

Black Sea Journal of Agriculture Open Access Journal e-ISSN: 2618 - 6578

BSPublishers

Research Article

Volume 2 - Issue 3: 146-155 / July 2019

PHENOTYPIC CHARACTERIZATION AND ASSESSMENT OF GENETIC DIVERSITY FOR AGRO-MORPHOLOGICAL TRAITS OF ETHIOPIAN CHICKPEA (*CICER ARIETINUM L.*) LANDRACES

Awol MOHAMMED^{1*}, Bulti TESSO²

¹Sirinka Agricultural Research Center, PO Box 74, Woldia, Ethiopia ²Haramaya University, P. O. Box 138, 138, Dire Dawa, Ethiopia

Received: January 30, 2019; Accepted: April 16, 2019; Published: July 01, 2019

Abstract

Ethiopia has a large number of Desi type chickpea landraces. However, limited information is available about the landraces character. This experiment was conducted in 2016 at Sirinka and Jari, under rainfed condition to characterize and assess genetic diversity among the Ethiopian chickpea landraces. Two hundred two new germplasm accessions were grown in an alpha lattice design with three replications. Data on 16 traits were collected and analyzed. Differences among the genotypes were highly significant (p<0.01).The genotypes were grouped into five clusters with different sizes. Genetic distances among the clusters were significant. The highest diversity indices pooled over characters within zones were recorded for accessions from South West Shewa ($H=2.03 \pm 0.05$) followed by Gurage ($H=0.81 \pm 0.08$), West Shewa ($H=0.73 \pm 0.04$) and North Gonder ($H= 0.72 \pm 0.05$). The existence of wider morpho-agronomic diversity among the chickpea collections implies the potential to improve the crop and the need to conserve the diversity. Future collecting operations of chickpea germplasm should strategically focus on areas with relatively large variation. From genetic conservation point of view, it appears that South West Shewa, Gurage, West Shewa and North Gonder, could be suitable as in situ conservation sites.

Keywords: Landraces, Morpho-Agronomic, Diversity, Clustering

*Corresponding author: Sirinka Agricultural Research Center, P. O. Box 74, Woldia, Ethiopia E mail: mawol50@yahoo.com (A. MOHAMMED) Awol MOHAMMED 10 https://orcid.org/0000-0002-5690-7635 Bulti TESSO 10 https://orcid.org/0000-0002-9341-2287 Cite as: Mohammed A, Tesso B. 2019. Phenotypic characterization and assessment of genetic diversity for agro-morphological traits of Ethiopian chickpea (*Cicer arietinum L.*) landraces. BSJ Agri, 2(3): 146-155.

1. Introduction

Chickpea (*Cicer arietinum L.*) is, self-pollinated diploid (2n=2X=16), annual leguminous plant in the family Fabacea with a genome size of 738.09 Mbp (Varshneyet al., 2013). Southwest Asia and the Mediterranean region

particularly Southeast Turkey and Syria are considered as the two primary centers of origin of chickpea (Singh et al., 1997). Ethiopia is considered as one of the centers of secondary diversity for chickpea (van der Maesen, 1987). In Ethiopia, chickpea is the third largest food legume crop in sowing area and production (CSA, 2015). There are two types of chickpea depending on seed color, shape, and size. The Kabuli type has large, round or ram head and cream-colored seeds, and is grown in temperate regions. The desi type chickpea is grown in the semi-arid tropics (Muehlbauer and Singh, 1987), and is characterized by relatively small angular shaped seeds with light brown, yellowish or black colour.

Ethiopia has a large number of desi type chickpea landraces cultivated by farmers through traditional method of selection over centuries. These landraces have high capacity to tolerate biotic and abiotic stress; resulting in high yield stability under low input agricultural systems (Zeven, 1998). Thus, characterization of landraces and knowledge on the pattern of variation for important morpho-agronomic traits is needed for a proper management and a better exploitation of this gene pool (Jain et al., 1975; Gebrekidane, 1982; Assefa 2003). The existence of genetic diversity has special significance for he maintenance and enhancement of productivity in agricultural crops in a country like Ethiopia, which is characterized by highly varied agro-climates and diverse growing conditions (Worede, 1993; Woredeet al., 2000; Brush 2000).The Ethiopian landraces have tremendous genetic diversity in both domesticated and wild relatives (Edwards, 1991). The extent and pattern of genetic diversity in Ethiopian landraces is not yet systematically studied (Hailuet al., 1991).

Geographical separation with physical barriers and genetic barriers to cross ability is believed to give rise to genetic diversity among genetic materials (Singh, 2001). However, whether differences in geographic origin necessarily imply genetic distance in parental selection for hybridization is still a matter of some controversy. Joshi and Dhawan (1966) suggested the concept that geographic diversity may serve as an index of genetic diversity in parental selection. Others argue that genetic divergence was not apparently related to geographic diversity in some crops (Durgaet al., 1985; Nadafet al., 1986; Katuleet al., 1992). If the former holds true, it is logical to expect that the physical barriers might have resulted in distinct genetic diversity of chickpea accessions growing in different parts of Ethiopia. Thus, the Ethiopian Biodiversity Institute (EBI), Debre Zeit Agricultural Research Center (DZARC) and ICRISAT collected 202 new landraces from different areas of the country. Phenotypic characterization of these landrace collections is essential for their utilization in breeding programs for the improvement of the crop, and for gene bank curators for efficient and effective management of the collections. Therefore, the objectives of this experiment were to evaluate the genetic diversity among the Ethiopian chickpea landraces, the relative contribution of various morpho-agronomic traits to the total diversity in the associations and to study the association of geographic origin with genetic diversity.

2. Materials and Method

The experiment was executed under rain fed condition at Sirinka and Jari. The former one is located at11°45' North latitude and 39° 36' 36" East longitudes. The altitude of Sirinka is 1850 meter above sea level in North Wollo Zone. The annual rainfall of this site is 1006.3mm with 13.6°C minimum and 26.7 °C maximum temperature. The second site, Jari, is located at 11°21' North latitude and 39° 47' East longitudes and at an altitude of 1680 meter above sea level in South Wollo Zone. The annual rainfall of this site is 987.3 mm with 14.2 °C minimum and 28.7 °C maximum temperature. According to Sirinka Agricultural Research Center soil classification (unpublished), the soils of the sites are classified as Vertisols.

In general, 202 desi type chickpea landrace collections from Amhara, Oromiya and Southern Nations, Nationalities and Peoples' Regional States were used for this study with two released varieties as checks, Fetenech (early maturing variety, 78 - 90 days to mature) and Minjar variety (high yielder, 2.2 - 5 tons and recommended for this tested area) The landraces were collected from elevations ranging from 1174 to 2660meter above sea level. The collections were made in 2013 (42) and in 2016 (160). Generally, 90 landraces were collected from Amhara region and 91 and 24 landraces were collected from Oromiya and SNNP, respectively. These landraces with two released varieties as checks were tested and characterized for agromorphological traits. The experiment was setup on 02 September 2016 by using alpha lattice designwith three replications at Sirinka under rainfed conditions. Each landrace was sown in two rows at 60 cm, 30 cm, and 10 cm spacing between plots, rows, and plants, respectively; with 1 m row length. All agronomic practices were done uniformly for all accessions as required. There was no fertilizer application.

The data of morphological, phenological, and agronomical traits were collected during the growth period of the crop, depending on the descriptors for chickpea (IBPGR, ICRISAT and ICARDA, 1993). The data of plant height, stem colour, number of leaflet per leaf, plant canopy width, number of primary branches, number of secondary branches, pod length, number of pods per plant, number of seeds per pod, days to 50% flowering, days to 50% pod setting, pod filling period, days to 75% maturity, total biomass, hundred seed weight, seed yield, harvest index, seed coat colour, seed shape, diseases and insect damage score (1-9) and seed testa texture were determined.

Analysis of variance (ANOVA) was performed for the quantitative data using SAS computer software (SAS, 2004) as per the following linear model for alpha lattice design.

Cluster analysis is important to group genotypes into homogeneous sets based on their response to the environments considered. Elements within the same group or cluster are relatively homogeneous and elements in different clusters are relatively heterogeneous. The clustering was done using proc cluster of SAS and the average linkage option was used. Genetic distance between clusters was calculated using the generalized Mahalanobis's D2 statistics.

Frequency distribution of the various categories of qualitative traits was studied in to the zones and altitude from which the accessions were collected. The Shannon-Weaver diversity index (H') was computed using the phenotypic frequencies to assess the overall phenotypic diversity for each character by zones and altitude ranges. The altitude was arbitrarily classified in to three altitude classes, <1801, 1801 - 2200, and >2200 meter above sea level. The Shannon-Weaver diversity index as described by Hutchenson (1970) was used to calculate phenotypic diversity.

3. Result and Discussion

Analysis of variance revealed highly significant differences (p<0.001) among the landraces for the most of the studied traits except for the number of seeds per pod at both locations (Table 1). This indicates that there was adequate genetic variability among the genotypes for most of the traits at both locations. However, differences among the genotypes determined over pooled data were mostly non-significant. Similarly, previous studies on chickpea landraces indicated significant variations for most of the traits like plant height, days to flowering, days to maturity, number of pods per plant, hundred seed weight and grain yield (Tesfamickael et al., 2014; Uday et al., 2012).

| Table 1. Mean squares, signification | ance and CV% of morpho-ag | ronomic characters of chickpea landraces |
|--------------------------------------|------------------------------|--|
| rubic 1. Mean Squares, Significa | ance and GV /0 of morpho agi | i ononne enaracters or emerged fandraces |

| Turita | | Mean square (CV %) | | | | | | | |
|--------|-------------------------|----------------------------|-----------------|--|--|--|--|--|--|
| Traits | Sirinka | Jari | Combined | | | | | | |
| DF | 24.25**(6.56) | 25.83**(5.04) | 4.24 ns(8.2) | | | | | | |
| DP | 31.23**(6.46) | 15.06**(4.89) | 9.73 ns(4.98) | | | | | | |
| PFP | 18.70**(6.07) | 34.74**(5.02) | 5.99 ns (6.23) | | | | | | |
| DM | 42.32**(4.28) | 22.08**(5.68) | 5.39 ns(4.52) | | | | | | |
| CW | 38.83**(8.15) | 132.25**(11.5) | 14.03*(22.31) | | | | | | |
| NLtL | 0.87**(5.97) | 3.08*(15.78) | 0.79*(16.23) | | | | | | |
| РН | 31.12**(9.85) | 86.37**(17.60) | 8.61**(20.13) | | | | | | |
| РВ | 0.72**(19.65) | 0.05 ^{ns} (21.5) | 0.1(28.9) | | | | | | |
| SB | 11.11**(17.16) | 0.92 ^{ns} (20.42) | 1.88*(25.61) | | | | | | |
| NPP | 520.46**(21.02) | 167.32*(28.6) | 50.90**(30.2) | | | | | | |
| NSP | 0.08 ns (12.06) | 0.02 ^{ns} (15.23) | 0.029(11.6) | | | | | | |
| PL | 0.24**(26.5) | 0.85*(29.42) | 0.25*(31.2) | | | | | | |
| HSW | 8.34**(13.23) | 5.27**(12.52) | 0.43(8.96) | | | | | | |
| GYKH | 441140.22**(28.66) | 193086.47**(24.56) | 23558.56(30.2) | | | | | | |
| ВМКН | 1936174.5**(25.97) | 734469.2*(29.62) | 123242.6(32.21) | | | | | | |
| HI | 240.00**(21.48) | 0.55(18.96) | 1.42(28.9) | | | | | | |

DF = Days to flowering, DP = Days to pod setting, PFP = Pod filling period, DM = Days to maturity, CW = Canopy width, NLtL = Number of leaflets per leaf, PH = Plant height, PB = Primary branches, SB = Secondary branches, NPP = Number of pods per plant, NSP = Number of seeds per pod, PL = Pod length, HSW = Hundred seed weight, GYKH = Grain yield kilo gram per hectare, BM = Biomass kilo gram per hectare, HI = Harvest index,

*= significant at 5% probability level, ** = significant at 1% probability level

Clustering of landraces was performed using 16 traits that were significantly different among the accessions. Clustering was done based on average linkage method using SAS software. The pseudo F- statistic and the pseudo t2 statistic were examined to decide the number of clusters. This suggested that about 5 clusters would be an appropriate classification for the 204 chickpea genotypes. The accessions were grouped into five diversity classes (Table 2), different members within a cluster being assumed to be more closely related with each other than those members in the other clusters in terms of the traits under consideration. Similarly, accessions in clusters with non-significant distance were assumed to have more close relationships with each other than they are with those in significantly distant clusters. The cluster grouping is given on the dendrogram is depicted in Figure 1.

Cluster I was the largest with 96 accessions or constituting closer to 47% of the total population, followed by cluster II with 68 accessions or 33% of the total population. These two clusters (I and II) comprised accessions with low yielding potential, larger seed size and early maturity. Cluster III constituted 36 (17%) accessions while cluster IV and cluster V had 3 and 1 accessions; respectively. Cluster V consisted of solitary genotype, entry 118 (Acc. IE-16- 118). Accession IE-16-118 had the unique nature of having the highest values of secondary branches, grain yield and biomass yield than all accessions in the other clusters (Table 2).

The frequency of accessions collected from the different zones in a specific cluster is given in Table 3. This was done to see if accessions from one region group into the same cluster. The result showed that, even though the clusters contained accessions from different regions, accessions from three zones, namely North Gonder, North Shewa and East Shewa, were highly represented in clusters I, II, and III with a total number of (10, 27 and 21), (18, 10 and 4) and (26, 7 and 3) accessions; respectively (Table 3).

The pair wise generalized square distance (D2) among the five clusters is presented in Table 4. The maximum distance was found between clusters IV and I (D2 = 82.38). The second most divergent clusters were cluster V and I (D2 = 67.18), and the third divergent clusters were cluster V and IV (D2 = 53.94). The genetic divergence between clusters V and III (D2 = 37.91), V and II (D2 = 42.02), IV and II (D2 = 41.39), III and I (D2 = 32.39) were also highly significant (p<0.001). According to Chandel

and Joshi (1983), populations from areas distantly separated geographically and having complex environments is expected to accumulate large genetic diversity. In this study, some accessions from the same region were scattered over different clusters, indicating that genetic diversity in chickpea is not uniformly distributed over the regions. Accessions from North Gonder and West Shewa were distributed in four clusters out of five with irregular pattern. The distances between accessions from North Gonder (IV and I, IV and II, and III and I) from West Shewa (V and I, V and II, V and III, and III and I) were highly significant. This implies that crosses between parents selected from these pairs of clusters are expected to result in good level of genetic recombination and generate desirable segregants with broad genetic base.

Table 2. Distribution of accessions of the zones into 5 clusters

| Zones | | | Clusters | | |
|--------------------|----|----|----------|----|---|
| | Ι | II | III | IV | V |
| East Gojam | 1 | 2 | 0 | 0 | 0 |
| East Shewa | 26 | 7 | 3 | 0 | 0 |
| Gurage | 8 | 11 | 4 | 0 | 0 |
| North Gonder | 10 | 27 | 21 | 3 | 0 |
| North Shewa | 18 | 10 | 4 | 0 | 0 |
| South West Shewa | 9 | 7 | 1 | 0 | 0 |
| West Shewa | 24 | 4 | 1 | 0 | 1 |
| Fetenech (check 1) | 0 | 0 | 1 | 0 | 0 |
| Minjar (check 2) | 0 | 0 | 1 | 0 | 0 |
| Total | 96 | 68 | 36 | 3 | 1 |

The minimum and maximum distances between pairs of clusters shown in Table 3 are 8.44 (between clusters I and II) and 82.38 (between clusters I and IV). Maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances between them. There was non-significant distance between cluster I and II (D2 = 8.44) and cluster II and III (D2 = 8.77), suggesting close relationship among the accessions.

Generally, clustering has exposed similarities of accessions collected from adjacent zones and dissimilarities of accessions collected from regions

geographically located apart. This may indicate that more frequent exchange of chickpea genotypes between adjacent zones than between regions very far from. However, some accessions did not follow that pattern, which may witness the fact that genotypes could be shared among regions very far apart. For example, three genotypes from East Shewa were grouped in cluster III with accessions from North Gonder. It is also worthy to note that cluster II contained 39.7% of the accessions from North Gonder were classified with accessions from Gurage zone which is far from North Gonder. The North Gonder accessions were also scattered in four clusters; I, II, III, and IV.

| Table 3. Genetic distance matrix for 5 clusters formed by 204 chickpea genotypes | |
|---|--|
| | |

| Clusters | Ι | II | III | IV | V |
|----------|---|---------|------------|-------------|-------------|
| Ι | 0 | 8.44499 | 32.93084** | 82.37948*** | 67.17946*** |
| II | | 0 | 8.77427 | 41.39001*** | 42.02385*** |
| III | | | 0 | 15.24779 | 37.91786*** |
| IV | | | | 0 | 53.94062*** |
| V | | | | | 0 |

The same is true for the few accessions representing zones such as West Shewa, North Shewa and Gurage. This might witness the fact that some genotypes are scattered overlarge ecological zones, which could be due to exchange of genetic material over larger distances.

Generally, the clustering did not show clear pattern of grouping based on geography or physical distances.

Cluster means are presented in Table 5. The solitary cluster V consisting of entry 118 (accession IE-16- 118) had the highest plant height, secondary branches, grain yield and biomass. Cluster IV included genotypes that are early flowering (40 days), early pod setting (47) and early maturing (83 days).Members of clusters I, and IV were dwarf genotypes with plant height of 35 and 36 cm.

Clusters II, III, and IV had intermediate values for almost all traits, although genotypes of cluster V gave the highest grain yield of 2794kg per hectare. The lowest grain yield was obtained from members of cluster I with 1192kg per hectare.

Crossing of best accessions from distant clusters is expected to produce transgressive segregants, which exceed both parents (Gemechu et al., 2003). Hence, maximum variation in the subsequent generations is expected from crosses that involve parents from the clusters characterized by maximum inter-class distances. Therefore, pedigree selection in segregating generations of these crosses seems to give promising results. For example, crossing genotype 118 of cluster V with genotype such as 19, 24, 26, 27, 55, 109, 158, and 181 of cluster III is expected to produce interesting segregants. Crossing entry 60 in cluster IV with those in cluster II (entry 1 and 35) is also expected to give high yielding crosses. Due to the disproportional representations of chickpea accessions from the three altitudinal regions (96, 68, and 34 accessions from altitudes A, Band C, respectively), the composition of the five clusters is not as distinct as it was for the zones (Table 6). Clusters I, II and III had most of the genotypes from altitude B and C.

Altitude A constituted small number of genotypes from clusters I, II, and III. The rest clusters grouped in Altitude B (Table 4).

Table 4. Distribution of the chickpea accessions from the3 altitudinal classes into 5 clusters.

| Altitude | | | Cluster | ſS | |
|----------|----|----|---------|----|---|
| Annuae | Ι | II | III | IV | V |
| А | 9 | 13 | 4 | 0 | 0 |
| В | 44 | 45 | 23 | 3 | 1 |
| С | 43 | 10 | 7 | 0 | 0 |
| Total | 96 | 68 | 34 | 3 | 1 |

Altitude A= less than 1801 masl, Altitude B= between 1801 and 2200 masl, Altitude C = greater than 2200 masl

Qualitative traits are less influenced by environmental factors unlike quantitative traits (Singh et al., 2008). Qualitative morphological traits of chickpea that were considered in this study include growth habit, stem color, seed coat color, seed shape, seed testa texture, pod dehiscence, flower color, and leaf type. From the traits studied, only growth habit, stem color, and seed coat color showed wide differences among genotypes while the other traits like pod dehiscence, flower color, seed shape, seed testa texture and leaf type were monomorphic. Five types of growth habit were observed; namely erect, semi erect, semi- spreading, spreading and prostrate. Semi spreading (46%) was the most predominant among the genotypes, followed by spreading (35.8%) and prostrate (11.3%), while the semi - erect and erect types were the least frequent (5.9%) and (1%), respectively (Figure 2).

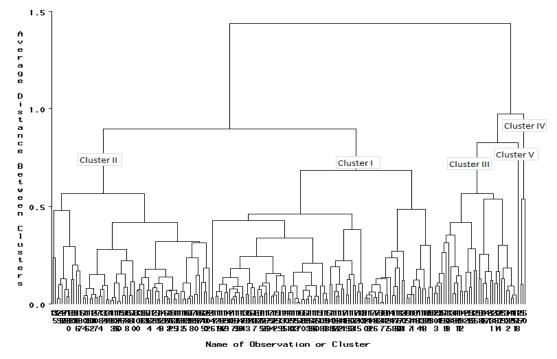


Figure 1. The dendrogram illustrating the distribution of 204 desi type chickpea genotypes into five clusters

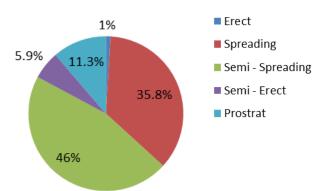


Figure 2. Growth habit frequencies of 204 chickpea genotypes.

Genotypes varied for stem color and showed four types of color categories. The majority of genotypes had predominantly purple stem color (48.0%) followed by partly purple (35.3%) and highly purple (11.8%), while the minimum frequency was observed for the dark green type (4.9%) (Figure 3).

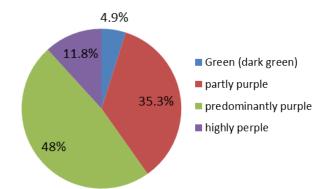


Figure 3. Stem color frequency of 204 genotypes

The studied genotypes showed substantial differences for seed coat color, which had 10 different categories. The maximum frequency was recorded for light brown (59.8%) followed by brown (26.0%), green light yellow (9.8.0%), while light brown with black, brown with black, yellow with black and green light yellow with black occurred with the least frequency (4.4%) (Figure 4).

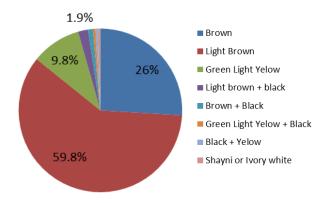


Figure 4. Seed coat color frequency of 204 genotypes

In addition to the morphological traits, the responses of the accessions to the diseases root rot and Fusarium wilt, and to the insect pest pod borer were scored. The accession varied significantly for their response to these biotic factors. For root rot, 184 of the 204 genotypes (90.1%) scored 1 or resistant, two genotypes (1%) scored 3 or moderately resistant, three genotypes (1.5%) scored 5 or average reaction, 13 genotypes (6.4%) scored 7 or moderately susceptible, and two genotypes (1%) scored 9 or highly susceptible (Figure 4).

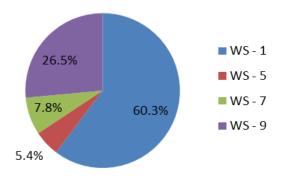


Figure 5. Fusarium wilt disease response of 204 chickpea genotypes (WS= Wilt Score)

Fusarium wilt was another disease scored in this study. It is a soil borne fungal disease, which has been reported to cause yield losses ranging between 10 and 15% worldwide, and is the most important disease affecting chickpea yield in Ethiopia; causing up to 100% crop loss in extreme cases (Tebkew and Chris, 2016). According to the observation, 123 genotypes (60.3%) scored 1 or resistant, 11 genotypes (5.4%) scored 5 or average reaction, 16 genotypes (7.8%) scored 7 or moderately susceptible and 54 genotypes (26.5%) scored 9 or highly susceptible.

Pod borer is a noxious pest of chickpea causing heavy damage to the crop. It damages almost all the pods in case of severe damage, but causes nearly 20-30% annual yield losses in Ethiopia (ICRISAT, 2012). This study also assessed the response of the genotypes to this noxious pest.

The result showed that 61 genotypes (29.9%) scored 1 or resistant, 18 genotypes (8.8%) scored 3 or moderately resistant, 66 genotypes (32.4%) scored 5 or average reaction, 21 genotypes (10.3%) scored 7 or moderately susceptible, and 38 genotypes (18.6%) scored 9 or highly susceptible to this pest.

3.1. Estimates of Diversity for Each Zones Depending on Qualitative Traits

The Shannon-Weaver diversity index was calculated to compare phenotypic diversity index (H) among characters and groups. Table 5 shows the estimates of Shannon-Weaver diversity index for 8 discrete characters by zones.

Black Sea Journal of Agriculture

This index was previously used to determine the range of variation in several crop species including wheat (Jain et al., 1975; Getachewet al., 1997), barley (Fassilet al., 2001) and finger millet (Dagnachewet al., 2012).

| Zones | GH | SC | SCC | SSh | STT | RRR | FWR | PB | Mean (H ± SE) |
|------------------|------|------|------|------|------|------|------|------|-----------------|
| East Gojam | 0.64 | 0.64 | 1.1 | 0 | 0 | 0 | 1.1 | 1.1 | 0.57 ± 0.07 |
| East Shewa | 1.14 | 0.92 | 0.46 | 0 | 0 | 0.52 | 0.72 | 1.34 | 0.64 ± 0.06 |
| Gurage | 1.36 | 1.15 | 1.02 | 0 | 0 | 0.46 | 1.05 | 1.45 | 0.81 ± 0.08 |
| North Gonder | 1.14 | 0.85 | 1 | 0.08 | 0.08 | 0.2 | 0.92 | 1.47 | 0.72 ± 0.07 |
| North Shewa | 0.94 | 0.79 | 1.04 | 0 | 0 | 0.38 | 0.93 | 1.48 | 0.70 ± 0.05 |
| South West Shewa | 0.47 | 0.99 | 4.42 | 0 | 0 | 0.6 | 4.02 | 5.72 | 2.03 ± 0.05 |
| West Shewa | 1.06 | 1.13 | 0.76 | 0 | 0 | 0.46 | 1.06 | 1.34 | 0.73 ± 0.04 |
| Over all | 0.96 | 0.92 | 1.40 | 0.01 | 0.01 | 0.37 | 1.40 | 1.99 | 0.88 ± 0.06 |

Table 5. Estimates of the Shannon-Weaver diversity index (H), for eight qualitative traits and 7 zones

GH= growth habit, SC= stem color, SCC= seed coat color, SSh= seed shape, STT= seed testa texture, RRR= root roat response, FWR= Fusarium wilt response, PB= Pod Borer and SE= standard error of the mean.

Over all, all characters revealed diversity ranging from 0.37 for root rot to 1.99 for pod borer response. Monomorphism (H< 0.10) was observed in all accession collected from all Zones for the traits seed shape and testa texture (Table 5). The highest diversity indices pooled over characters within zones were recorded for accessions from South West Shewa (H= 2.03 ± 0.05) followed by Gurage (H= 0.81 ± 0.08), West Shewa (H= 0.73 ± 0.04), North Gonder (H= 0.72 ± 0.05), and North Shewa(H= 0.70 ± 0.05) whereas genotypes from East Gojam showed relatively lower diversity estimates of (H= 0.57 ± 0.07)

3.2. Cluster Analysis Based on Qualitative Traits

The 204 chickpea genotypes were clustered into five distinct groups based on eight qualitative traits. The traits used for clustering were growth habit, stem color, seed coat color, seed shape, seed testa texture, root rot response, Fusarium wilt response, and pod borer response. The number of genotypes belonging to each cluster varied from one in cluster V to 111 in cluster I.

The largest cluster (Cluster I) included chickpea genotypes with all types of growth habits except erect type, and all stem color types except light green. The genotypes in this cluster have angular seed shape with rough texture. Almost all genotypes (99%) in this cluster are totally free from Fusarium wilt and root rot diseases. Compared to others clusters, the genotypes in cluster I had better response to pod bore; 43.2% of the genotypes in this cluster being free this pest.

Cluster II comprised 77 genotypes (37.8%) and have four types of growth habit namely; semi- spreading, spreading, prostrate and semi-erect. The genotypes in this cluster have predominantly purple stem color (52% of the genotypes), followed by partly purple stem color (29.9%). Most of the genotypes in cluster II have light brown seed coat color (64.9%), and 20.8%, 10.4%, 3.9% and 1.3% of the genotypes have brown, green light yellow, brown with black, and yellow with black seed coat color, respectively. All genotypes in this cluster have angular seed shape, except one genotype, with rough testa texture. Genotypes in this cluster are the most susceptible to Fusarium wilt

(63.6% highly susceptible, 18.2% susceptible and 18.9% average reaction); while 96.1% and 15.6% of the genotypes are free from root rot and pod borer, respectively.

Cluster III consisted of 10 genotypes (4.9%) with four types of growth habits and stem color. Their seed coat color is light brown, brown and green light yellow with angular shape and rough texture. 50% and 40% of the genotypes in cluster III have brown and light brown seed color, respectively. All genotypes in this cluster were from Fusarium wilt, but were highly susceptible to root rot (80% susceptible and 10% highly susceptible).

Cluster IV consisted of five genotypes (2.5% of all genotypes) that have semi spreading, spreading and prostrate types of growth habits. Most of the genotypes have predominantly purple stem color (80%) and light brown seed coat color (60%). All genotypes were susceptible to root rot and Fusarium wilt with average reaction to pod borer.

Cluster V is the solitary group, it consisted only one genotype. This genotype has semi spreading growth habit and highly purple stem color. Its seed shape and testa texture is angular and rough, respectively. Diseases and insect pest response of this genotype was relatively the same as the genotypes in cluster IV.

In both quantitative and qualitative cluster analysis the accessions were grouped into five clusters with different number of accessions, the highest number of accessions grouped in cluster I and there is solitary cluster, cluster five. The major difference is that in the quantitative cluster analysis, cluster I comprised the lowest yielding accessions relative to the other clusters, while in the case of qualitative cluster analysis, 75% of the twelve highest yielding accessions were grouped in cluster I.

In the quantitative cluster, the solitary cluster V consisted entry 118 (accession IE-16- 118), which had the tallest plant height, the highest number of secondary branches, highest grain yield and biomass, and relatively late maturity than the other clusters. However, entry 165 (accessions DLRWAA005), which had semi spreading, highly purple, and a unique seed coat color of brown with black, and susceptible to Fusarium wilt and root rot diseases, was included in the qualitative solitary cluster V.

3.3. Altitudinal Trait Distribution

Table 8 summarizes the phenotypic frequencies of individual traits for altitudinal classes as percentages of the number of genotypes from each altitude class. In this study, altitudes were classified into three ranges; altitude A = <1801, B = 1801 - 2200, and C = >2200masl. All types of growth habit sub classes were observed fully in altitude class A (< 1801masl). Especially, accessions with erect growth habit were observed only in this altitudinal range. In addition to this, the three seed coat colors were also appeared proportionally in this altitude class. Moreover, the highest frequency of green (dark green) stem color was observed in this altitude class (<1801masl).

Genotypes with semi-spreading and spreading growth habit and light brown seed coat color were highly frequent in altitude class B (1801 to2200masl). The highest frequency of partly purple stem color and semi spreading type of chickpea were altitude class B.

Except spreading growth habit and predominantly purple stem color, most of the traits were less frequent altitude class C (>2200masl).In addition to this, the frequency of semi-erect and prostrate growth habits decreased at the higher altitudes. Genotypes from the higher altitudes were more susceptible to Fusarium wilt, but less susceptible to pod borer. The highest number of Fusarium wilt resistant genotypes were from the altitudinal class B (74 genotypes) followed by altitude class C (31 genotypes) and (14genotypes) in lower elevation (altitude class C).

3.4. Diversity Index for Altitudinal Class

The estimates of diversity index (H) for each trait at the three altitudinal classes is shown in Table 6. Polymorphism was common in varying degrees for most traits, implying the existence of variation in the accessions. The H value ranged from monomorphic for seed shape and seed testa texture to highly polymorphic for growth habit. The diversity indices for seed shape and seed testa texture were nearly the same in all altitude classes. Pod borer response was also very high in all classes. The growth habit traits, stem color, seed coat color, and pod borer response were observed most frequently in altitude class I. Compared with zonal estimates, H values pooled over all traits for altitudinal classes showed higher variation. Altitude classes A (<1801masl) followed B (1801 - 2200masl) and C (>2200masl) showed relatively from high to low diversity indices.

Table 6. Estimates Shannon-Weaver diversity index (H) for eight qualitative traits and three altituide classes

| Altitude | GH | SC | SCC | SSh | STT | RRR | FWR | PBR | Mean (H ± SE) |
|----------|------|------|------|------|------|------|------|------|-----------------|
| А | 2.6 | 0.98 | 1.07 | 0.0 | 0.0 | 0.43 | 0.94 | 1.57 | 0.95 ± 0.23 |
| В | 1.15 | 1.01 | 0.86 | 0.05 | 0.05 | 0.45 | 0.70 | 1.29 | 0.71 ± 0.17 |
| С | 1.0 | 0.97 | 0.90 | 0.0 | 0.0 | 0.29 | 0.93 | 1.38 | 0.68 ± 0.18 |

Altitude A= < 1801, B= 1801 – 2200, C= >2201, GH= growth habit, SC= stem color, SCC= seed coat color, SSh= seed shape, STT= seed testa texture, RRR= root rot response, FWR= Fusarium wilt response, PBR= Pod borer response and SE= standard error of the mean.

4. Conclusion

The chickpea accessions clustered into five distinct groups of different sizes based on 16 quantitative traits. The clustering pattern indicated that the number of genotypes in each cluster varied from 1 in cluster V and 96 in cluster I. The maximum distance was found between clusters IV and I (D2 = 82.38). The second most divergent clusters were cluster V and I (D2 = 67.18), and the third divergent clusters were cluster V and IV (D2 = 53.94). Maximum variation in the subsequent generations is expected from crosses that involve parents from the clusters characterized by maximum inter-class distances. The accessions varied also for qualitative traits. The proportion of accessions with the semi - spreading, spreading, prostrate, semi - erect and erect growth habits were 47%, 33.2%, 12.4%, 6.4% and 1% respectively. Most of the genotypes had light brown seed coat color (58.9%) followed by brown (26.2%) and green light yellow (9.9%). There were other seed coat colors that are not described in chickpea descriptor. The proportion of genotypes with Fusarium wilt response were (59.3%), (26%) and (7.4) for resistance, highly susceptible, and susceptible, respectively.

The highest diversity indices pooled over characters within zones were recorded for accessions from South West Shewa (H= 2.03 ± 0.05) followed by Gurage (H= 0.81 ± 0.08), West Shewa (H= 0.73 ± 0.04) and North Gonder (H= 0.72 ± 0.05). These zones can be used for in situ conservation of chickpea landraces as representatives of chickpea producing areas of Ethiopia.

Altitudinal trait distribution indicated that all types of growth habit sub classes were observed fully in the lower elevation, less than 1801masl. Especially, accessions with erect growth habit were observed only in this group. In addition, the different seed coat color classes were also appeared proportionally in this altitude class. According to this study, genotypes from the higher altitudes were more susceptible to Fusarium wilt, but less susceptible to African boll worm. In addition, the frequency of semierect and prostrate accessions decreased at the higher altitudes.

The diversity index (H) ranged from monomorphic for seed shape and seed testa texture to highly polymorphic for growth habit. The diversity indices for seed shape and seed testa texture were nearly the same in all altitude classes. The altitude class with the highest diversity index was below 1801masl (Altitude A).

There is high genetic diversity in the Ethiopian chickpea landraces even though it was not uniformly distributed across the regions. The existence of wider agromorphological diversity among the chickpea collections implies the potential to improve the crop and the need to conserve the diversity. Accessions from different regions of origin might be closely related regardless of their geographic origin and accessions from the same regions of origin also might have different genetic background. There was no definite correspondence between geographic origin and genetic diversity. Parental selection should, therefore, not be based on geographic difference but it should rather be made based on systematic assessment of genetic distance in a specific population.

Future collecting operations of chickpea germplasm should strategically focus on areas with relatively large variation. From genetic conservation point of view, it appears that South West Shewa, Gurage, West Shewa and North Gonder, with appropriate altitudinal focus, could be suitable as in situ conservation sites.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

First of all, the authors deepest gratitude and acknowledge goes to Amhaera Agricultural Research Institute and/or Sirinka Agricultural Research Center for providing research budget and facilitate the process. We would like also to express sincere thanks to Sirinka Agricultural Research Center pulse case team members for contributing their great effort this successful accomplishment of the experiment.

References

- Assefa A. 2003. Genetic variability and breeding potential of barley (*Hordeum vulgare L.*) Landraces from North Shewa in Ethiopia. PhD Thesis, Faculty of Natural and Agricultural Sciences University of Free State, Bloemfontein, South Africa. 226 pp.
- Belay G, Bechere E, Mitiku D, Merker A, Tsegaye S. 1997. Patterns of morphologigal diversity in tetraploid wheat (*Triticum turgidum L.*) landraces from Ethiopia. Acta Agric Scand, Sect B Soil Plant Sci, 47: 221-228.
- Brush SB. 2000. The issue of in situ conservation of crop genetic resources. Genes in the field on farm conservation of crop diversity. IBBNO-88936-884-8 International Development Research Center.
- Chandel KP, Joshi BS. 1983. Multivariate analysis in green seeded pea. Indian J Agric Sci, 53(4): 198 200.
- CSA (Central Statistical Authority). 2015. Agricultural Sample Survey, Volume I: Report on Area and Production for major crops (*Meher Season*) Statistical Bulletin No. 578 Addis Ababa, Ethiopia.
- Durga Prasad MMK, Arunachachalam V, Bandy Opadyay A. 1985. Diversity pattern elucidating parents for hybridization in varieties of groundnut. Arachis hypogaea L. Trop Agric, 62:

237-242

- Edwards SB. 1991. Crops with wild relatives found in Ethiopia. In: Engels J.M.M., Hawkes J.G and Worede Melaku (Eds.), Plant genetic resources of Ethiopia. Cambridge University Press pp. 42-74.
- Gebrekidane B. 1982. Utilization of sorghum germplasm in sorghum improvement: Sorghum in the eighties. p. 335-345. In Proc. of the international symposium on sorghum, Pantanchiru, A.P. India. ICRISAT.
- Hutchenson K. 1970. A test for comparing diversities based on the Shannon formula. J Theoret Boil, 29: 151-154.
- IBPGR, ICRISAT and ICARDA. 1993. Descriptor for chickpea (*Cicer arietinum L.*). International Board for Plant Genetic Resources, Italy; International Crop Research Institute for the Semi-Arid Tropics, Patancheru, India and International Center for Agricultural Research in Dry Areas, Aleppo, Syria.
- Jain SK, Qualset CO, Bhatt GM, Wu KK. 1975. Geographical pattern of phenotypic diversity in a world collection of durum wheats. Crop Sci, 15: 700-704.
- Joshi AB, Dhawan NL. 1966. Genetic improvement in yield with special reference to self fertilizing crops. Indian J Genet, 26: 101-113.
- Katule BK, Thombare MV, Dumbre AD, Pawar BB. 1992. Genetic diversity in bunch groundnut. J Maharashtra Agric Univer, 17: 302-303.
- Kebebew F, Tsehaye Y, McNeilly T. 2001. Morphological and farmers cognitive diversity of barley (*Hordeum vulgare L. [Poaceae]*) at Bale and North Shewa of Ethiopia. Genetic Res Crop Evol, 48: 1-10.
- Lule D, Tesfaye K, Fetene M. 2012. Qualitative traits diversity and eco-geographical distribution in finger millet (Eleusine coracana GURE subsp. Coracana) landraces from eastern and southeastern Africa: An implication for germplasm collection and conservation. African J Plant Sci, 6(13): 346-354.
- Mekibeb H, Demissie A, Tullu A. 1991. Pulse crops of Ethiopia. In: Engels JMM, Hawkes JG, Worede M (eds), Plant genetic resources of Ethiopia, Cambridge University Press, pp. 328-343.
- Muehlbauer FJ, Singh KB. 1987. Genetics of chickpea, In: Saxena M. C. and Singh K.B. (eds.): the chickpea. CAB International. p. 99/125.
- Mullu ST, Mwangi SG, Nyende AB, Rao NVPRG, Odeny DA, Rathore A, Kumars A. 2014. Assessment of genetic variation and heritability of agronomic traits in chickpea (*Cicer arietinum L*). Inter J Agron Agri Res, 5(4): 76-88.
- Nadaf HL, Habia AF, Groud JV. 1986. Analysis of diversity in bunch groundnut. J Oilseeds Res, 3: 37-45
- SAS Institute 2004 SAS/STAT guide for personal computers, version 9.0 edition, SAS Institute Inc. Cary, NC.
- Singh BD, 2001. Plant Breeding: Principles and methods. Kalyani Publishers, New Delhi. pp. 896.
- Singh RK, Chaudhary BD. 1977. Biometrical methods in quantitative genetic analysis. Kalyani Publisher, New Delhi, Ludhiana, India, pp.300.
- Uday Chand Jha, Singh DP, Lavanya R. 2012. Assessment of genetic variability and correlation of important yield related traits in chickpea (*Cicer arietinum L.*). Legume Res, 35 (4): 341-344.
- Van der Maesen LJG. 1987. Origin, history and taxonomy of chickpea. In: Saxena MC, Singh KB (eds) The Chickpea. Wallingford: C.A.B International, pp. 11-34.
- Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, et al. 2013. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat Biotechnol, 31:

240-246.

Worede M, Tesemma T, Feyissa R. 2000. Keeping diversity alive: an Ethiopian perspective. In Stephen B.Brush. (eds.) Genes in the field: on farm conservation of crop diversity. IBBNO-88936-884-8. International Development Research Center.

Worede M. 1993. The role of Ethiopian farmers in the

conservation and Utilization of crop genetic resources.In: International crop science society of America, D.R. Buxton et al (eds.). Madison, WI: Crop Sci Soc America.

Zeven AC. 1998. Landraces: A review of definitions and classifications. Euphytica, 104(2): 127-139.