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# Genetic diversity and variability for protein and micro nutrients in advance breeding lines and chickpea varieties grown in Andhra Pradesh

**V. Jayalakshmi*, A. Trivikrama Reddy and K.V. Nagamadhuri[1]**

Regional Agricultural Research Station,
Nandyal-518 501, Andhra Pradesh, India.
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**ABSTRACT**

An investigation was taken up to study the nutritional value, extent of genetic variability and genetic diversity in advance breeding lines and chickpea varieties grown in Andhra Pradesh, India. Protein and micronutrient content (iron, zinc, copper and manganese) varied significantly among 54 genotypes. Protein content ranged from 9.5% to 24.9% while micro nutrients varied from 2.6 mg/100 g to 14.6 mg/100 g for iron, 3.5 mg/100 g to 7.7 mg/100 g for zinc, 0.5 mg/100 g to 3.2 mg/100 g for copper and 1.6 mg/100 g to 3.4 mg/100 g for manganese. Moderate to high genotypic variability for protein and micro nutrient content with high heritability and genetic advance indicated the scope for enhancement of traits through selection. Genetic diversity studies revealed five different clusters and that high protein lines are grouped in cluster I and lines with higher concentration of micro nutrients are grouped in clusters IV and 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 programmes intended to breed for superior chickpea cultivars.

**Key words:** *Cicer arietinum* L., Copper, Genetic diversity, Genetic variability, Iron, Manganese, Zinc.

## INTRODUCTION

Globally chickpea (*Cicer arietinum* L.) is the second most important pulse crop occupying 14.80 million ha of area with 14.23 million tons of production (FAO STAT, 2016). India is the largest producer and consumer of chickpea in the world with cultivable area of 8.84 million ha and 8.29 million tons of production (http://dpd.dacnet.nic.in/). Chickpea has high nutritional value and can be a best supplement for meat. Apart from being a good source of protein, chickpea consumption is known to have potential beneficial effects on lowering risk of some of the important human diseases such as cardio vascular diseases, type 2 diabetes, digestive diseases and some types of cancers (Jukanti *et al.,* 2012). Chickpea also contributes significant quantities of several minerals in the human diet. Bio-fortification of crops can be addressed through mineral fertilization (Kayan *et al.*, 2015) and conventional plant breeding approaches (Mayer *et al.*, 2008). Although chickpea has the potential to supply ~40% of the adult Recommended Dietary Allowance for manganese and copper, or ~15% for iron and zinc, it was observed that seed concentrations can vary across genotypes (Wood and Grusak, 2007). The identification of chickpea accessions rich with protein and micronutrients help breeders to identify donors for targeted breeding to breed for elevated levels of protein and micronutrient bio-fortification. Considering the importance of this crop as a dietary element across a major part of global population, protein and micronutrient rich chickpeas have a potential to address global health issues. Therefore, in the present study an attempt has been made to study the genetic variability for protein and micronutrient content in chickpea varieties grown in Andhra Pradesh and also advance breeding lines of chickpea developed at Regional Agricultural Research Station (RARS), Nandyal, Andhra Pradesh, India to assess their possible use as donors in improving nutritional quality of chickpea.

## MATERIALS AND METHODS

Twenty six *desi* and twenty *kabuli* high yielding advance breeding lines bred at RARS, Nandyal along with eight popular and high yielding varieties grown in Andhra Pradesh, India were studied for their genetic variability for protein and micronutrient composition *viz*., iron, zinc, copper and manganese. Fifty four genotypes were grown in a randomized block design with three replications during *rabi* 2016-17 at RARS, Nandyal. The *desi* check varieties are NBeG 3, NBeG 47, NBeG 49, JG 11 and JAKI 9218 where as *kabuli* check varieties are KAK 2, Vihar and NBeG 119. The soil at RARS, Nandyal is a calcareous vertisol with PH 8.3. The soils are low in available N, medium in phosphorous availability and high in potassium. The micro nutrients iron and zinc are below critical level where as copper and manganese status is above critical level.

Mature dry seeds from each genotype were harvested from each replication and powdered seed samples were utilized for estimation of protein and micro nutrients,

*Corresponding author's e-mail: veera.jayalakshmi@gmail.com
[1]Regional Agricultural Research Station, Tirupati-517 502, Andhra Pradesh, India.

iron, zinc, copper and manganese. Protein content was measured by estimation of nitrogen in seed samples using a single digest (sulfuric acid selenium digestion). Aliquots of digests were used to determine nitrogen by distillation with sodium hydroxide using Kjeldahl method (Kjeldhal, 1883). Total nitrogen content in powdered seeds was multiplied with a factor 6.25 to arrive at protein content (%) of grains (Jones, 1941). The micronutrients in the chickpea seeds (iron, zinc, copper and manganese) were analyzed by Atomic Absorption Spectroscopy (AAS) by measuring absorbance of the species at its resonance wavelengths. One gram of oven dried powdered seeds was digested with 10 ml of triacid mixture ($HNO_3$ : $H_2SO_4$ : $HCLO_4$ @ 9:4:1). The volume of digested samples was made up to 100 ml. The filtered extract was used to measure the concentration of various elements by relative method using analytical grade solutions of elements of interest (Tandon, 1993).

Genetic variability parameters *viz.*, coefficients of variation, hetitability and genetic advance were estimated according to Singh and Chaudhary (1977) where as genetic divergence analyses were carried out as per Mahalanobis (1936) and Rao (1952).

## RESULTS AND DISCUSSION

**Genotypic variation for protein:** Analysis of variance for protein in 54 genotypes revealed highly significant sum of squares due to genotypes which indicated that there is substantial genetic variability among lines for protein content. This is also supported by a wide range as observed in the mean values of these characters (Table 1). In the present study, the protein content of the seed ranged from 9.5% (NBeG 805) to as high as 24.9% (NBeG 507). Two *desi* breeding lines NBeG 507 (24.9%), NBeG 506 (23.8%) and three *kabuli* genotypes NBeG 399 (24.7%), NBeG 458 (23.4%), NBeG 724 (23.5%) were characterized by significantly higher protein content than the relevant popular varieties JG 11 and KAK 2 in *desi* and *kabuli* groups, respectively which recorded a protein content of 20%. Sharma *et al.* 2013 and Jadhav *et al.,* 2015 reported wide variability in protein content of chickpea genotypes. Five chickpea genotypes of the present study with higher protein content have already been found to be high yielders, can be tested for their stability in performance across locations and years and may be utilized for commercial cultivation or may be deployed in breeding programme to further improve protein content of chickpea and to enhance the crop’s protein contribution to the human diet.

The intrinsic genetic variability for protein content amenable to selection and associated genetic gain can be predicted based on heritability and expected genetic advance (Table 2). Genotypic and phenotypic coefficients of variation, pcv and gcv values (20.99% and 21.97%), heritability (91%) and also expected genetic advance (41.32%) were high for protein. Kozgar *et al.* (2012) and Gaikwad *et al.* (2011) and several others reported high values of broad sense heritability estimates for protein content. The high values 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.

**Genetic variability for micro nutrients:** Chickpea seeds provide important minerals to the human diet, e.g. iron, magnesium, zinc, manganese, selenium and chromium (Wood and Grusak 2007; Jukanti *et al.* 2012). Selection and use of chickpea genotypes with higher potential uptake of minerals is one of the viable options to enhance mineral concentration of seeds and increased supply of minerals through food is one of the best options proposed for sustainable food based solution to global malnutrition. Mineral composition of chickpea with respect to iron, zinc, copper and manganese in 54 chickpea lines is presented in Table 1. The observed variability in 54 genotypes is significant and varied from 2.6 mg/100 g (NBeG 829) to 14.6 mg/100 g (NBeG 47) for iron, 3.5 mg/100 g (NBeG 699, NBeG 873) to 7.7 mg/100 g (Vihar) for zinc, 0.5 mg/100 g (NBeG 873, NBeG 731) to 3.2 mg/100 g ( NBeG 49) for copper and 1.6 mg/100 g (NBeG 806) to (NBeG 3, N BeG 47) 3.4 mg/100 g (NBeG 3, NBeG 47) for manganese.

Among *desi* genotypes the highest iron content was observed in NBeG 47 (14.6 mg/100g) followed by NBeG 49 (13.2 mg). These two are recently released high yielding varieties from RARS, Nandyal, the former being a tall semi erect plant type, suitable for mechanical harvesting (Muniratnam *et al*., 2015). NBeG 47 (7.6 mg), JAKI 9218 (7.2 mg) and NBeG 785 (7.0 mg) are characterized by significantly higher zinc concentration. In *kabuli* genotypes, KAK 2 (11.8 mg, 7. mg per 100g) and Vihar (11.6 mg, 7.0 mg per 100g), the two popular varieties of chickpea, extensively grown in Andhra Pradesh have recorded high iron as well zinc content. Recent studies (Diapari *et al.*, 2014, Torutaeva *et al.* 2014 and Upadhyaya *et al.,* 2016) investigated genetic diversity and nutritive value of chickpea germplasm and indicated scope for molecular breeding for improvement of nutritive value of chickpea. The average copper content was significantly higher in NBeG 49 (3.2 mg) among *desi* genotypes and Vihar (3.0 mg) in *kabuli* genotypes. The average manganese content was higher in three varieties released by RARS, Nandyal *viz.,* NBeG 47 (3.4 mg), NBeG 3 (3.4 mg) and NBeG 49 (3.2 mg) and also in two advance breeding lines NBeG 785 (2.8 mg) and NBeG 779 (3.1 mg). Among *kabuli*, Vihar had higher manganese (3.2 mg) and advance *kabuli* breeding lines NBeG 399 (2.6 mg), NBeG 440 (2.6 mg), NBeG 471 (2.4 mg), NBeG 529 (2.4 mg) NBeG 731 (2.8 mg), NBeG 732 (2.5 mg) were also promising and as good as Vihar. These chickpea genotypes of the present study can be considered as good source for these four essential mineral micronutrients and

**Table 1:** Protein and micronutrient content in 54 chickpea genotypes.

| Genotype | Protein % | Iron | Zinc mg/100 g | Copper | Manganese |
|---|---|---|---|---|---|
| NBeG 147 | 15.3 | 4.7 | 4.8 | 0.8 | 2.4 |
| NBeG 399 | 24.7 | 4.9 | 4.3 | 1.0 | 2.6 |
| NBeG 440 | 19.9 | 7.6 | 5.3 | 0.9 | 2.6 |
| NBeG 452 | 20.5 | 5.7 | 4.4 | 1.0 | 2.2 |
| NBeG 458 | 23.4 | 5.2 | 4.7 | 0.9 | 1.7 |
| NBeG 460 | 21.4 | 4.4 | 4.3 | 0.9 | 2.3 |
| NBeG 471 | 22.3 | 4.7 | 4.4 | 0.8 | 2.4 |
| NBeG 506 | 23.8 | 5.0 | 4.3 | 0.8 | 2.6 |
| NBeG 507 | 24.9 | 5.4 | 4.9 | 0.8 | 2.6 |
| NBeG 510 | 22.5 | 5.3 | 4.5 | 0.7 | 2.4 |
| NBeG 511 | 14.0 | 3.9 | 4.3 | 0.7 | 2.2 |
| NBeG 529 | 20.2 | 4.4 | 4.3 | 0.7 | 2.4 |
| NBeG 620 | 20.2 | 4.5 | 4.8 | 0.9 | 1.9 |
| NBeG 699 | 18.1 | 3.5 | 3.5 | 0.6 | 1.9 |
| NBeG 723 | 19.6 | 4.4 | 4.4 | 0.9 | 2.0 |
| NBeG 724 | 23.5 | 4.1 | 4.4 | 0.7 | 1.9 |
| NBeG 731 | 20.4 | 6.2 | 4.4 | 0.5 | 2.8 |
| NBeG 732 | 21.1 | 6.6 | 5.1 | 0.8 | 2.5 |
| NBeG 738 | 21.3 | 4.9 | 5.0 | 0.8 | 2.2 |
| NBeG 740 | 20.9 | 7.2 | 5.0 | 1.0 | 2.4 |
| NBeG 753 | 20.0 | 8.7 | 5.7 | 1.1 | 2.7 |
| NBeG 754 | 16.4 | 8.0 | 5.1 | 1.1 | 2.6 |
| NBeG 773 | 21.1 | 8.5 | 4.6 | 1.0 | 2.7 |
| NBeG 776 L | 18.0 | 8.3 | 4.4 | 1.0 | 2.3 |
| NBeG 776 S | 20.9 | 7.0 | 4.2 | 0.8 | 2.4 |
| NBeG 778 | 20.1 | 8.0 | 4.7 | 1.0 | 2.3 |
| NBeG 779 | 17.5 | 12.4 | 6.6 | 1.1 | 3.1 |
| NBeG 785 | 14.9 | 10.2 | 7.0 | 1.1 | 2.8 |
| NBeG 786 | 14.8 | 10.3 | 5.8 | 1.0 | 2.6 |
| NBeG 789 | 13.0 | 7.5 | 4.9 | 0.9 | 2.4 |
| NBeG 790 | 13.0 | 4.2 | 4.6 | 0.8 | 1.9 |
| NBeG 798 | 12.5 | 5.9 | 3.8 | 0.7 | 2.0 |
| NBeG 801 | 13.1 | 6.3 | 3.7 | 0.7 | 2.0 |
| NBeG 805 | 9.5 | 3.5 | 3.8 | 0.7 | 2.6 |
| NBeG 806 | 15.9 | 2.7 | 3.6 | 0.7 | 1.6 |
| NBeG 807 | 12.2 | 4.0 | 4.2 | 0.9 | 1.9 |
| NBeG 810 | 10.9 | 3.4 | 3.9 | 0.7 | 2.0 |
| NBeG 829 | 14.5 | 2.6 | 4.0 | 0.7 | 1.8 |
| NBeG 833 | 11.3 | 3.8 | 3.8 | 0.7 | 2.3 |
| NBeG 835 | 10.9 | 4.7 | 4.2 | 0.7 | 2.2 |
| NBeG 837 | 10.8 | 3.4 | 4.5 | 0.7 | 2.0 |
| NBeG 844 | 14.9 | 3.5 | 4.3 | 0.7 | 2.1 |
| NBeG 863 | 20.5 | 3.6 | 4.2 | 0.7 | 2.1 |
| NBeG 864 | 20.5 | 5.3 | 4.5 | 0.8 | 2.1 |
| NBeG 873 | 21.0 | 5.1 | 3.5 | 0.5 | 2.1 |
| NBeG 1004 | 19.3 | 6.9 | 4.4 | 0.7 | 1.9 |
| NBeG- 3 | 18.5 | 9.2 | 6.5 | 2.7 | 3.4 |
| NBeG-119 | 18.8 | 8.0 | 6.0 | 2.2 | 2.0 |
| NBeG-47 | 18.4 | 14.6 | 7.6 | 2.5 | 3.4 |
| NBeG-49 | 16.8 | 13.2 | 6.5 | 3.2 | 3.2 |
| KAK 2 | 20.0 | 11.8 | 6.9 | 1.6 | 2.0 |
| Vihar | 19.1 | 11.3 | 7.7 | 3.0 | 3.0 |
| JG-11 | 20.0 | 12.4 | 5.2 | 2.2 | 2.4 |
| JAKI 9218 | 17.6 | 11.9 | 7.2 | 2.6 | 2.5 |
| **General Mean** | 18.06 | 6.54 | 4.86 | 1.07 | 2.35 |
| S.EM± | 1.17 | 0.66 | 0.33 | 0.13 | 0.24 |
| C.D. 5% | 3.28 | 1.86 | 0.93 | 0.36 | 0.66 |
| C.D. 1% | 4.35 | 2.46 | 1.24 | 0.47 | 0.87 |
| C.V. 1% | 11.24 | 17.58 | 11.86 | 20.69 | 17.38 |

**Table 2:** Estimates of genetic parameters for protein and micronutrient content in 54 chickpea genotypes.

| Character | Mean | Range | GCV(%) | PCV(%) | Heritability(%) | GA as % of mean |
|---|---|---|---|---|---|---|
| Protein (%) | 18.06 | 9.5 - 24.9 | 20.99 | 21.97 | 0.91 | 41.32 |
| Iron (mg/100 g) | 6.54 | 2.6 – 14.6 | 44.68 | 45.82 | 0.95 | 89.77 |
| Zinc (mg/100 g) | 4.86 | 3.5 – 7.7 | 20.75 | 21.85 | 0.90 | 40.60 |
| Copper (mg/100 g) | 1.07 | 0.5 – 3.2 | 59.31 | 60.50 | 0.96 | 119.7 |
| Manganese (mg/100 g) | 2.35 | 1.6 – 3.4 | 13.99 | 17.21 | 0.66 | 23.42 |

**Table 3:** Genetic diversity in 54 genotypes of chickpea as determined by protein and micronutrient contents.

| Cluster Number | Number of genotypes | Genotype(s) |
|---|---|---|
| **I** | 29 | NBeG 147, NBeG 399, NBeG 440, NBeG 452, NBeG 458, NBeG ,460,NBeG 471, NBeG 506, NBeG 507, NBeG 510, NBeG 529, NBeG,620,NBeG 699, NBeG 723, NBeG 724, NBeG 731, NBeG 732, NBeG 738,NBeG 740, NBeG 754, NBeG 773, NBeG 776 L, NBeG 776, NBeG 778, NBeG 844, NBeG 863, NBeG 864, NBeG 873, NBeG 1004 |
| **II** | 13 | NBeG 511, NBeG 789, NBeG 790, NBeG 798, NBeG 801, NBeG 805, NBeG 806, NBeG 807, NBeG 810, NBeG 829, NBeG 833, NBeG 835, NBeG 837 |
| **III** | 1 | NBeG 753 |
| **IV** | 4 | NBeG 779, NBeG 785, NBeG 786, KAK 2 |
| **V** | 7 | NBeG- 3, NBeG119, NBeG47 , NBeG49, Vihar , JG11 , JAKI 9218 |

hold promise in addressing micro nutrient malnutrition of population. However, chemical composition of different chickpea genotypes can vary due to influence of climatic, environmental (e.g. soil composition) or physiological factors (Iqbal *et al.* 2006). Therefore, the nutritional value of promising genotypes has to be further verified by growing them again in the same field. Further, PCV and GCV (table 2) were high for iron, zinc and copper content (> 20%) where as for manganese, moderate values were noticed. Heritability values for iron, zinc, copper and manganese contents were higher in magnitude (66% - 96%). This reflected that selection could be effective for improvement of the traits which is also indicated by estimates of high expected genetic advance (above 20 %).

**Genetic diversity studies:** The promising chickpea varieties identified among fifty four genotypes should be deployed in plant breeding programmes to further enrich nutritional quality of chickpeas. In breeding programmes crossing between genetically diverse parents having better combining ability are more likely to give better segregants. Though fifty four genotypes were clustered into five distinct groups (Table 3), cluster I has accommodated as many as twenty nine genotypes where as thirteen advance breeding lines formed cluster II. The third cluster was the smallest with single genotype NBeG 753. Cluster four had three advance breeding lines (NBeG 779, NBeG 785, NBeG 786) and one *kabuli* variety KAK 2. All the recently released varieties as well as popular varieties in Andhra Pradesh were grouped into fifth cluster. Torutaeva *et al* (2014) reported a relatively rich genetic diversity and good nutritional value of chickpea landraces grown in Kyrgyzstan. In this study, protein content is the major determinant of genetic diversity among 54 genotypes with 34.8% contribution where as iron content contributed 32.6% for genetic diversity. Copper content contributed 21.59% followed by small contribution of 6.78% by zinc and 4.26% by manganese (Table 5).

**Table 4:** Average inter and intra cluster distances in 54 chickpea genotypes.

| Cluster | I | II | III | IV | V |
|---|---|---|---|---|---|
| I | 1.72 | 2.9 | 2.38 | 4.16 | 6.14 |
| II | | 1.52 | 3.65 | 4.79 | 6.78 |
| III | | | 0 | 2.29 | 4.65 |
| IV | | | | 2.14 | 4.53 |
| V | | | | | 2.64 |

**Table 5:** Relative contribution of protein and micronutrient content for genetic diversity in 54 chickpea genotypes.

| Character | Times ranked First | Contribution (%) |
|---|---|---|
| Protein | 498 | 34.80 |
| Iron | 466 | 32.56 |
| Zinc | 97 | 6.78 |
| Copper | 309 | 21.59 |
| Manganese | 61 | 4.26 |

An insight into genetic diversity of promising chickpea genotypes for protein revealed that chickpea genotypes with high protein content were grouped into diverse clusters (NBeG 399, NBeG 458, NBeG 506, NBeG 507, NBeG 724 clustered in cluster I and KAK 2 in cluster IV and JG11 in cluster V). Similarly KAK 2, NBeG 785, NBeG 47, NBeG 49, Vihar and JAKI 9218 with higher iron and zinc were grouped in clusters IV and V. Three clusters *viz.*, cluster I (NBeG 399, NBeG 440, NBeG 471, NBeG 529), cluster V (NBeG 49 and Vihar and NBeG 3) and cluster IV (NBeG 785) included genotypes with higher manganese and copper content (Table 3 and 4).

## CONCLUSION

This study has revealed substantial genetic variability for protein and micronutrients ( iron , zinc, manganese and copper) in chickpea varieties and advance breeding lines. Promising chickpea varieties and breeding lines must be retested for confirmation of nutritional value of high performing lines. Genetic diversity studies indicated that high protein lines are grouped in cluster I and lines with higher concentration of micro nutrients are grouped in clusters IV and 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 programmes intended to breed for enhanced levels of protein and micronutrients in chickpea.

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