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**Full Length Research Paper**

# Study the Genetic Diversity in Protein, Zinc and Iron in Germplasm Pools of Desi Type Chickpeas as Implicated in Quality Breeding

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**Abstract**

*Chickpea (Cicer arietinum L.) is an important pulse crop with a wide range of potential nutritional benefits due to its chemical composition which is of immense importance. This study aimed to evaluate the variability of protein and micro nutrients concentrations among chickpea genotypes. Eighty-one genotypes were collected and evaluated under the field at Jari, Sirinka and Kobo research field of Sirinka Agriculture research center, Amhara regional state, Ethiopia. A simple lattice design with two replications was used. Seeds were collected, dried and the powder was used for protein determination using near infrared reflectance spectroscopy and micro nutrients atomic absorption spectrophotometer. Analysis of variance showed significant differences ($p<0.01$) among the genotypes, environment, and genotype by environment interaction. Protein content ranged from 14.15% to 23.08% while micro nutrients varied from 3.07 mg/100 g to 10.40 mg/100 g for iron and 1.34 mg/100 g to 3.47 mg/100 g for zinc. Moderate to high genotypic variability for protein and micro nutrient content with high heritability and genetic advance mean indicated the scope for enhancement of traits through selection. Genetic diversity studies revealed five different cluster and that high proteins lines are grouped in cluster IV and lines with higher concentration of micro nutrients are grouped in clusters V. Systematic hybridization between promising lines for protein and micronutrients chosen from these clusters is suggested to study their combining ability and subsequent use in breeding programs intended to breed for superior chickpea cultivars.*

## Introduction

Chickpea (*Cicer arietinum* L.) is self-pollinating crop with diploid chromosome number (2n= 2x =16), and genus Cicer, tribe Cicereae, family Fabaceae, and subfamily Papilionaceae. Chickpea is the third leading legume grain in the world after dry bean and field pea (Tesfamichael Semere *et al.*, 2015) and third in the area and production of pulses following faba bean (V*icia faba* L.) and haricot bean (*Phaseolus vulgaris* L.) in Ethiopia (CSA, 2015).

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Chickpea research has contributed towards increased productivity of the crop thereby increasing the availability of food and improving the economic and social wellbeing of the producers by developing and transferring of improved cultivars suitable for different agro-ecologies along with appropriate crop management practices. Within fifty years, about 30 chickpea varieties of different market class were released from different agricultural research centers and these varieties vary depending on their yield performance, seed color, seed size and other response to biotic factors. Less attention has been given for nutritious chickpea cultivar development directed toward improving protein and micronutrient concentration in seeds. In an effort to develop protein and micro nutrient- dense chickpea lines, an entry point could be to study the level of variability in protein, zinc and iron among germplasm sources. The chickpea breeding programs have so far released some 30 varieties, none of which were justified in their nutritional profile. There is a need to assess genetic variability available in the germplasm of cultivated and wild species for various quality traits. Studies are also needed on establishing genetics, linkage relationships and G × E interactions for many of these traits (Aliyi Robsa, 2017).

Chickpea is an excellent food as a source of dietary proteins and micronutrients (Jukanti *et al*., 2012). It is the third most important grain legume protein source in the world and its protein quality is better than other legumes (Kaur and Singh, 2005). The crude protein content varied from 18 to 31% (Sharma *et al*., 2013). Chickpea has an average of 2.2 - 20 mg of zinc per 100g edible portion and iron 3.0 - 14.3 mg/100gm and 334 - 446 Kcal per 100g edible portion (Ray *et al.*, 2014). The protein and micronutrient contents of food vary greatly from sample to sample, reflecting a variety of factors, such as differences in soil and climatic conditions (Iqbal *et al*., 2006).

Ethiopian farmers have been cultivating chickpea accessions for a long period of time because of the high potential of genetic diversity, they hold, better adaptation and resistance to diseases and insect pests. Exploration of genetic diversity in parental material is important for a successful breeding program (Annicchiarico *et al*., 2018). The study of genetic diversity provides an appropriate basis for the classification of genetic material and information on genetic diversity in parental material assists the breeders to identify and select the most suitable types from a mixed population (Agrawal *et al*., 2018). Genetic diversity serves as the most basic source for the production of new and valuable combinations and measurement of the extent of such variability and its source is thus of prime importance in breeding programs (Mahmood *et al*., 2016).

Despite the large number of chickpea germplasm collections held in Ethiopia, most of them have not been characterized at either morphological or molecular levels in terms of protein, iron, and zinc were not characterized (Aliyi Robsa, 2017). Although other publications have described the physicochemical and nutritional characteristics of chickpea, there is limited information relating its nutritional components to health benefits. Identification of chickpea genotypes rich in minerals help breeders to identify donors for targeted Fe and Zn bio-fortification breeding (Bhagyawant SS.*et al*., 2015). Despite the lack of cultivars registered in the national list based on nutrition, the development of variety with enhanced mineral concentration is one of the most sustainable and cost-effective approaches for alleviating malnutrition. Therefore, in the present study, an attempt has been made to study the genetic variability in protein and

micronutrient content in chickpea varieties grown in Jari, Sirinka and Kobo, to assess their possible use as donors in improving the nutritional quality of chickpea.

## Materials and Methods

### Description of Study Sites

The experiment was conducted at three locations in Eastern Amhara region, Ethiopia, that represent major agro-ecologies which are potential for the production of chickpea. The three locations were Jari, Sirinka, and Kobo, which are found along the road side from Addis Ababa to Mekele with a distance of 437, 508 and of 562 km from Addis Ababa, respectively. The detailed description of the experimental sites is indicated (Table 1)

**Table1**. Description of the experimental sites

| Location | Altitude (masl) | Soil type | Average-Rainf all (mm) | Temperature | | Global position | | env't code |
|---|---|---|---|---|---|---|---|---|
| | | | | Min (ºC) | Max. (ºC) | Latitude | Longitude | |
| Jari | 1680 | *Vertisol* | 1204.6 | 11.2 | 25.6 | $11^0$21' N | $39^0$38' E | 1 |
| Sirinka | 1850 | *Eutric fluvisol* | 945 | 13.6 | 27.3 | 11º45' N | 39º36'E | 2 |
| Kobo | 1450 | *Eutric fluvisol* | 850 | 15.8 | 29.1 | 11º21' N | 39º38'E | 3 |

*masl = meter above sea level, mm = millimetre, ºC = degree Celsius; Source: Sirinka Agricultural Research Center (SARC)*

### Experimental materials, design and procedure

The experimental material comprising eighty-one chickpea genotypes, which include cultivars, landrace and advanced lines having different genetic background were used, of chickpea was grown at the end of august 2018/19 in a Simple Lattice Design with two replications. The seeds were sown in row distance of 30 cm. The plot sizes were 0.6 × 1 m. Seeds were placed at 2-3 cm depth in each row, keeping 30 cm distance between the two rows. Two seeds were sown in each row. The excess plants were thinned out keeping one plant in each row 15 days after sowing. The seed yields were measured by harvesting each plot at crop maturity. Detailed description of the experimental materials indicated in the table 2 below.

**Table 2**. Detail description of chickpea materials

| SN. | Genotype | Description | SN. | Genotype | Description |
|---|---|---|---|---|---|
| **1** | IE-16-091 | Landrace | 42 | ICC-15614 | Advance breeding line |
| **2** | IE-16-044 | Landrace | 43 | ICCMABCA-23 | Advance breeding line |
| **3** | IE-16-148 | Landrace | 44 | ICCMABCA-36 | Advance breeding line |
| **4** | IE-16-146 | Landrace | 45 | ICCV-10107 | Advance breeding line |
| **5** | IE-16-078 | Landrace | 46 | ICCX-060045-F3-P113-BP | Advance breeding line |
| **6** | IE-16-072 | Landrace | 47 | ICC-6875 | Advance breeding line |
| **7** | IE-16-114 | Landrace | 48 | ICCV-09309 | Advance breeding line |

| | | | | | |
|---|---|---|---|---|---|
| **8** | IE-16-110 | Landrace | 49 | DZ-2012-CK-0231 | Advance breeding line |
| **9** | IE-16-121 | Landrace | 50 | MARIYE | Variety |
| **10** | IE-16-029 | Landrace | 51 | ICCV-4918 | Advance breeding line |
| **11** | IE-16-125 | Landrace | 52 | ICCV-10 | Advance breeding line |
| **12** | IE-16-080 | Landrace | 53 | ICCMABCD-21 | Advance breeding line |
| **13** | IE-16-115 | Landrace | 54 | ICC-9848 | Advance breeding line |
| **14** | IE-16-066 | Landrace | 55 | DALOTA | Variety |
| **15** | IE-16-030 | Landrace | 56 | IE-16-060 | Landrace |
| **16** | IE-16-040 | Landrace | 57 | ICC-1205 | Advance breeding line |
| **17** | IE-16-158 | Landrace | 58 | ICC-3391 | Advance breeding line |
| **18** | IE-16-062 | Landrace | 59 | DZ-2012-CK-240 | Advance breeding line |
| **19** | IE-16-149 | Landrace | 60 | ICCV-4958 | Advance breeding line |
| **20** | IE-16-127 | Landrace | 61 | ICC-15510 | Advance breeding line |
| **21** | IE-16-120 | Landrace | 62 | DZ-2012-CK-0277 | Advance breeding line |
| **22** | IE-16-147 | Landrace | 63 | ICCX-060039-F3-P152-BP | Advance breeding line |
| **23** | IE-16-133 | Landrace | 64 | ICCX-060045-F3-P152-BP | Advance breeding line |
| **24** | IE-16-150 | Landrace | 65 | NATOLI | Variety |
| **25** | IE-16-132 | Landrace | 66 | DZ-10-11 | Variety |
| **26** | IE-16-159 | Landrace | 67 | ICC-4418 | Advance breeding line |
| **27** | IE-16-156 | Landrace | 68 | ICCX-060045-F3-P225-BP | Advance breeding line |
| **28** | IE-16-032 | Landrace | 69 | Kutaye | Variety |
| **29** | IE-16-069 | Landrace | 70 | ICC-1230 | Advance breeding line |
| **30** | LOCAL CHECK | Landrace | 71 | IE – 16-058 | Landrace |
| **31** | Fetenech | Variety | 72 | IE – 16-060 | Landrace |
| **32** | ICC-7413 | Advance breeding line | 73 | MABC-13 | Advance breeding line |
| **33** | DZ-2012-CK-0235 | Advance breeding line | 74 | ICCMABCD-6 | Advance breeding line |
| **34** | ICC-1882 | Advance breeding line | 75 | MABC-7 | Advance breeding line |
| **35** | ICCRIL-04-0044 | Advance breeding line | 76 | DZ-2012-CK-0254 | Advance breeding line |
| **36** | DZ-2012-CK-20115-16-0058 | Advance breeding line | 77 | MABC-18 | Advance breeding line |
| **37** | ICC-12537 | Advance breeding line | 78 | ICCV-11108 | Advance breeding line |
| **38** | ICC-11903 | Advance breeding line | 79 | ICCMABCD-18 | Advance breeding line |
| **39** | ICCV-96836 | Advance breeding line | 80 | ICCMABCD-7 | Advance breeding line |
| **40** | MINJAR | Variety | 81 | ICCMABCA-27 | Advance breeding line |
| **41** | ICCX-060045-F3-P139-BP | Advance breeding line | | | Advance breeding line |

**Methods of Data Collection**

The data was taken on the plot and finally laboratory analysis was conducted at Ethiopian Institute Agri-cultural Research and Holetta Soil laboratory for iron and zinc analysis.

**Data collected on Plot Basis**

***Seed Yield*:** The seed yield was weighed using an electronic sensitive balance for each plot.

**Data collected on laboratory Basis**

***Protein*:** The protein content was determined by near infrared reflectance (NIRs) (Zhu Z.*et al*., 2018). Chickpea grain samples were cleaned manually and milled using a cyclotec mill (Foss Tecator Cyclotec) with a 1mm sieve and stored in a glass cup for NIRS analysis. About 3 grams of homogenized chickpea flour was analysed in duplicate using a Foss NIRS Systems 6500 spectrometer equipped with a spinning module and small ring cup. Spectra were recorded as log (1/R) of diffuse reflectance from 400nm to

2500nm, in 2 nm steps. Proximate compositions (list of parameters) were predicted using plant-based global calibration (infrasoft international) from the collected spectra.

***Zinc and Iron*:** The micronutrients; Zinc and Iron (in the chickpea seeds vis-à-vis, Zn, and Fe) were analyzed by Atomic Absorption Spectroscopy (AAS) method using AACC (117) (20). It is known for measuring the absorbance of the species at its resonance wavelengths. Flour sample 0.5g which was ashed using a maffle furnace at 550°C for five hours. Briefly, the samples in the powdered form were accurately weighted and digested in a mixture of nitric acid and perchloric acid (5:1) (Herber *et al.*, 1994). After digestion, a few drops of concentrated HCl were added. The solution was heated gently and then filtered. The residue was again subjected to digestion and the filtrate was collected. The entire filtrate was diluted suitably with deionized water. The diluted filtrate was used for analysis of Zn and Fe in all the the accessions by AAS using suitable hollow cathode lamps, in Holetta Agricltural Research Center. The filtered extract was used to measure the concentration of various elements by a relative method using analytical grade solutions of the elements of interest (Tandon, 1993).

Adjust the AAS Osborne and Voogt (1978) in accordance with the manufacturer's instructions and optimize the response of the instrument to an oxidizing air-acetylene flame at the following wavelengths:

Fe: 248.3 nm

Zn: 213.8 nm

Calculation

Using a calibration curve (Preparation of Calibration Curves), the trace element concentration in the solution was calculated, based on (AAS model SP9)

$$Cs = \frac{Abs - b}{m}$$

Where,

*Cs* = element concentration of the sample solution [µg/ml],

*Abs* = absorbance value of the sample solution,

*b* = y-intercept of the regression line, and

*m* = slope of the regression line.

Element content of the sample in mg/100g considering dilution step is calculated as:

Element (mg/100g) = $\frac{CS \times V \times F}{W}$

Where,

V = volume of the sample solution [ml],

F = dilution factor, and

w = sample weight [g].

**Data analysis**

All statistical analyses were performed using SAS Computer Statistical Package version 9.2 (SAS Institute Inc., 2008). The analysis of variance and Least Significance Difference Test (LSDT) were performed to test differences between means. Mean values of the nutritional traits for genotypes were standardized and used for computing Euclidean distances between them. Cluster analyses were used to obtain Euclidean distances between genotypes and to characterize the relation to the most discriminating traits. Genetic variability parameters viz., genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) coefficients of variation were computed for all traits were according to Singh and Chaudhary (2004) using the equations:

GCV (%) = $\{(\sqrt{\sigma^2 g}) / x\} \times 100$

PCV (%) = $\{(\sqrt{\sigma^2 p}) / x\} \times 100$

Where $\sigma^2 g$ = genotypic variance, $\sigma^2 p$ = phenotypic variance, and x = grand mean for the trait.

The GCV and PCV were considered low when less than 10%, moderate when 10 to 20%, and high when

greater than 20% as explained by Deshmukh *et al.* (1986).

Broad-sense heritabilities were estimated using variance ratios as explained by Hallauer *et al.* (2010) using the following equations:

$H^2 = \{\sigma^2g/ (\sigma^2g+ \sigma^2ge/e + \sigma^2/re)\} \times 100$

Where $\sigma^2g$ = genotypic variance, $\sigma^2$ = environmental variance, $\sigma^2ge$ = variance due to genotype by environment interaction, $\sigma^2p$ = phenotypic variance = $\sigma^2g+ \sigma2ge+ \sigma2$, r = number of replicates and e = number of environments.

Heritability estimates were categorized into low (less than 40%), medium (40-59%), moderately high (60-79%), and very high (80% and above) as described by Singh (2001).

Genetic advance expressed as percentage of the mean was estimated as described by Souza *et al.* (2009) as follows:

GA (%) = GA/x× 100 Where x = grand mean of all for the trait.

Genetic distance between clusters as standardized Mahalanobis $D^2$ (Mahalanobis, 1936) statistics were calculated as:

$D^2_{ij} = (x_i– x_j)' \ cov^{-1} (x_i– x_j)$;

Where $D^2_{ij}$ = the distance between cases i and j;

$x_i$ and $x_j$ = vectors of the values of the variables for cases i and j and $Cov^{-1}$ = pooled groups variance-covariance matrix.

## RESULTS AND DISCUSSIONS

### Analysis of Variance

***Genotypic variation for proteins*:** Analysis of variance for proteins in eighty-one genotypes revealed a highly significant sum of squares due to genotypes, which indicated that there is substantial genetic variability among lines for protein content (Table 3). The observed variability in eighty-one genotypes is significant and varied from 14.15% (ICCX-060045-F3-P225-BP) to 23.08% (local check) (Table 3). Genotypes that have the highest protein content was observed in local check (23.08%) followed by ICC-1882 (22.70%). In agreement with the current findings, Sharma *et al.* (2013) and Jadhav *et al.* (2015) reported wide variability in protein content, chickpea cultivars have a wide range of variation, from 12 to 30 %, existing in chickpea germplasm. This variation can provide sufficient scope for further selection and improvement on these chickpea genotypes (Table 3). The results, combined analysis of variance for protein content revealed significant differences not only for genotypes but also a high magnitude of genotype-environment interaction, reflecting genetic variability in experimental material as well as the difference in the environmental conditions (Table 5). In agreement with the present results, Iqbal *et al*. (2006) reported that both the quantity and the quality of protein vary considerably depending on soil and climatologically conditions (location).

In the present study with higher protein content (13% higher in comparison to the rest varieties), has already been found to be high protein content, can be tested for their stability in performance across locations and years and may be utilized for commercial cultivation or may be deployed in a breeding programmes to further improve the protein content of chickpeas and to enhance the crop's protein contribution to the human diet. The intrinsic genetic variability of protein content amenable to selection and associated genetic gain can be predicted based on heritability and expected genetic advance (Table 4). Genotypic and phenotypic coefficients of variation, PCV and GCV values (16.16% and 15.87%), heritability (96.47%), and expected genetic advance (31.91%) were high for protein (Table 4). Kozgar *et al*. (2012) and Gaikwad *et al.* (2011) and several others reported high values of broad sense heritability estimates for protein content. The characters which showed high

heritability estimates indicate that the environmental influence on them was lower than the genetic influence. Therefore, the success of crop improvement through selection could be easier. The medium value of genotypic variability coupled with high heritability and genetic advance suggests that protein content is under the influence of additive genes and can be improved by phenotypic selection.

There are limited breeding efforts in enhancing protein content in chickpea, identification of adapted chickpea lines with higher protein content will help in food fortification and in utilizing promising lines in further breeding programmes. Five favourable genotype local checks, ICC-1882, IE-16-078, IE-16-121 and IE-16-080 of the present study can be utilized in this direction.

**Table 3**. Mean performance of protein content in 81 chickpea genotypes

| SN. | Genotype | PC | SN. | Genotype | PC |
|---|---|---|---|---|---|
| 1 | IE-16-091 | 19.59 | 43 | ICCMABCA-23 | 16.71 |
| 2 | IE-16-044 | 20.32 | 44 | ICCMABCA-36 | 17.41 |
| 3 | IE-16-148 | 19.12 | 45 | ICCV-10107 | 18.88 |
| 4 | IE-16-146 | 19.22 | 46 | ICCX-060045-F3-P113-BP | 17.68 |
| 5 | IE-16-078 | 22.63 | 47 | ICC-6875 | 17.95 |
| 6 | IE-16-072 | 20.15 | 48 | ICCV-09309 | 16.17 |
| 7 | IE-16-114 | 19.64 | 49 | DZ-2012-CK-0231 | 17.35 |
| 8 | IE-16-110 | 18.91 | 50 | MARIYE | 16.34 |
| 9 | IE-16-121 | 22.56 | 51 | ICCV-4918 | 15.36 |
| 10 | IE-16-029 | 19.95 | 52 | ICCV-10 | 16.66 |
| 11 | IE-16-125 | 19.95 | 53 | ICCMABCD-21 | 19.29 |
| 12 | IE-16-080 | 22.55 | 54 | ICC-9848 | 15.51 |
| 13 | IE-16-115 | 20.42 | 55 | DALOTA | 17.33 |
| 14 | IE-16-066 | 19.90 | 56 | IE-16-060 | 18.33 |
| 15 | IE-16-030 | 18.54 | 57 | ICC-1205 | 17.77 |
| 16 | IE-16-040 | 20.21 | 58 | ICC-3391 | 15.88 |
| 17 | IE-16-158 | 18.42 | 59 | DZ-2012-CK-240 | 15.10 |
| 18 | IE-16-062 | 20.08 | 60 | ICCV-4958 | 17.61 |
| 19 | IE-16-149 | 19.98 | 61 | ICC-15510 | 16.05 |
| 20 | IE-16-127 | 19.21 | 62 | DZ-2012-CK-0277 | 15.53 |
| 21 | IE-16-120 | 20.05 | 63 | ICCX-060039-F3-P152-BP | 14.23 |
| 22 | IE-16-147 | 20.23 | 64 | ICCX-060045-F3-P152-BP | 18.47 |
| 23 | IE-16-133 | 20.41 | 65 | NATOLI | 19.27 |
| 24 | IE-16-150 | 20.25 | 66 | DZ-10-11 | 20.44 |
| 25 | IE-16-132 | 19.16 | 67 | ICC-4418 | 15.83 |
| 26 | IE-16-159 | 19.81 | 68 | ICCX-060045-F3-P225-BP | 14.15 |
| 27 | IE-16-156 | 19.86 | 69 | Kutaye | 16.31 |
| 28 | IE-16-032 | 18.94 | 70 | ICC-1230 | 16.58 |
| 29 | IE-16-069 | 14.94 | 71 | IE – 16-058 | 19.63 |
| 30 | LOCAL CHECK | 23.08 | 72 | IE – 16-060 | 18.79 |
| 31 | Fetenech | 18.03 | 73 | MABC-13 | 17.64 |
| 32 | ICC-7413 | 15.40 | 74 | ICCMABCD-6 | 16.25 |
| 33 | DZ-2012-CK-0235 | 17.26 | 75 | MABC-7 | 16.48 |
| 34 | ICC-1882 | 22.70 | 76 | DZ-2012-CK-0254 | 17.09 |
| 35 | ICCRIL-04-0044 | 16.21 | 77 | MABC-18 | 17.01 |
| 36 | DZ-2012-CK-20115-16-0058 | 16.84 | 78 | ICCV-11108 | 17.34 |
| 37 | ICC-12537 | 17.09 | 79 | ICCMABCD-18 | 18.77 |
| 38 | ICC-11903 | 17.90 | 80 | ICCMABCD-7 | 17.36 |
| 39 | ICCV-96836 | 17.93 | 81 | ICCMABCA-27 | 19.67 |
| 40 | MINJAR | 17.70 | | G.M | 18.33 |
| 41 | ICCX-060045-F3-P139-BP | 16.50 | | LSD | 0.63 |
| 42 | ICC-15614 | 18.93 | | C.V % | 3.04 |

Where G.M = grand mean, LSD = least significance difference, C.V% = coefficient variation.

**Table 4**. Estimates of genetic parameters for protein and micronutrient content in 81 chickpea genotypes

| Character | Mean | Range | | GCV (%) | PCV (%) | $H^2$ | GAM |
|---|---|---|---|---|---|---|---|
| | | Max. | Min. | | | | |
| PC (%) | 18.33 | 23.08 | 14.15 | 15.87 | 16.16 | 96.47 | 31.91 |

Where *Max. = maximum, min. = minimum, PCV **(%)** = phenotypic coefficient of variation, GCV **(%)** = genotypic coefficient of variation, $H^2$ = heritability, GAM (%) = genetic advance a percentage of mean,* PC (%) = *protein content.*

Bartlett's test showed that the homogenous error variance for the protein which allowed to proceed further for pooled analysis across environments. The combined analysis of variance for the protein exhibited differences P<0.01 among environments, genotypes, and genotype by environment interaction, indicating differences in environments and the presence of genetic variability among genotypes (Table 5).

**Table 5**. Combined mean squares for different sources of variation and the corresponding coefficient of variation (CV) for one trait of chickpea genotypes studied at Jari, Sirinka, and Kobo in 2018/19

| Traits | Source of variation | | | | | | |
|---|---|---|---|---|---|---|---|
| | MSL df (2) | MSG Df (80) | MSGL Df (160) | MSBR Df (16) | MSRL Df (2) | MSE Df (224) | CV % |
| PC(%) | 368.29** | 17.23** | 4.48** | $0.18^{NS}$ | $0.27^{NS}$ | 0.31 | 3.04 |

Where *PC = protein content, MSE = Mean square of error, MSL = Mean square by location, MSG = Mean square by genotype or Treatmnt, MSGL = Mean square genotype by location interaction, MSBR = Mean square block by replication, MSRL = Mean square replication by location, ns = non - significant and ** significant at 1% probability level, respectively.*

**Genetic variability for micro nutrients:** Selection and use of chickpea genotypes with higher potential uptake of minerals is one of the viable options to enhance the minerals concentration of seeds and increased supply of minerals through food is one of the best options proposed for a sustainable food-based solution to global malnutrition. Iron and zinc content results revealed significant differences not only for genotypes but also a high magnitude of genotype-environment interaction, reflecting genetic variability in experimental material as well as a difference in the environmental conditions (Table 8). Significant genotype location interaction for Fe and Zn implied that there is location-specific adaptation of the genotypes. In a single-year multilocation study, Kumar *et al.* (2013) also reported significant genotype-by-location interaction for both micronutrients. The observed variability in eighty one genotypes is significant and varied from 3.07 mg/100 g (ICCV-11108) to 10.40mg/100 g (ICCV-96836) for iron and 1.34 mg/100 g (ICCMABCD-21) to 3.47 mg/100 g (ICCV-96836) for zinc (Table 6). Genotypes that have highest iron content was observed in ICCV-96836 (10.40 mg/100g) followed by DZ-2012-CK-0277 (8.95 mg/100g). ICCV-96836 (3.47 mg/100g) and IE-16-080 (3.09 mg/100g) are characterized by

significantly higher zinc concentration. Recent studies Upadhyaya *et al.*, 2016) investigated the genetic diversity and nutritive value of chickpea germplasm and indicated the scope for molecular breeding for improvement of the nutritive value of chickpea.

Therefore, the nutritional value of promising genotypes has to be further verified by growing them again in the same field. The GCV values computed were high for the traits zinc (24.80%) and iron (37.58%) (Table 7). Heritability values for iron and zinc contents were higher in magnitude (96.99% - 99.6%) (Table 7). This reflected that selection could be effective for the improvement of the traits, which is also indicated by estimates of high expected genetic advance (above 20%). This suggests that the influence of environmental factors on the expression of these traits is low and they show a higher response to selection. High heritability with high genetic advance as per cent of the mean was noticed for Zn and Fe. This could be due to additive gene action and selection pressure could effectively be exerted on these traits for their improvement. In harmony with the present results, Jayalakshim *et al*. (2018) reported a high genotypic coefficient of variation high for iron (44.68) and zinc (20.75) and the phenotypic coefficient of variation for iron (45.82) and zinc (21.85) and heritability between iron (95%) and zinc (90%).

**Table 6**. Mean performance of zinc and iron content in 81 chickpea genotypes

| SN. | Genotype | Zn | Fe | SN. | Genotype | Zn | Fe |
|---|---|---|---|---|---|---|---|
| 1 | IE-16-091 | 2.35 | 8.30 | 43 | ICCMABCA-23 | 1.79 | 4.06 |
| 2 | IE-16-044 | 2.10 | 6.18 | 44 | ICCMABCA-36 | 2.03 | 4.92 |
| 3 | IE-16-148 | 2.41 | 7.45 | 45 | ICCV-10107 | 1.92 | 4.92 |
| 4 | IE-16-146 | 2.20 | 6.39 | 46 | ICCX-060045-F3-P113-BP | 1.40 | 3.56 |
| 5 | IE-16-078 | 1.87 | 6.32 | 47 | ICC-6875 | 1.38 | 4.95 |
| 6 | IE-16-072 | 1.88 | 5.62 | 48 | ICCV-09309 | 1.74 | 5.64 |
| 7 | IE-16-114 | 2.08 | 6.60 | 49 | DZ-2012-CK-0231 | 1.90 | 7.14 |
| 8 | IE-16-110 | 1.97 | 5.18 | 50 | MARIYE | 1.64 | 4.80 |
| 9 | IE-16-121 | 2.47 | 6.99 | 51 | ICCV-4918 | 2.38 | 8.59 |
| 10 | IE-16-029 | 2.06 | 6.38 | 52 | ICCV-10 | 1.84 | 5.84 |
| 11 | IE-16-125 | 2.18 | 7.05 | 53 | ICCMABCD-21 | 1.34 | 4.53 |
| 12 | IE-16-080 | 3.10 | 8.15 | 54 | ICC-9848 | 1.41 | 4.66 |
| 13 | IE-16-115 | 2.22 | 6.47 | 55 | DALOTA | 2.71 | 6.15 |
| 14 | IE-16-066 | 2.00 | 6.44 | 56 | IE-16-060 | 2.06 | 5.71 |
| 15 | IE-16-030 | 1.65 | 3.42 | 57 | ICC-1205 | 2.27 | 7.82 |
| 16 | IE-16-040 | 2.31 | 4.82 | 58 | ICC-3391 | 1.76 | 5.81 |
| 17 | IE-16-158 | 2.04 | 5.69 | 59 | DZ-2012-CK-240 | 1.72 | 5.92 |
| 18 | IE-16-062 | 2.03 | 5.40 | 60 | ICCV-4958 | 2.18 | 7.93 |
| 19 | IE-16-149 | 2.20 | 5.74 | 61 | ICC-15510 | 1.74 | 5.82 |
| 20 | IE-16-127 | 2.12 | 5.81 | 62 | DZ-2012-CK-0277 | 2.27 | 8.95 |
| 21 | IE-16-120 | 2.41 | 8.73 | 63 | ICCX-060039-F3-P152-BP | 1.96 | 5.58 |
| 22 | IE-16-147 | 2.36 | 5.58 | 64 | ICCX-060045-F3-P152-BP | 1.98 | 4.57 |
| 23 | IE-16-133 | 1.78 | 6.02 | 65 | NATOLI | 2.09 | 5.40 |
| 24 | IE-16-150 | 1.66 | 5.01 | 66 | DZ-10-11 | 2.31 | 6.53 |
| 25 | IE-16-132 | 2.13 | 4.45 | 67 | ICC-4418 | 1.87 | 5.36 |
| 26 | IE-16-159 | 2.30 | 6.01 | 68 | ICCX-060045-F3-P225-BP | 2.07 | 4.69 |
| 27 | IE-16-156 | 1.86 | 4.22 | 69 | Kutaye | 2.02 | 6.50 |
| 28 | IE-16-032 | 2.19 | 5.97 | 70 | ICC-1230 | 2.01 | 5.86 |
| 29 | IE-16-069 | 2.28 | 4.37 | 71 | IE – 16-058 | 1.96 | 6.30 |
| 30 | LOCAL CHECK | 2.03 | 5.19 | 72 | IE – 16-060 | 1.79 | 4.79 |
| 31 | Fetenech | 1.83 | 4.83 | 73 | MABC-13 | 1.80 | 4.39 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 32 | ICC-7413 | 1.83 | 3.77 | 74 | ICCMABCD-6 | 1.75 | 4.32 |
| 33 | DZ-2012-CK-0235 | 2.18 | 4.41 | 75 | MABC-7 | 1.88 | 4.30 |
| 34 | ICC-1882 | 1.96 | 5.67 | 76 | DZ-2012-CK-0254 | 2.26 | 6.12 |
| 35 | ICCRIL-04-0044 | 1.72 | 3.65 | 77 | MABC-18 | 2.26 | 6.38 |
| 36 | DZ-2012-CK-20115-16-0058 | 2.47 | 6.71 | 78 | ICCV-11108 | 1.76 | 3.07 |
| 37 | ICC-12537 | 2.08 | 5.88 | 79 | ICCMABCD-18 | 1.97 | 7.39 |
| 38 | ICC-11903 | 2.13 | 6.51 | 80 | ICCMABCD-7 | 1.87 | 4.83 |
| 39 | ICCV-96836 | 2.4 | 10.40 | 81 | ICCMABCA-27 | 2.18 | 4.31 |
| 40 | MINJAR | 1.95 | 4.57 | | G.M | 2.04 | 5.75 |
| 41 | ICCX-060045-F3-P139-BP | 1.86 | 5.13 | | LSD | 0.072 | 0.1405 |
| 42 | ICC-15614 | 2.06 | 6.01 | | C.V | 3.12 | 2.15 |

Where G.M = grand mean, LSD = least significance difference, C.V = Coefficient variation

**Table 7.** Estimates of genetic parameters for zinc and iron content in 81 chickpea genotypes

| **Element content** | **Mean** | **Range** | | **GCV (%)** | **PCV (%)** | **$H^2$** | **GAM** |
|---|---|---|---|---|---|---|---|
| | | Max. | Min. | | | | |
| Zn (mg/100g) | 2.04 | 3.47 | 1.34 | 24.80 | 25.57 | 96.99 | 51.08 |
| Fe (mg/100g) | 5.75 | 10.40 | 3.07 | 37.58 | 37.66 | 99.6 | 77.28 |

Where *Max. = maximum, min. = minimum, PCV* ***(%)*** *= phenotypic coefficient of variation, GCV* ***(%)*** *= genotypic coefficient of variation,* $H^2$ *= heritability, GAM (%) = genetic advance a percentage of mean.*

**Table 8**. Combined mean squares for different sources of variation and the corresponding coefficient of variation (CV) for the two traits of chickpea genotypes studied at Jari, Sirinka, and Kobo in 2018

| **Traits** | **Source of variation** | | | | | | |
|---|---|---|---|---|---|---|---|
| | MSL df (2) | MSG Df (80) | MSGL Df (160) | MSBR Df (16) | MSRL Df (2) | MSE Df (224) | CV % |
| Zn(mg/100g) | 2.11** | 0.54** | 0.41** | $0.01^{NS}$ | 0.19** | 0.004 | 3.12 |
| Fe(mg/100g) | 301.28** | 9.36** | 10.28** | $0.02^{NS}$ | 0.24** | 0.02 | 2.15 |

Where *Zn = zinc, Fe = iron, MSE = Mean square of error, MSL = Mean square by location, MSG = Mean square by genotype or Treatmnt, MSGL = Mean square genotype by location interaction, MSBR = Mean square block by replication, MSRL = Mean square replication by location, ns = non - significant and ** significant at 1% probability level.*

***Genetic diversity studies*****:** In breeding programmes, crossings between genetically diverse parents having better combining ability are more likely to give better segregants. Tocher's method of genetic diversity studies grouped eighty-one genotypes into five clusters. The high number of clusters indicated that the presence of wide genetic variability among the tested chickpea genotypes. Distribution of the genotypes revealed that the maximum genotypes grouped in Cluster I (38) shared 46.91% of the genotypes, followed by Cluster II comprised 35 genotypes shared 43.21%. Other two Clusters; Clusters III and IV comprised 2 and 5 genotypes, respectively, which constituted 8.64% of the total genotypes (Table 9.) One

standalone cluster; cluster V contributed 1.23% of genotypes from the total distribution. Cluster means were found higher in Cluster IV for protein content; while for Zn and Fe the mean was higher in Cluster V (Table 10). Maximum inter-Cluster distance was observed between Cluster III and IV (73.49) followed by II and IV (54.23) and Cluster IV and V (53.10) (Table 11). The minimum inter-cluster distance was found between clusters I and II ($D^2$=11.82) (Table 11). Therefore, crossing between clusters III and IV would produce maximum segregation at F2. Therefore, hybridization between genotypes from cluster III and cluster IV could produce better segregants in segregating populations for the studied characters and crossing between genotypes from clusters I and II can produce minimum segregants. The grouping pattern had a clear demarcation for entries with high concentrations of different micronutrients and proteins. Similarly, the recent studies of Aliu *et al.* (2016) on genetic diversity in Kosovan chickpea, genotypes for nutritive traits revealed a wide range of variation, and the genotypes were grouped into four clusters. The present study in chickpea indicated substantial genetic variability for protein, zinc, and iron contents and promising genotypes like local check (it is a landrace) with higher protein in cluster IV and Zn and iron concentrations in Cluster V, ICCV-96836 in Cluster V with higher iron and zinc concentration, while DZ-10-11 having high concentration of protein in cluster I and Zinc was in Cluster IV and iron in cluster III along with two other entries were found diverse. Torutaeva *et al.* (2014) reported a relatively rich genetic diversity and good nutritional value of chickpea landraces grown in Kyrgyzstan. An insight into the genetic diversity of promising chickpea genotypes for protein revealed that chickpea genotypes with high protein content were grouped into diverse clusters IE-16-078, IE-16-121, IE-16-080, ICC-1882, Local check clustered in cluster IV and DZ-10-11 and IE-16-115 in cluster I. Similarly, ICCV-96836, DZ-2012-CK-0277, IE-16-120 with higher iron were grouped in clusters V, III, and I and zinc ICCV-96836, IE-16-080 and dalota were grouped in clusters V, IV and II respectively (Figure 1). To hasten biofortification in chickpea, systematic hybridization followed by studies on combining ability should be initiated among these promising and diverse genotypes for genetic improvement of protein and micronutrient.

**Table 9**. Genetic diversity in 81 genotypes of chickpea as determined by protein and zinc and iron contents

| Cluster Number | Number of genotypes | Genotype(s) |
|---|---|---|
| I | 38 | 17,56,10,14,13,66,29,81,28,42,45,72,57,60,6,18,7,71,19 |
| | | 26,20,8,2,23,27,22,4,3,79,64,1,21,25,16,24,11,65,53 |
| II | 35 | 58,61,44,80,52,70,40,73,74,75,76,77,48,41,50,37,43,31 |
| | | 47,67,33,55,35,46,78,36,69,38,49,63,68,32,54,15,19 |
| III | 2 | 51,62 |
| IV | 5 | 5,9,12,30,34 |
| V | 1 | 39 |

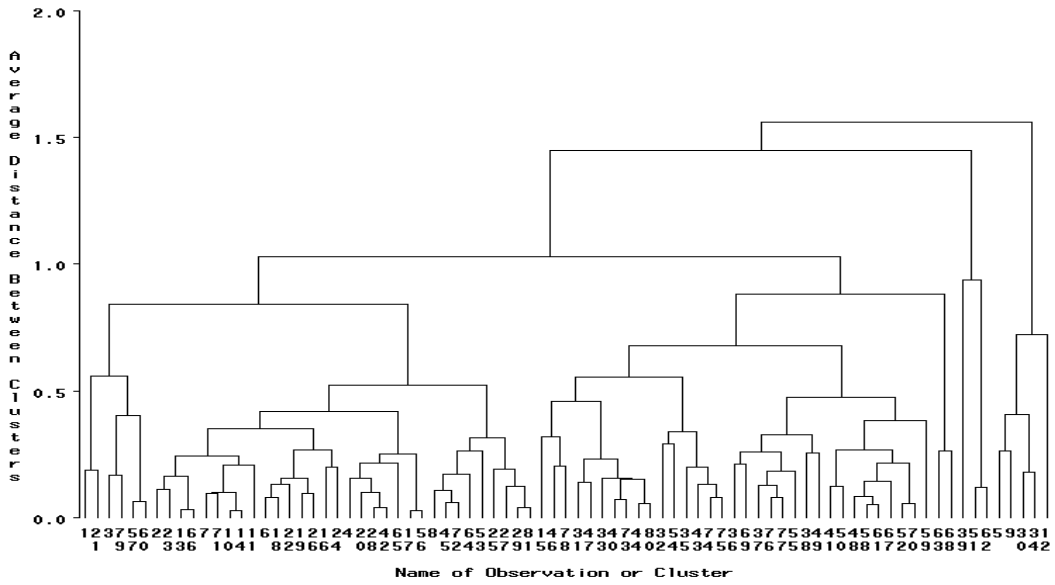


**Figure 1**. Dendrogram constructed using **3** traits of 81 chickpea accessions used in the study at Jari**,** Sirinka and Kobo 2018/19

**Table 10**. Cluster means of 3 element contents of the 81 chickpea genotypes tested

| Cluster Number | Pro-tein | Zinc | Iron |
|---|---|---|---|
| I | 19.45 | 2.08 | 5.95 |
| II | 16.68 | 1.89 | 5.13 |
| III | 15.44 | 2.33 | 8.77 |
| IV | 22.70 | 2.29 | 6.46 |
| V | 17.93 | 3.47 | 10.40 |

**Table 11**. Intra cluster (bold diagonal) and Inter cluster (off diagonal) Pair wise generalized squared distance ($D^2$) among 5 clusters constructed from 81 chickpea genotypes tested.

| Cluster | I | II | III | IV | V |
|---|---|---|---|---|---|
| I | **2.16** | 11.82* | 26.30*** | 15.50** | 32.47*** |
| II | | **2.34** | 12.30* | 54.23*** | 41.75*** |
| III | | | **1.65** | 73.94*** | 26.34*** |
| IV | | | | **3.62** | 53.10*** |
| V | | | | | **3.14** |

*, ** and *** stand for Cluster significant at 5, 1, and 0.1%, respectively. $X^2$ = 9.49, 13.3 and 18.5 at 5, 1 and 0.1% respectively.

**Conclusion**

This study showed substantial genetic variability for protein, iron, and zinc in chickpea varieties (cultivars), landrace and advanced breeding lines. Genetic diversity studies indicated that high protein lines are grouped in cluster IV and lines with higher concentration of micronutrients are grouped in cluster V. Systematic hybridization between promising lines for

proteins and micronutrients chosen from these clusters is suggested to study their combining ability and subsequent use in breeding programmes intended to breed for enhanced levels of protein, iron, and zinc in chickpea. Promising chickpea varieties, landraces and advanced breeding lines must be retested for their stability in performance across locations and years and may be utilized for commercial cultivation or may be deployed in the breeding programmes to further improve the protein, zinc and iron content of chickpea and to enhance the crop's protein, zinc and iron contribution to the human diet. Generally, three results and findings from this research suggest a great chance for genetic improvement of chickpea in different breeding programs for the development of desirable genotypes through hybridization.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

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