Phenotypic variability of chickpea (*Cicer arietinum* L) germplasm with temporally varied collection from the Amhara Regional State, Ethiopia

Sintayehu Admas, Kassahun Tesfaye, Teklehaimanot Haileselassie, Eleni Shiferaw and K. Colton Flynn

*Cogent Food & Agriculture* (2021), 7: 1896117
Phenotypic variability of chickpea (*Cicer arietinum* L) germplasm with temporally varied collection from the Amhara Regional State, Ethiopia

Sintayehu Admas¹,²*, Kassahun Tesfaye², Teklehaimanot Haileselassie², Eleni Shiferaw¹ and K. Colton Flynn³

**Abstract:** Information on the diversity changes occurring in farmers’ field overtime is very important for effective genetic resource conservation and use. Thus, this study was initiated to investigate the phenotypic diversity changes between the current (2017) and previous chickpea (*Cicer arietinum* L) collections (1979–1983) of the Amhara Regional State, Ethiopia. An experiment was conducted using simple lattice design with two replications at Debra Zeit Agricultural Research Center for two consecutive years (2018/2019 to 2019/2020). Genetic erosion of 30.4% to 100% was recorded in chickpea for the past 35 years in the study areas. Shannon-Weaver diversity index estimates of black seeded and ivory white seeded chickpea types decreased from 1.99 to 0.69 and 1.33 to 0.0 in the past 35 years, respectively. In previous collections, 25.8% of black and 6.5% of white–coated genotypes were observed, while these chickpea types were rare (black 6.5%) or unobtainable (white 0.0%) in current collections. There was a significant difference (*p* < 0.05) between

© 2021 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.
mean of current and previous collections for plant height, days to 50% flowering and podding, days to 90% maturity, number of primary and secondary branches, number of pods per plant, thousand Seed weight, and grain yield tested at the individual site. Cluster analysis showed that genotypes were clustered with respect to the time of collections and irrespective of their source of origins. Generally, chickpea genotypes of current and previous collections were distinct from one another. Black and white seeded chickpea landraces were vulnerable to genetic erosion and it is recommended to implement immediate restoration of chickpea landraces to recover and maintain the lost chickpea landraces in the studied region.

**Subjects:** Genetics; Molecular Biology; Biotechnology

**Keywords:** Chickpea; chickpea landrace; genetic erosion; landraces; phenotypic diversity

1. Introduction

Exploration and identification of plant genetic resources' hot spot areas across the world have led to the identification of the twelve Vavilovian centers of origin/diversity for several domesticated crops and their wild and weedy relatives (Vavilov, 1951). In order to safeguard food and nutrition security for both the present and the future, genetic resources are conserved in more than 1,700 gene banks established around the world (Singh et al., 2012), with the Ethiopian Biodiversity Institute (EBI) representing one of the most important gene banks for sub-Saharan Africa. These gene banks, including EBI, conserve more than 7.5 million accessions of various plant species collected from different parts of the world in ex-situ gene banks (FAO, 2010). The plant genetic resources have been promoting human health for centuries by delivering food, fiber, medicine, shelter, etc. In addition, conserved plant genetic resources have the ability to contain genes that confer resistance to disease, insect pests, and improved agronomic traits which can give an opportunity to plant breeders for a variety of crop improvement programs.

Ethiopia posses diverse soil characteristics, climatic conditions, and cultures which provides a suitable region to develop a rich genetic resource variation within crop species (Worede, 1992). Because of this, Ethiopia is considered as one of the major Vavilovian centers of origin/diversity for several domesticated crops and their wild and weedy relatives (Vavilov, 1951). Chickpea (*Cicer aritienum* L) is among the crops, where Ethiopia is proposed as the secondary center of diversity (Van Der Maesen, 1987). To date, 1,223 chickpea accessions have been conserved in the EBI gene bank, of which 69% were collected from 1977 to 1991 and 14% were collected from 1992 to 2015. The remaining 17% of genotypes lack information for collection years. Regular chickpea diversity monitoring for Ethiopian agriculture is equally important to indicate the detrimental evolutionary patterns, set conservation priorities and assesses the impact of conservation policies (Barry et al., 2008). Though the genetic diversity created in the farmers’ fields over millennia provides the raw material for crop variety improvement (Upadhyaya et al., 2008), the conservation strategies that have been employed to date are not sufficient resulting in a significant loss of plant genetic resources among major crop landraces (i.e. Genetic erosion) (Van De Wouw et al., 2009).

Mazhar (1997) estimated that three-fourths of the genetic diversity of major food crops have been exposed to genetic erosion with an annual rate of reduction by one or 2%. In Ethiopia, Tsegaye and Berg (2007) estimated a 77% and 95% loss of tetraploid wheat landraces diversity in Ejeri and Akaki districts in Ethiopia, respectively. Teklu and Hammer (2006) reported reduction in
the use of wheat landraces by farmers in Eastern Ethiopia. Mekbib (2008) also reported that there was a genetic erosion of sorghum germplasm amongst individual farmers in Eastern Ethiopia. Shewayrgra et al. (2008) indicated that some important sorghum landraces have disappeared either locally or regionally in the past 30 years. The major factors that cause genetic erosion in Ethiopia include: displacement of landraces by other crops, introduction and expansion of improved varieties, expansion of economical crops, lack of a mechanism to re-supply seeds of landraces, decline in size of land holdings, changes in land use and cropping patterns, and lack of policy support (Mekbib, 2008; Shewayrgra et al., 2008; Teklu & Hammer, 2006; Tsegaye & Berg, 2007).

A significant amount of changes in genetic diversity has occurred in major crops in Ethiopia. In chickpea, though a considerable effort has been practiced for ex-situ conservation work, information regarding the diversity changes that occur overtime amongst Ethiopian agriculture is minimal. Genetic diversity change can be assessed by comparing the level of diversity occurring at time zero and a subsequent time at species, varietal, agromorphological and molecular diversity level (Barry et al., 2008). Therefore, the objectives of the present study were to (1) investigate the level of phenotypic diversity change within and among the current (2017) and previous (1979 to 1983) chickpea collections and (2) to examine the presence of chickpea landraces loss in the Amhara Regional State, Ethiopia. The finding of this study will help to design the appropriate conservation strategies to conserve and maintain chickpea germplasm for sustainable use.

2. Material and methods

Twenty-seven chickpea accessions from EBI’s collection were selected based on the year of collection from three zones (East Gojam, North and South Gondar) of Amhara Regional state and their subsequent districts representing the major chickpea growing areas of the region. These germplasms were collected from 1979 to 1983 and conserved ex-situ for over 35 years. These germplasms were not subjected to multiplication or regeneration. Another collection program was executed in 2017 in these three zones to recollect chickpea germplasm and 29 chickpea germplasms were collected in the locations that match the previous collection conducted between 1979 and 1983. To ensure the matching of locations, reference to the gene bank passport data containing information on administrative region, district name, altitude, latitude, and longitude of collection sites, including the distance from the nearby town was used. The geographical distribution of the germplasm is indicated in Figure 1. Two improved medium-sized varieties (Mastewal and Worku) of chickpea were also included in the study (see Supplementary Table S1 for further details).

The chickpea accessions of current and previous collections naturally exist as a mixture of different lines. Before determining characterization, each accession was sorted into nearly homogenous genotypes based on seed color, texture, size, and shape. Forty-one homogenous genotypes were developed from the 29 accessions of current collections, while 93 genotypes were developed from 27 accessions of previous collections. These homogenous genotypes (134) were used for qualitative trait analysis (For further details see Supplementary Table S2). Eighty one (32 from current, 47 previous collections and two varieties) selected from 134 homogenous genotypes were used for quantitative traits analyses.

The experiment was conducted at Debre Zeit Agricultural Research Center (DZARC) for two consecutive cropping seasons (2018/19 to 2019/20). The experimental site is located 47 km South East of Addis Ababa (8° 44’N, 38° 58’ E) at an altitude of 1,860 meter above sea level (m. a.s.l) (Figure 1). It receives an annual rainfall ranging from 412.9 to 926.9 mm with an annual mean precipitation of 682.08 mm. The temperature ranges from 11.23°C to 25.19°C with mean annual temperature of 19°C. The dominant soil type is vertisol.

Simple Lattice Design with two replications was used. Each genotype was sown in two rows with 3 m row length and 0.2 m spacing between rows and 0.1 m between plants. DAP (100 Kg ha⁻¹) and
other management practices were applied. Five individual plants were tagged randomly from each genotype per plot and they were used for morphological data collection. Chickpea descriptor (IBPGR, ICRISAT & ICARDA, 1993) was used to score five qualitative (stem/foliage pigmentation, seed shape, seed testa texture, seed color and growth habit) and nine quantitative (plant canopy height, days to 50% flowering, days to 50% podding, days to 90% maturity, number of primary branches, number secondary branches, number of pods per plant, thousand seed weight and grain yield) traits.

2.1. Data analyses
The genetic erosion of chickpea genotypes was estimated using the formula suggested by Hammer and Laghetto (2005). The phenotypic frequencies of the qualitative traits were analyzed by the Shannon-Weaver diversity index (H) to assess the diversity changes of each trait over time at farmers’ fields by zones and region. H was computed using GenStat 16th edition versions 4.2–7.1 14 statistical software (Payne et al., 2013). The performances of genotypes were tested for significance by performing an analysis of variance (ANOVA), using a General Linear Model (GLM) in a simple lattice design, using R statistical package (META-R (Multi Environment Trial Analyses with R for Windows), 2016). Mean separation at 1% or 5% probability level was conducted using Dunkan’s Multiple Range Test (DMRT) following Gomez and Gomez (1984). Mean comparison test between current and previous collections was conducted for each of the quantitative traits for each collection site (eight sites in total) separately using t-tests with 95% confidence interval and cluster analysis was computed using the Wards method of hierarchical clustering technique (Ward, 1963) after standardization by using MINTAB 14 statistical package (MINITAB, 1998).
3. Results

3.1. Qualitative traits diversity analyses
The distribution of seed color classes was assessed in three districts from East Gojjam, four districts from North Gondar and one district from South Gondar. East Gojjam and North Gondar were represented by nearly equal number of genotypes. South Gondar was represented by less number of genotypes (five genotypes for current and six genotypes for previous) and because of this South Gondar was included in North Gondar.

Qualitative trait diversity was assessed on the 134 homogenous genotypes. Result from the score of leaf/stem pigmentation, seed shape and texture, and growth habit, showed that the majority of the genotypes exhibited similar score for the respective traits. One hundred twenty-eight germplasm (95.5%; 41 from current, 87 from previous collections) had a pigmented leaf or stem (score 5), angular seed shape (score 1), rough texture (score 1) and semi-erect growth habit (score 4). Hence, these qualitative traits were uniform for most genotypes and were abundant in both zones in both collection times. Six genotypes (4.5%: 41,324-B, 41,026-A, 41,267-A, 207,170-A, 41,306-B and 41,311-A) showed no leaf/stem pigmentation had white smooth coat and showed pea-shaped seeds (Figure 2). These characteristics were not observed in current collections and occurred at low frequencies in previous collections of the two zones (East Gojjam and North Gondar).

The distribution and frequencies of seed color classes and their genetic erosion by zones over years are indicated in Table 1. A range of 30.4% to 100% genetic erosion in chickpea genotypes was recorded at regional level. Highest genetic erosion was recorded for genotypes with white seed coat (100%) followed by black seed coat (91.7%) from 1979 to 2017. Brown, light brown and dark brown seed color classes were dominant and distributed with more or less similar frequency at regional level. Black and ivory white seed class type occurred in low/nil frequencies in both zones. The pattern in the distribution of black (25.8% to 4.9%) and ivory white seed (6.5% to 0.0%) class type decreased overtime, while the remaining phenotypic classes increased and occurred in more or less similar frequency at regional level overtime.

The Shannon-Weaver diversity index estimates (H) for individual traits, and zones are presented in Table 2. The estimates of H were variable for individual traits and zones for current and previous collections. In East Gojjam zone, the H estimates ranged from 0.64 for ivory white to 1.08 for light brown in previous collection and 0.0 for ivory white to 1.06 for light brown in current collection. In North Gondar zone, the H estimates ranged from 0.64 for ivory white to 1.52 for light brown in previous collection and 0.0 for ivory white to 1.56 for brown in current collection. The estimate of diversity was higher in North Gondar (0.95) than in East Gojjam (0.63).

3.2. Quantitative traits diversity analyses
The group means values of each quantitative trait were tested for the current and previous collections of each individual collection site at 95% confidence interval (Supplementary Table S3 for further details). There was a significant difference between current and previous collections for the mean value of plant canopy height, days to 50% flowering, days to 50% podding, days to 90% maturity, number of primary branches, number of secondary branches, number of pods per plant, thousand seed weight, and grain yield.

The result of ANOVA indicated that there were significant differences among genotypes for plant height, number of primary branches, number of secondary branches, number of pod per plant, days to flowering, days to podding, days to maturity, thousand seed weight, and grain yield at p < 0.01 probability level (Table 3). The effect of genotype by season interaction on the performances of genotypes was significant at p < 0.01 probability level.

Mean result indicated that the differences among the means of most genotypes for given traits were significant (P < 0.05). Wide mean ranges were observed for all quantitative traits in the genotypes (see
Supplementary Table S4 for further details). The range for each measurement were as follows: 27.07 to 42.87 cm for plant height, 2.97 to 10.14 for number of primary branch, 3.14 to 11.09 for number of secondary branch, 25.29 to 79.88 for number of pod per plant, 41.82 to 51.46 for days to flowering, 52.21 to 62.18 for days to podding, 86.29 to 103.87 for days to maturity, 126.58 to 166.66 g for thousand seed weight and 1071.13 to 3264.4 g/ha for grain yield were recorded.

Mean separation test was done among means of quantitative traits for current and previous collections. Three genotypes 30,324-A, 30,319-A, and 30,310-A, all from current collections, produced grain yield above the mean value of the best standard checks (Mastewal, 2641 kg ha⁻¹) and, 22 (15 from current and 7 from previous collections) genotypes, the majorities from current collections, gave comparable grain yield with the best standard check. None of the genotypes matured later than the standard checks. Regarding thousand seed weight since the standard checks are medium-sized chickpea, they showed better thousand seed weight than the other which is desi chickpea type, however among the desi type, the majority of current collections produced better thousand seed weight than the previous chickpea collections. (see Supplementary Table S4 for further details). In all district, most current collected genotypes had shown a significant mean value difference ( p < 0.05) among previous collected genotypes for plant canopy height, days to flowering, days to 50% podding, days to maturity, number of primary branches, number of secondary branches, number of pods per plant, and thousand seed weight and grain yield. This further confirms that the existence of quantitative traits changes over time.

The result of hierarchical cluster analyses indicated that the 81 genotypes were grouped into six clusters (Figure 3) with variable number of accessions per cluster (Table 4). The highest number of genotypes grouped in Cluster IV (24 genotypes) and the least is in cluster VI (2 genotypes). Cluster I and III contained 19 and 9 genotypes respectively, and all of them were from the previous collections. Cluster II and V were composed of 7 and 20 genotypes respectively, and all were from current collections except 207,143-B, 241,801-C, 41,026-B from cluster II and 41,222-B from cluster V which were from previous collections. Cluster IV contained 16 genotypes from previous collections and 8 genotypes from current collections. Cluster VI was entirely consisted of improved genotypes.

4. Discussion
Loss of genetic diversity is a major concern especially for countries like Ethiopia which are considered as crop centers of origin or diversity. Previously, the amount and extent of genetic loss have been assessed for tetraploid wheat and sorghum and a significant amount of genetic erosion was reported in Ethiopia (Mekbib, 2008; Shewayra et al., 2008; Teklu & Hammer, 2006; Tsegaye & Berg, 2007). However, assessment of the amount and the extent of diversity change of chickpea landraces that occur over time have not been estimated. The aim of the present study was to assess the genetic variability of current (collected in 2017) and previous collections (collected from 1979 to 1983) of chickpea germplasm and to assess the existence of genetic loss in selected chickpea growing areas of the Amhara Regional State, Ethiopia.
Table 1. Seed color classes, weighted mean percentage, genetic integrity (GI), and genetic erosion (GE) of chickpea genotypes collections in two zones of the Amhara Regional State, Ethiopia of 1979–1983 and 2017

| Zones       | Seed color types | Number of genotypes collected in | GI   | GE       | Percentage of seed color class in |
|-------------|------------------|----------------------------------|------|----------|----------------------------------|
|             | 1979 to 1983     | 2017                             |      |          | 1979 to 1983 | 2017 |
| East Gojam  | Black            | 11                               | 0.0  | 100.0    | 26.2             | 0.0 |
|             | Brown            | 9                                | 8    | 88.9     | 11.1             | 47.1 |
|             | Light brown      | 12                               | 5    | 41.7     | 58.3             | 28.6 |
|             | Dark             | 7                                | 4    | 57.1     | 42.9             | 16.7 |
|             | Ivory white      | 3                                | 0    | 0.0      | 100.0            | 7.1  |
|             | Total            | 42                               | 17   |          |                   |      |
| North Gondar| Black            | 13                               | 2    | 15.4     | 84.6             | 25.5 |
|             | Brown            | 14                               | 8    | 57.1     | 42.9             | 27.5 |
|             | Light brown      | 12                               | 8    | 66.7     | 33.3             | 23.5 |
|             | Dark             | 9                                | 6    | 66.7     | 33.3             | 17.6 |
|             | Ivory white      | 3                                | 0    | 0.0      | 100.0            | 5.9  |
|             | Total            | 51                               | 24   |          |                   |      |
| Region      | Black            | 24                               | 2    | 8.3      | 91.7             | 25.8 |
|             | Brown            | 23                               | 16   | 69.6     | 30.4             | 24.7 |
|             | Light brown      | 24                               | 13   | 54.2     | 45.8             | 25.8 |
|             | Dark             | 16                               | 10   | 62.5     | 37.5             | 17.2 |
|             | Ivory white      | 6                                | 0    | 0.0      | 100.0            | 6.5  |
|             | Total            | 93                               | 41   |          |                   |      |

Based on seed color characterization, all genotypes did not show variation in stem/leaf pigmentation, seed shape, seed texture, and growth habit in both collection years, except very few genotypes. These traits are not sufficient enough to see the level of diversity within Ethiopian desi-type chickpea because the majority of the collections are uniform for these traits. Vishnyakova et al. (2017) also indicated that Ethiopian collections have a narrow genetic base amongst these qualitative traits, though the Ethiopian collections are unique, primitive type and endemic to Ethiopia.

Genetic erosion has occurred in all chickpea genotypes at different levels. The loss is more alarming for black and ivory white-coated seed genotypes in all study sites. The frequency and Shannon-Weaver diversity index estimates (H) have decreased over time. The current collections are more or less uniform in seed color diversity in comparison to the previous collections. In the recent collections, no entry with black and ivory white seeds were obtained in most studied areas. However, black chickpea seeds are frequently observed in the previous collections. Vavilov (1927) also reported the occurrence of black seeded chickpea type in Ethiopia in 1927. White seed desi-type chickpea existed in previous collection with very rare frequency. Vishnyakova et al. (2017) also reported their observation on the presence of white seeded desi-type chickpea from Ethiopian collections though it was rare. The black and ivory white chickpea seeds are likely nearing extinction because these types of seeds are either absent or have rare occurrence at farmers’ fields in the studied districts.

Loss of black seed chickpea from farmers within the studied areas would have consequences in health benefits that used to be obtained from black seeded chickpea. It is well known that desi-type chickpea with black seed coats had the highest total phenolic, flavonoid, monomeric anthocyanin and proanthocyanidin contents than desi chickpea with brown and green seed coats and...
Kabuli chickpea (Egan & Wood, 2016; Ghosh et al., 2019; Segev et al., 2010). Because of their chemical composition, black chickpea seeds have numerous health benefits which include reduction of cholesterol levels. They are also rich in iron, a powerhouse of phytochemicals which serve as antioxidants potentially preventing cancers, aids in digestion, regularizes blood sugar and multiple other health-related benefits (Abete et al., 2010; Jukanti et al., 2012; Mollard et al., 2012; Nestel et al., 2004; Wallace et al., 2016; Yang et al., 2007).

Though the black seeded chickpea has a paramount importance to human health benefits, it receives less attention among plant breeders in chickpea development programs of Ethiopia and, moreover, there is no improved variety released with black seed coat to date. Additionally, the EBI gene bank has not implemented the restoration of black seeded chickpea genotypes in its original place of collection. It is clear that black seed chickpea faced a major threat of genetic erosion at farmers’ level because of the substitution of a diverse set of genetically variable crop landraces with few genetically uniform improved varieties and partly because of a negative selection. Similar views were also observed during the collection time (in 2017) from traditional medicine healers. Traditional healers claim that the local farmers do not keep black seed chickpea. The local farmers sorted black seeds from chickpea populations to sell it at a higher price to traditional healers. This practice gradually affects the genetic composition of chickpea in the areas resulting in reduction of black seed from time to time which can be considered as a negative selection.

t-tests were used to compare two means having unequal variance and sample size (Armitage et al., 2002). Based on this test, the mean values of each quantitative trait of the current and previous collections were significant for individual sites. It is possible to draw information from this result that the populations mean values for quantitative traits of chickpea germplasm collected at different times are significantly different. This indicates that the current and previous chickpea populations are quite different and the level of diversity between the current collection and previous collection are also different.

Results from ANOVA and mean and range values of the analysed traits revealed the presence of significant difference among genotypes for the considered traits. The results have shown the existence of sufficient variability of agronomic traits among chickpea genotypes. Similar observation was reported in prior studies for chickpea (Archak et al., 2016; Keneni et al., 2013; Parameshwarappa et al., 2012).

Result from LSD showed that from current collections, three genotypes out yield and 22 genotypes gave comparable yield to the best standard check. The majority of current chickpea collections had better thousand seed weight than previous chickpea collections. The mean value of quantitative traits of current chickpea collections for each individual location is non-significant for most locations, while for previous chickpea collections some genotypes showed a significant mean difference in all quantitative traits considered. This indicated that genotypes of current collection had shown similar

---

**Table 2. Shannon diversity index (H) and mean Shannon diversity with standard errors of mean for two zones, region and seed color character of 1979–1983 (P) and 2017 (C) collected chickpea genotypes**

| Zones          | Black  | Brown | Light brown | Dark brown | Ivory white | H mean ±SE | H mean ±SE |
|----------------|--------|-------|-------------|------------|-------------|------------|------------|
|                | P      | C     | P           | C          | P           |            |            |
| East Gojam     | 1.07   | 0.00  | 1.00        | 1.04       | 2.06        | 0.96       | 1.04       | 0.64       | 0.0        | 0.95 ± 0.08 | 0.63 ± 0.26 |
| North Gondar   | 1.50   | 0.69  | 1.47        | 1.56       | 1.52        | 1.69       | 1.22       | 1.01       | 0.64       | 1.27 ± 0.17 | 0.95 ± 0.29 |
| Region         | 1.99   | 0.69  | 1.95        | 1.99       | 1.99        | 1.79       | 1.70       | 1.33       | 0.0        | 1.80 ± 0.13 | 1.27 ± 0.4   |
Performance while previous collection showed variable performance. From these results it is possible to conclude current and previous chickpea populations are quite different and in addition the genotypes of current chickpea collection are homogeneous, while the previous collections are heterogeneous. Low yield performances were observed in the previous collection. This might be due to the arrest of evolutionary process more than 35 years. Ex-situ conservation does not maintain an evolutionary process that created new germplasms (Hamilton, 1994), hence the climatic conditions might be new to the old accessions which result in adaptation problem. Regarding the current collections, the genotypes have been grown by the farmers for decades so that the genotypes may not face adaptation problem. However, due to environmental and human selection a loss in heterogeneity has been observed as management and preferential treatment among farmers often results in greater uniformity which results in germplasm loss. Because of these reasons gene bank managers recommend in-situ conservation be implemented side by side with ex-situ conservation. In-situ conservation will help the evolutionary process to continue and allow genetic resources adapt to the changing environments (Hawkes, 1991; Horovitz & Feldman, 1991). Similar findings were observed in other crops by Teklu and Hammer (2006); Tsegaye and Berg (2007) and Mekbib (2008).

Cluster analyses grouped all the chickpea genotypes into six distinct clusters having variable number of individuals in each cluster. Cluster II and V contained individual from the majority of current collections, while cluster I and III contained individual solely from previous collection. Cluster VI was made entirely from improved genotypes. Genotypes having similar characteristics were grouped into the same cluster, while genotypes with different characteristics were grouped at different cluster. This indicates that that genotypes grouped in the same cluster had little divergence from each other with respect to the traits considered than individuals from different clusters. Moreover genotypes are clustered relatively in respect to time of collection and irrespective of their source of origin. Similar grouping of genotypes irrespective of their origin was also observations reported for EBI collections by Keneni et al. (2013).

5. Conclusion and recommendation

Current collections (2017) and previous collections (1979–1983) are distinct from each other in terms of the degree of phenotypic variability and agronomic performances. The genotypes of the current collections were more or less homogeneous for the studied quantitative and quantitative
traits, while genotypes from the previous collection were heterogeneous. The ongoing human selections made the chickpea germplasm uniform which resulted in the loss of germplasm.

These conclusions were made based on data from three districts in one region. To provide a more holistic approach consideration of all major chickpea growing areas of the country are required to obtain information on the national genetic variability and/or genetic erosion status. Molecular studies are also necessary to complement the phenotypic studies to ascertain the genetic distinctness of currently and previously collected chickpea genotypes. Immediate implementation of a restoration program is required to restore the lost genotypes in the studied districts. Additionally, strengthening on-farm conservation by establishing community seed banks in representative chickpea growing areas is required to supply landrace seeds to the local farmers through farmer-to-farmer seed exchange.

**Acknowledgements**

Ethiopian Biodiversity Institute and Addis Ababa University for financial support and providing the chickpea genotypes and Debre Zeit Agricultural Research Center for allowing us to use the research station for field work. These initiatives are supported by the United States Department of Agriculture, which is an equal opportunity provider and employer.

**Funding**

The study is part of the first author PhD thesis funded by Ethiopia Biodiversity Institute and Addis Ababa University.

**Author details**

Sintayehu Admas

E-mail: sintayehu.admas@ebi.gov.et

ORCID ID: http://orcid.org/0000-0002-4590-9555

---

**Table 4. Clustering of 79 chickpea genotypes and two improved chickpea varieties into six clusters using means of nine quantitative traits**

| Cluster Name | No of genotypes | Genotypes |
|--------------|-----------------|-----------|
| Cluster I    | 19              | 207,143-A, 207,170-A, 227,158-C, 41,026-C, 41,267-A, 41,310-A, 41,324-B, 207,145-A, 225,887-A, 227,161-B, 41,078-A, 41,295-A, 41,310-B, 207,145-B, 227,158-A, 227,161-C, 41,229-A, 41,306-B, and 41,322-B |
| Cluster II   | 7               | 207,143-B, 241,801-C, 30,301-C, 30,311-A, 30,322-B, 30,341-A, and 41,026-B |
| Cluster III  | 9               | 207,166-A, 241,801-A, 41,223-A, 41,231-A, 41,231-B, 41,268-B, 41,269-B, 227,160-B and 41,046-A |
| Cluster IV   | 24              | 225,887-B, 30,301-A, 30,312-A, 30,331-B, 41,078-B, 41,268-A, 41,311-B, 236,493-A, 30,302-A, 30,323-A, 41,026-A, 41,222-A, 41,295-B, 41,322-A, 241,800-A, 30,304-A, 30,323-B, 41,046-B, 41,223-B, 41,311-A, 41,324-C, 241,800-B, 30,309-A, and 30,331-A |
| Cluster V    | 20              | 30,287-A, 30,293-A, 30,317-A, 30,320-A, 30,325-A, 30,340-A, 30,288-A, 30,308-A, 30,318-A, 30,321-A, 30,326-A, 30,332-A, 41,222-B, 30,289-A, 30,310-A, 30,319-A, 30,324-A, 30,327-A, and 30,339-A |
| Cluster VI   | 2               | Mastewal and Worku |

---

**Figure 3. Dendrogram constructed using Ward’s method based on quantitative morphological traits of 79 chickpea genotypes and two improved chickpea varieties.**

---

---

---

---
Kassahun Tesfaye1
Telekehaimonat Hailellassie2
Eleni Shiferaw3
K. Colton Flynn1
1 Ethiopian Biodiversity Institute, Crop and Horticulture Biodiversity Directorate PO Box 30726, Addis Ababa, Ethiopia.
2 Institute of Biotechnology, College of Natural Sciences, Addis Ababa University, Addis Ababa, 3285, Ethiopia.
3 USDA-ARS, PA, Grassland Soil and Water Research Laboratory, Temple, TX, 76502, USA.

Disclosure statement
The authors declare there are no competing interests.

Competing interests
The authors declare no competing interests.

Author’s Contributions
Conceptualization, SA, KT; Fieldwork/Data Collection: SA, TH, ES, KCF; Writing/Editing, SA, KT, TH, ES, KCF.

Data availability
Data can be shared upon request.

Ethics approval
Not applicable.

Informed consent
All authors agreed on the publication of the present work.

Cover Image
Source: Author.

Citation information
Cite this article as: Phenotypic variability of chickpea (Cicer arietinum L) germplasm with temporarily varied collection from the Amhara Regional State, Ethiopia, Sintayehu Admas, Kassahun Tesfaye, Telekehaimonat Hailellassie, Eleni Shiferaw & K. Colton Flynn, Cogent Food & Agriculture (2021), 7: 1896117.

References
Abete, I., Astrup, A., Martinez, J. A., Thorsdottir, L., & Zulet, M. A. (2010). Obesity and the metabolic syndrome: Role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. Nutrition Reviews, 68(4), 214–231. https://doi.org/10.1111/j.1753-4887.2010.00280.x
Archak, S., Tyagi, R. K., Harer, P. N., Mahase, L. B., Singh, N., Dahiya, O. P., Nizar, M. A., Singh, M., Tilekar, V., Kumar, V., Dutta, M., Singh, N. P., & Bansal, K. C. (2016). Characterization of chickpea germplasm conserved in the Indian National Genebank and development of a core set using qualitative and quantitative trait data. The Crop Journal, 4(5), 417–424. https://doi.org/10.1016/j.cj.2016.06.013
Armstrong, P., Berry, G., & Matthews, J. N. S. (2002). Ratio and other functions. Statistical methods in medical research (Fourth ed). Blackwell Science Ltd.
Berry, M. B., Pham, J. L., Be ovogui, S., Ghesquiere, A., & Ahmadi, N. (2008). Diachronic (1979–2003) analysis of rice genetic diversity in Guinea did not reveal genetic erosion. Genetic Resources and Crop Evolution, 55, 723–733. https://doi.org/10.1007/s10722-007-9280-z
Egan, N., & Wood, J. (2013). Investigating the diversity of chickpea seed coat phenolics. 66th Australasian Grain Science Association (AGSA) Conference “From the soil to the supermarket”. Australasian Grain Science Association. https://doi.org/10.13140/RG.2.2.24960.02562
FAO. (2010). ‘Chapter 3 The state of ex situ conservation’ The second report on the state of the world’s plant genetic resources for food and agriculture, pp. 54–90
Ghosh, A., Dadich, A., Bhardwaj, P., Babu, J. N., & Kumar, V. (2019). Comparative analysis of metabolites in contrasting chickpea cultivars. Journal of Plant Biochemistry and Biotechnology, 29, 253–265. https://doi.org/10.1007/s13562-019-00530-2
Gomez, K. A., & Gomez, A. A. (1984). Statistical procedures for agricultural research. John Wiley & Sons.
Hamilton, M. B. (1994). Ex situ conservation of wild plant species: Time to reassess the genetic assumptions and implications of seed banks. Conservation Biology, 8(1), 39–49. https://doi.org/10.1046/j.1523-1739.1994.08010039.x
Hammer, K., & Laggetti, G. (2005). Genetic erosion – Examples from Italy1,2. Genetic Resources and Crop Evolution, 52(5), 629–634. https://doi.org/10.1007/s10722-005-7902-x
Hawkes, J. G. (1991). International workshop on dynamic in-situ conservation of wild relatives of major cultivated plants: Summary of final discussion and recommendations. Israel Journal of Botany, 40, 529. http://www.ask-force.org/web/Africa-Harvest-Sorghum-Lit-1/Hawkes-Landraces-Workshop-1991.pdf
Horowitz, A., & Feldman, M. (1991). Evaluation of the wild-wheat study at Ammadi: Population dynamics of the wheat progenitor, Triticum turgidum var. dicoccoides, in a natural habitat in Eastern Galilee. Israel Journal of Botany, 40(5–6), 501–508. http://www.ask-force.org/web/Africa-Harvest-Sorghum-Lit-1/Hawkes-Landraces-Workshop-1991.pdf
IBPGR, ICRIAT & ICARDA. (1995). Descriptors for Chickpea (Cicer arietinum L.). In International board for plant genetic resources, Rome, Italy; International Crops Research Institute for the semi-arid tropics, Patancheru, India and International Center for Agriculture Research in the Dry Areas. 31.
Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.): A review. British Journal of Nutrition, 108(51), S11–S26. https://doi.org/10.1017/S0007114512000797
Kenen, G., Bekele, E., Assefa, F., Itmiaz, M., Debele, T., Daghe, K., & Getu, E. (2013). Evaluation of Ethiopian chickpea (Cicer arietinum L.) germplasm accessions for sumbo-argonomic performance. Renewable Agriculture and Food Systems, 28(4), 338–349. https://doi.org/10.1017/ S1742170512000221
Mazhar, F. (1997). Nayakrishni Andolani: An initiative of the Bangladesh peasants for a better living. In L. Sperling & M. Lovelsohn (Eds.), Using diversity: Enhancing and maintaining genetic resources on-farm. International Development Research Centre.
Mekbib, F. (2008). Genetic erosion of sorghum (Sorghum bicolor (L.) Moench) in the centre of diversity. Ethiopia. Genetic Resources and Crop Evolution, 55(3), 351-364. https://doi.org/10.1007/s10722-007-9240-7
META-R (Multi Environment Trial Analyses with R for Windows). (2016). META-R users guide, Version 6.0 Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT).
MINITAB. (2018). MINITAB users guide, release 14.
Mollard, R. C., Luhovvy, B. L., Panahi, S., Nunez, M., Hanley, A., & Anderson, G. H. (2012). Regular consumption of pulses for 8 weeks reduces metabolic
syndrome risk factors in overweight and obese adults. British Journal of Nutrition, 108(S1), S111–S112. https://doi.org/10.1017/S0007114512000712

Nestel, P., Cehuri, M., & Chronopoulos, A. (2004). Effects of long-term consumption and single meals of chickpeas on plasma glucose, insulin, and triacylglycerol concentrations. The American Journal of Clinical Nutrition, 79(3), 390–395. https://doi.org/10.1093/ajcn/79.3.390

Parameshwarappa, S. G., Salimath, P. M., Upadhyaya, H. D., Patil, S. S., & Kajjidoni, S. T. (2012). Genetic variability studies in mincore collection of chickpea (Cicer arletinum L.) under different environments. Karnataka Journal of Agricultural Sciences, 25(3), 305–308. http://oar.cris.org.in/di/6606

Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. B., & Soutar, D. (2013). GenStat for Windows (16th Edition) Introduction, VSN International.

Segev, A., Badani, H., Kapulnik, Y., Shomer, L., Oren- Shamir, M., & Galili, S. (2010). Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (Cicer arletinum L.). Journal of Food Science, 75(2), S115–S119. https://doi.org/10.1111/j.1502-7687.2010.01477.x

Shewryra, H., Jordan, D. R., & Godwin, I. D. (2008). Genetic erosion and changes in distribution of sorghum (Sorghum bicolor L. (Moench)) landraces in north-eastern Ethiopia. Plant Genetic Resources, 6(1), 1–10. https://doi.org/10.1037/S1479262108923789

Singh, A. K., Varapradosad, K. S., & Venkateswaran, K. (2012). Conservation costs of plant genetic resources for food and agriculture: Seed genebanks. Agricultural Research, 1(3), 223–239. https://doi.org/10.1007/s40003-012-0029-3

Teklu, Y., & Harmer, K. (2008). Farmers’ perception and genetic erosion of tetraploid wheats landraces in Ethiopia. Genetic Resources and Crop Evolution, 55(6), 1099–1113. https://doi.org/10.1007/s10722-005-1145-8

Tsegaye, B., & Berg, T. (2007). Genetic erosion of Ethiopian tetraploid wheat landraces in Eastern Shewa, Central Ethiopia. Genetic Resources and Crop Evolution, 54(4), 715–726. https://doi.org/10.1007/s10722-006-0016-2

Upadhyaya, H. D., Dwivedi, S. L., Baum, M., Vashney, R. K., Udutha, S. M., Gowda, C. L., Hoisington, D., & Singh, S. (2008). Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (Cicer arletinum L). BMC Plant Biology, 8, 106. https://doi.org/10.1186/1471-2229-8-106

Van De Vouw, M., Chris Kik, C., Van Hintum, T., Van Treuren, R., & Visser, B. (2009). Genetic erosion in crops: Concept, research results and challenges. Plant Genetic Resources: Characterization and Utilization, 8(1), 1–15. https://doi.org/10.1017/S1479262109900062

Van Der Maesen, L. J. G. (1988). Origin, history and taxonomy of chickpea. In M. J. Saxena & K. B. Singh (Eds.), The chickpea (pp. 11–34). CAB International.

Vavilov, I. (1931). The Origin. Variation, Immunity and Breeding of Cultivated Plants Translated from the Russian by K.Starchester. Ronlind Press. New York.

Vavilov, N. I. (1927). Geographical regularities in the distribution of genes of cultivated plants. Priroda (Moscow), 10, 764–773.

Vishnyakova, M. A., Burlyova, M. O., Bulytshev, S. V., Seferova, I. V., Plekhanova, E. A., & Nuzhdin, S. V. (2017). Phenotypic diversity of chickpea (Cicer arletinum L.) landraces accumulated in the Vavilov collection from the centers of the crop’s origin. Russian Journal of Genetics: Applied Research, 7(7), 763–772. https://doi.org/10.1134/S2079059717070097

Wallace, T. C., Murray, R., & Zelman, K. M. (2016). The nutritional value and health benefits of chickpeas and hummus. Nutrients, 8(12), 766. https://doi.org/10.3390/nu8120766

Ward, J. H. (1963). Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association, 58(301), 236–244. https://doi.org/10.1080/01621459.1963.10500845

Woreda, M. (1992). Ethiopia: A genebank working with farmers. In Cooper, D.; Bellville, R & Hobbelink, H. (eds), Growing diversity: Genetic resources and local food security. (pp. 78–94).

Yang, Y., Zhou, L., Gu, Y., Zhang, Y., Tang, J., Li, F., Shang, W., Jiang, B., Yue, X., & Chen, M. (2007). Dietary chickpeas reverse visceral adiposity, dyslipidaemia and insulin resistance in rats induced by a chronic high-fat diet. British Journal of Nutrition, 98(4), 720–726. https://doi.org/10.1017/S0007114507750870
