conceptualization, Data curation, Formal Analysis Funding acquisition, Investigation, Methodology Resources, Software, Writing – original draft, Writing – review & editing

Ahadu Menzir: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing

Availability of Data Statements

The records used and/or analyzed during the current finding are existing from the conforming authors upon rational request.

Conflicts of Interest

The authors declare no conflicts of interest.

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