Electronic Journal of Plant Breeding, 9(4): 1370-1377 (Dec 2018) DOI: 10.5958/0975-928X.2018.00171.0
ISSN 0975-928X

Research Article

Genetic diversity assessment of Indian chickpea varieties for protein and micronutrient composition

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(Received: 01 Aug 2018; Revised: 01 Dec 2018; Accepted: 02 Dec 2018)

Abstract
To address the feasibility of developing protein rich and micronutrient dense varieties of chickpea, it is imperative to assess the existing variability of protein as well as micronutrients. The present efforts were made with 27 cultivars grown in India to ascertain the variability and diversity of these genotypes with respect to nutritive traits protein, iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). Among the varieties, Virat had higher concentration of protein, Zn and Mn; CSJ 515 had higher concentration of protein and Fe whereas, Kripa had higher concentration of Cu. Higher heritability with high genetic advance as per cent of mean was observed for Cu, Zn and Mn indicating that these traits are under additive gene action and selection pressure could effectively be applied for their improvement. With the genetic diversity analysis, it is inferred that crosses can be made between genotypes Virat with higher protein and Zn concentration from Cluster IV, Kripa in Cluster V with higher copper concentration, while CSJ 515 having high concentration of protein and Fe in Cluster III to realize better segregants for simultaneous improvement of more than one nutrient.

Key words
Chickpea, copper, zinc, iron, manganese, protein, genetic improvement

Introduction

Green revolution in India was fruitful in increasing the production and productivity of food grain crops. Consequently, self-sustain was achieved in majority of food crops and now it's high time to focus on quality food rather than mere high yield per unit area. "Bio-fortification" purposes to enhance the micronutrient content of many staple food crops through conventional breeding or metabolic engineering. In India, chickpea is grown annually in an area of 10.74 million ha with a total production of 9.88 million tons and average productivity of 919.9 kg ha^{-1} (FAO, 2016). India ranks top in production and consumption of chickpea in the world followed by Pakistan, Mexico, Turkey, Canada, Iran, Australia, Tanzania, Ethiopia, Spain and Burma. In India, it is cultivated to a large extent in Madhya Pradesh and Southern states like Andhra Pradesh, Telangana and Karnataka. Among pulses, chickpea is an excellent source of complex carbohydrates, proteins, dietary fiber and energy (Ereifez *et al.*, 2001 and Wang *et al.*, 2010). Also, it is an important source for micronutrients like Fe, Zn, Mg and Cu (Ereifez *et al.*, 2001 and Abbo *et al.*, 2000). Since, cereals combined with pulses are a major diet for billons of people and targeting chickpeas could some extent alleviate the malnutrition problem. Studies on mining the protein and micro - nutrients level is fundamental in developing bio-fortified varieties as this helps to ascertain the existing variability levels, genetic and non-genetic causes for the variation observed. Significant genetic variation for seed size, iron, zinc and protein content was reported in chickpea (Thavarajah *et al.*, 2009 and Singh *et al.*, 2014). Effect of genotype, geographical location, and temperature and soil factors on micronutrients and protein accumulation was reported in other pulse crop like lentil (Thavarajah *et al.*, 2010 and Thavarajah *et al.*, 2011). Chickpea may not be an exception to this and limited coherent studies on levels of micronutrient and protein content are available in Indian sub-continent conditions. Availability of such information can pave the way to design a sound breeding strategy for enhancing the nutrient load in chickpea. Thus, the present study is taken up with the objectives to determine the protein and micronutrient levels in different genotypes; to estimate the genetic variability, heritability and genetic advance as per cent of mean for different traits and to identify suitable parents that can prospect in evolving better genotypes with tailored traits.

Material and Methods

Twenty seven high yielding and popular chickpea varieties of India were subjected to genetic variability for protein and micronutrient content *viz*., iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). The selected varieties were grown in a randomized block design with three replications during *rabi* 2016-17 at RARS, Nandyal. The soil at RARS, Nandyal is a calcareous vertisol with P^H 8.3. Soil nutrient status indicated that the soils are low in available nitrogen, medium in phosphorous and high in potassium availability. The micro nutrients iron and zinc are below critical level where as copper and manganese status is above critical level.

Matured dry seeds from each variety were harvested from each replication and powdered seed samples were utilized for estimation of protein and micro nutrients *viz*., iron, zinc, copper and manganese. Protein content was estimated by analyzing the nitrogen in seed samples using a single digest (sulfuric acid selenium digestion). Aliquots of digests were used to determine nitrogen using sodium hydroxide by Kjeldahl distillation method (Kjeldahl, 1883). Total nitrogen content in powdered seeds was multiplied by a factor 6.25 to arrive at seed protein content (%) (Jones, 1941). The micronutrient (iron, zinc copper and manganese) content of chickpea seeds were analyzed using 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, heritability and genetic advance as per cent of mean were estimated according to Singh and Chaudhary (1977) whereas genetic divergence analysis was carried out as per Mahalanobis (1936) and Rao (1952).

Results and Discussion

The seed protein and micro - nutrient values for iron, zinc, copper and manganese contents of 27 chickpea varieties were subjected to analysis of variance and it revealed (Table 1) highly significant differences for all the traits.

The range of protein content in 27 genotypes is quite high and varied from 15.6% (JG 36) to 23.3 % (CSJ 515). Considerably high protein content was recorded in CSJ 515 (23.3) and Virat (21.8). The protein content of currently available chickpea cultivars generally ranges between 20 and 22 %, while a wide range of variation, from 12 to 30 %, exists in chickpea germplasm (Sharma *et al*., 2013; Jadhav *et al*. 2015). Of late, there is an increasing awareness of the nutritive value and health benefits of chickpea. (Jukanti *et al*., 2012). The *in vitro* protein digestibility of chickpea seeds was found to be higher compared with those for pigeonpea, mungbean, urdbean and soybean (Chitra *et al*., 1995). Since 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 also in utilizing promising lines in further breeding programmes. Two favourable genotypes Virat and CSJ 515 of the present study can be utilized in this direction.

Delivering micro nutrients through commonly eaten foods have greater role in correcting micro nutrient malnutrition than dietary supplementation. In addition to protein, chickpea is a good source of carbohydrates, dietary fibre, minerals (molybdenum, manganese, copper, phosphorus, iron and zinc) and vitamins (riboflavin, niacin, thiamin, folate and the vitamin A precursor beta-carotene) (Jukanti *et al*., 2012). The mineral nutrient composition of 27 varieties varied significantly for each of the four nutrients studied (Table 1). The variability of these genotypes for Fe, Zn, Cu and Mn ranged from 8.0 mg/ 100g (NBeG 119) to 14.7 mg/ 100g (JG 36); 3.7 mg/ 100g (Vishal) to 10.1 mg/ 100g (Virat); 1.5 mg/ 100g (JG 130, JG 315) to 7.4 mg/ 100g (Kripa) and 1.5 mg/ 100g (MNK 1) to 3.9 mg/100g (JG 63) respectively. These resulted are supported by findings of other researchers and in the literature, the concentration of micronutrients were reported to vary from 3 to 14.3 for Fe, 2 to 20 for Zn, 0.09 to 9.4 for Mn and 0.27 to 11.6 for Cu (Iqbal *et al.,* 2006, Petterson *et al*., 1997). Among 27 genotypes, JG 36 (14.7) had higher Fe concentration followed by JG 26 (14.6) and NBeG 47 (14.6). Many other entries like GNG 1969 (13.8), JG 130 (13.7), Kripa (13.2), GNG 1958 (13.2), CSJ 515 (13.2) and eight other genotypes had on par Fe concentration with the best genotype JG 36. Genotype Virat had significantly higher concentration of Zn (10.1) in comparison with other genotypes tested. Kripa (7.4), a large seeded kabuli chickpea had higher concentration of Cu. JG

Electronic Journal of Plant Breeding, 9(4): 1370-1377 (Dec 2018) DOI: 10.5958/0975-928X.2018.00171.0
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63 (3.9) and eleven other genotypes had recorded higher Mn concentration. Chickpea varieties in this study are worthy sources of protein, Fe and other micro nutrients .Virat had higher concentrations of protein, Zn and Mn while CSJ 515 had higher concentration of protein and Fe. Recent studies of other researchers (Thavarajah *et al.,* 2012 and Jayalakshmi *et al.*, 2018) have revealed better nutritive value of chickpea genotypes for protein and micronutrient content. Identification of chickpea genotypes with higher quality protein and trace elements will help in delivering better quality infant foods (Rincon *et al*., 1998) and also will have implications in breeding quality chickpeas.

Extent of genetic variability and genetic diversity in the breeding material, and the expected progress through selection are the key to initiate planned breeding programmes aimed at improvement of protein and other quality traits under consideration. The genotypic and phenotypic coefficients of variation were higher for Cu concentration and moderate to low for Fe, Zn, Mn and protein concentration (Table 2). Genotypic coefficient of variation along with heritability estimates would give a better idea of genetic gain though phenotypic selection (Burton, 1952). Characters having higher heritability estimates are less influenced by environment and they are under the influence of more number of fixable factors. Heritability (broad sense) was recorded higher for all the traits and the Cu and Zn concentrations topped among them followed by Fe, Mn and Protein. High heritability with high genetic advance as per cent of mean was noticed for Zn, Mn and Cu. This could be due to additive gene action and selection pressure could effectively be exerted on these traits for their improvement. High heritability with moderate genetic advance as per cent of mean was observed for Fe and protein content revealed that non-additive gene action could also be governing these traits; hence, selection would be effective at later generations. These findings are in confirmation of the findings of Aliu *et al.,* 2016; Ray *et al*., 2014 and Bueckert *et al*., 2011.

Genetic diversity as revealed by relative contribution of all characters was presented in (Table 3). Among protein and four micro -nutrients, Zn concentration contributed maximum towards genetic diversity of the genotypes followed by Cu and Mn. Tocher's method of genetic diversity studies grouped twenty seven genotypes into five clusters (Table 4). Cluster I was the largest and constituted by twenty one genotypes followed by cluster III with three genotypes and clusters II, IV and V with one genotypes each (Table 4). Cluster means were found higher in Cluster IV for protein content, Zn and Mn; while for Fe, the mean was higher in Cluster III and Cluster II for Cu (Table 6). Maximum inter Cluster distance was observed between Cluster III and V (6.61) followed by V and IV (6.29) and Cluster IV and III (5.91) (Tables 5). The grouping pattern had clear demarcation for entries with high concentration of different micronutrients and protein. 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 and nutrient contents and promising genotypes like Virat with higher protein and Zn concentration in Cluster IV, Kripa in Cluster V with higher copper concentration, while CSJ 515 having high concentration of protein and Fe was in Cluster III along with two other entries were found diverse. To hasten bio-fortification 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 micro nutrient.

Acknowledgements
Authors are grateful to University Grants commission for providing financial assistance in the form of UGC Research Award 2016-18 to the first author for carrying out this research work.

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Table 1. Protein and micronutrient content in 27 chickpea genotypes

S.No	Genotype	Protein (%)	Fe (mg / 100 g)	Zn (mg / 100 g)	Cu (mg / 100 g)	Mn (mg / 100 g)
1	Kripa	18.8	13.2	7.4	7.4	2.3
2	JG 14	19.5	11.9	5.6	2.8	2.5
3	JG 36	15.6	14.7	7.3	1.9	3.0
4	JAKI 9218	17.6	11.9	7.2	2.6	2.5
5	JG 315	18.4	10.4	7.5	1.5	3.2
6	KAK 2	20.0	11.8	6.9	1.6	2.0
7	JG 16	17.0	11.5	6.7	1.7	3.1
8	JG 130	16.7	13.7	5.5	1.5	3.2
9	VIJAY	16.0	11.5	4.9	2.8	2.8
10	VISHAL	19.7	11.6	3.7	1.9	1.8
11	DIGVIJAY	16.5	11.2	5.4	2.2	2.4
12	JG 11	20.0	12.4	5.2	2.2	2.4
13	VIHAR	19.1	11.3	7.7	3.0	3.0
14	MNK 1	20.0	9.6	5.4	2.6	1.5
15	JG 322	17.3	10.0	7.1	2.7	3.1
16	GNG 1958	15.9	13.2	7.2	2.4	3.6
17	JG 63	18.0	12.0	7.4	3.1	3.9
18	NBeG 3	18.5	9.2	6.5	2.7	3.4
19	GNG 1581	19.7	9.3	7.5	2.4	2.1
20	CSJ 515	23.3	13.2	4.4	1.9	2.3
21	JG 6	17.6	9.7	6.5	2.6	3.4
22	VIRAT	21.8	11.4	10.1	2.7	2.9
23	NBeG 119	18.8	8.0	6.0	2.2	2.0
24	NBeG 47	18.3	14.6	7.6	2.5	3.4
25	NBeG 49	16.7	13.2	6.5	3.2	3.2
26	GNG 1969	19.1	13.8	7.7	2.6	2.1
27	JG 26	19.9	14.6	7.3	2.0	2.7
General Mean		18.5	11.8	6.6	2.5	2.7
S.Em±		1.1	1.1	0.3	0.3	0.3
C.D @5 %		3.1	3.1	1.0	0.9	1.0
C.V. %		10.3	15.9	9.1	21.9	22.0

Table 2. Genetic variability for protein and micronutrient content in 27 chickpea genotypes

S. No	Characters	Mean	Range	GCV (%)	PCV (%)	Heritability (%)	GA as % of mean
1	Protein (%)	18.51	15.6 -23.3	7.69	9.71	62.0	12.56
2	Fe	11.80	8.0 – 14.7	12.01	15.12	63.0	19.66
3	Zn	6.60	3.7 – 10.1	19.03	19.74	92.0	37.81
4	Cu	2.54	1.5 – 7.4	40.99	42.89	91.0	80.68
5	Mn	2.73	1.5 – 3.9	18.20	22.20	67.0	30.73

Table 3. Relative contribution of protein and micronutrient content for genetic diversity in 27 chickpea genotypes

Character	Times ranked First	Contribution (%)
Protein	19	5.41
Fe	51	14.53
Zn	164	46.72
Cu	64	18.23
Mn	53	15.10

Table 4. Genetic diversity in 27 genotypes based on protein and micronutrient content

Cluster Number	Number of genotypes	Genotype(s)
I	21	JG 14, JG 36, JAKI 9218, JG 315, KAK 2, JG 16, DIG VIJAY, JG 11, VIHAR, MNK 1, JG 322, GNG 1958, JG 63, NBeG 3, GNG 1581, JG 6, NBeG 119, NBeG 47, NBEG 49, GNG 1969, JG 26
II	1	VIJAY
III	3	VISHAL, CSJ 515, JG 130
IV	1	VIRAT
V	1	Kripa

Table 5. Average inter and intra cluster distances in 27 chickpea genotypes

Cluster	I	II	III	IV	V
I	1.93	2.61	3.11	3.79	5.62
II		0	2.25	5.73	5.49
III			2.2	5.91	6.61
IV				0	6.29
V					0

Table 6. Cluster means for protein and micronutrient content in 27 chickpea genotypes

Cluster Number	Protein (%)	Fe (mg/ 100 g)	Zn (mg/ 100 g)	Cu (mg/ 100 g)	Mn (mg/100 g)
I	18.27	11.62	6.78	2.4	2.79
II	16.04	11.53	4.87	2.83	2.83
III	19.89	12.82	4.54	1.76	2.42
IV	21.82	11.37	10.1	2.67	2.93
V	18.84	13.2	7.37	7.43	2.27