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Exploiting Genomic Resources for Efficient Utilization of Chickpea (*Cicer arietinum* L.) Crop Improvement based on Agro-morphological, Yield and Nutritional Traits

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ABSTRACT

Background: In an effort to develop micronutrient-rich chickpea lines, a study to examine the variability in yield and nutritional traits was conducted.

Methods: 99 genotypes were studied, the data was recorded and analyzed on yield traits, protein, iron and zinc content in *Rabi* 2019-20.

Result: Maximum variability was recorded in plant yield followed by iron concentration (mg/kg), hundred seed weight and number of pods per plant. Of the total entries, nine entries namely, RGH4, RGH56, RG2016-84, ICC251762, RGH53, IPC98-12, RG2016-03, ICC1053 and RGH58 recorded high protein content (>20%). All these entries possessing high protein had pink flower. One chickpea accession (RGH53) accumulated the highest concentration for both protein and zinc, with an average of 21.86% and 73.00 ppm, respectively, but it showed low iron concentration (20.77 ppm). The first six principal components provided a reasonable summary of the data and explained 80.19% of the total variation. Ninety nine genotypes were grouped into ten clusters. Maximum inter cluster distance was observed between clusters VI and IX; VI and VII and IV and VI. The genotypes of these clusters are suggested for utilization in the crossing programs to breed varieties of chickpea for high yield with amenability to nutrients.

Key words: Amenability, Clusters, Characterization, Nutrition.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) popularly known as Gram, Bengal gram, Egyptian pea, Chana and Garbanzo bean is one of the first grain legumes to be domesticated by humans in old world. The genus *Cicer* belongs to the sub-family Papilionaceae of the family Leguminoceae, (Bentham and Hooker, 1970) now known as Fabaceae. The origin of the crop is considered to be Western Asia, from where it has been transferred in India and to the other parts of the world. Importance of pulses is relatively more in our country as its contribution in nutrient supply is far more in Indian diet than that in Asia and world as a whole (Ali, 2002). Two main types of cultivated chickpea are Kabuli (white seeded) and desi (brown seeded), representing two diverse gene pools. Practically, a variety must show (DUS) variations in the characters that are adopted for use in varietal identification. Emphasis on characterization and varietal identification of chickpea cultivars is very important to the certification officers, seed production officers and seed growers. Therefore, for keeping the purity of cultivars, stable visual diagnostic characters of seed, seedling and plant morphology are utmost essential to know (Lalitha, 2007).

Among legumes, chickpea ranks third worldwide and its seeds contain >20% protein that is an important energy source for human. In addition to protein, chickpea is a good source of carbohydrates, dietary fiber, minerals (iron and zinc) and vitamins (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). 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 (Jadhav *et al*. 2015). Thus, it seems feasible to develop cultivars with 20-25% higher protein content than the existing cultivars. These include control of seed size by a single gene (Argikar, 1956),

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two genes (Upadhyaya *et al.,* 2006; Hossain *et al*., 2010) and polygenes.

MATERIALS AND METHODS

The present research work was conducted at Research cum Instructional farm Department of GPB, CoA, IGKV, Raipur, Chhattisgarh, during the *Rabi* season of 2019-20. Raipur, the capital of Chhattisgarh, a tribal dominant state lies at 21°16'N latitude and 81°36'E longitude with an altitude of (289.60m) above mean sea level. The monthly mean of maximum and minimum temperature was 30.4°C and17.6°C and total rainfall during crop growing period (October, 2019 to April, 2020) was 252.8 mm. Ninety nine genotypes of chickpea comprised of germplasm, released varieties and segregating populations were grown in RBD with two (in RBD model only 2 replication not recommended. Why augment or any suitable model was not adopted for such a huge population?) replications. Date of sowing was 29th November, 2019. Each plot comprised of 1 row of 4 m length; row x row and plant x plant distance of 30.0 cm and10 cm. Seeds were pre-treated with Bavistin, Trichoderma, Rhizobium and PSB culture. Fertilizer dose @ of 20:40:20 kg per hectare (NPK) was applied. Two irrigations were given to the crop one month after sowing and another at the time of flower initiation. Data was collected on agromorphological, yield and nutritional traits.

Protein content of grains harvested from each plant was estimated by using standardized procedure through the estimation of nitrogen using a single digest method. 0.5 g of finely ground sample was taken and 14ml of concentrated sulfuric acid containing 0.5% selenium powder was added. Five grams of Se was added to 500 ml of sulfuric acid and heated. After adding the cooled digestion mixture to plant materials, digestion tubes were transferred to a block digester preheated to 370°C for 2.5 hr completing the digestion. Digests were adjusted by adding distilled water. The aliquots were used to determine nitrogen by distillation with sodium hydroxide, using an atomic absorption spectrophotometer (Sahrawat *et al*., 2002). Protein content was obtained by multiplying the total nitrogen content in the seeds by the correction factor 6.25 (Jones, 1941).

For iron and zinc estimation, the seeds were washed with distilled water and oven-dried at 60°C for 48hr before grinding. 20g of dried and powdered samples were kept overnight in an oven at 60°C. One g of sample was transferred to a digestion tube having nitric acid, sulfuric and perchloric acid in the ratio of 10:0.5:2 (v/v) and left overnight. Samples were digested initially at 120°C for one hr, then at 230°C for about 2 hr to get clear and colourless digests. Aliquots were taken from the digests and analyzed for Fe and Zn concentrations by AAS and expressed as mg kg^{-1} (ppm). Diverse statistical parameters, PCA, cluster analysis and Pearson's correlation coefficient were measured using SPSSv17.0.

RESULTS AND DISCUSSION

Based on variation in agro morphological, yield and nutritional traits variations, it was attempted to group the chickpea genotypes and identify each one of them through descriptors (Table 1). Anthocyanin pigmentation was classified into two classes as pigmentation absent in 3 and present in 96 genotypes. Variations were observed among genotypes for time of 50% flowering with at least one open flower in 50% plant population was noted and categorized into two *viz*., early (40-60 days) and medium (61-80 days). 67 genotypes flowered early as compared to 32 genotypes of medium duration and none was recorded in the category of extra early and late duration. Plant growth habit is a distinguishing feature in characterization. 18 genotypes were observed as erect type, 81 genotypes showed semi-erect type of growth habit. Four groups were made based on intensity of green colour of foliage in which 3 genotypes showed light green colour, 94 genotypes showed medium, 1 genotype showed dark green and greenish purple colour. With regard to size of leaflets, the variation was observed and categorized into two groups; 44 genotypes recorded small size (10 mm), 55 genotypes recorded medium (10-15 mm). For leaf pattern, 98 genotypes had pinnate leaves and one genotype had simple leaf. No variation was found among the genotypes for number of flowers per peduncle as all genotypes had single flower per peduncle. Three groups were made based on variation in flower colour; one genotype had white, 97 genotypes showed pink, only one genotype showed blue colour flower. Genotypes were examined for the stripes on standard petal of the flower, the stripes were absent in 1 and present in 97 genotypes. Morphological features of genotypes have been a major component of varietal identification. However, it is not possible to identify varieties using any single parameter. A detailed morphological description of plants and seeds should therefore be prepared. Utilization of these features in sequential fashion is useful and convenient to distinguish different genotypes. Similarly, genotypes identification based on distinguishable morphological characters were reported by Lalitha (2007) in chickpea.

Significant variability differences were observed in the yield traits. Maximum variability was recorded in plant yield followed by iron concentration (mg/kg), hundred seed weight and number of pods / plant. Mean of DTF was 58.13 days which ranged from 47 days to 63 days. Maturity days ranged from 97 to 108 days with the mean of 103.63 days. Plant height was classified into two classes, 75 having short (<45 cm), 24 with medium (45-65 cm) height. The trait possessed a mean of 42.47 cm ranging from 22.40 cm to 60.00 cm. Primary branches and secondary branches also emphasized on yield, thus, had a mean of 2.30 and 7.75, respectively.

Considerable variation did exist in traits namely, pods/ plant, hundred seed weight, plant yield, protein (%), zinc and iron concentration. Genotype (ICC12539) showed high number of pods (19.40). Seed index is the important character, it influences seed yield to a great extent. Seventy

five accessions had seed weight less than 20 g (very small); nineteen fell into small (20-25 g) and remaining five in medium class (26-35 g). Plant yield was recorded highest in RG 2016-117 (83 g) ranging from 7 g to 83 g with the mean of 24.94 g.

Protein

Highest protein % was observed in RGH- 4 with variability of 14.48%. RGH-4, RGH-56, RG2016-84, ICC251762, RGH53, IPC98-12, RG2016-03, ICC1053 and RGH58 recorded high protein content (>20%). These entries possessing high protein had pink flower. ICC251811 beard blue flower with 18% protein. Research says, high protein content in blue flowered, could be because of their reduced seed size compared to pink flowered and white flowered. RGH-4 and RG-2016-84 showed high protein with pink flower and recorded small seed size followed by RGH-56, ICC251762, RGH 53, IPC 98-12, RG 2016-03, ICC1053 and RGH 58 with very small seed size (<20 g). It is observed

Table 1: Agromorphological variation in chickpea germplasm.

Quantitative traits	Mean ± SE	Range	CV (%)
Days to 50% flowering	58.13±0.36	47.00-63.00	6.11
Days to maturity	103.63±0.36	97.00-108.00	3.46
Plant height (cm)	42.47±0.55	22.40-60.00	12.94
Primary branches	2.30±0.03	1.00-2.60	12.43
Secondary branches	7.75±0.14	1.40-11.20	18.55
Number of pods/plant	8.95±0.28	3.20-19.40	30.74
Hundred seed weight (g)	15.97±0.52	5.00-28.00	32.32
Plant yield (g)	24.94±1.35	7.00-83.00	54.04
Protein (%)	17.46±0.25	10.16-25.85	14.48
Zinc (mg/kg)	50.07±1.39	21.70-136.00	27.64
Iron (mg/kg)	35.65±1.75	10.00-73.65	48.81
Qualitative traits	**Descriptor states**	**Shanon Weaver Diversity Index (H')**	
Stem: Anthocyanin colouration	Before flowering	0.126	
Time of flowering (50% of the plants with at least one open flower)	First flowering	0.834	
Plant: Growth habit	DTF	0.469	
Plant: colour of foliage	DTF	0.239	
Leaflet: Size	DTF	0.684	
Leaf: Pattern	DTF	0.046	
Flower: Number per peduncle	DTF	0.010	
Flower: Colour	DTF	0.083	
flower: Stripes on standard	DTF	0.046	
Plant: Height	Fully developed green pod	0.549	
Seed: Size (weight of 100 seeds at 10% moisture content)	30 days after harvest	0.675	

Table 2: Correlation coefficients of eight yield and yield attributing traits in chickpea genotypes.

	DTF	DM	PH	PB	SB	NPP	HSW	PY	Protein	ZN	FE
DTF	1.000										
DM	0.917[a]	1.000									
PH	-0.151	-0.148	1.000								
PB	-0.083	-0.151	0.321[a]	1.000							
SB	0.006	-0.017	0.284[a]	0.420[a]	1.000						
NPP	0.027	0.022	0.375[a]	0.285[a]	0.406[a]	1.000					
HSW	0.113	0.117	0.284[a]	0.191	0.319[a]	0.205[a]	1.000				
PY	0.122	0.097	0.367[a]	0.114	0.077	0.357[a]	0.415[a]	1.000			
Protein	0.019	0.047	-0.275[a]	-0.019	-0.158	-0.178	-0.076	-0.095	1.000		
ZN	0.078	0.096	0.054	-0.026	-0.091	-0.053	0.086	0.167	-0.003	1.000	
FE	-0.252[a]	-0.250[a]	0.036	0.255[a]	0.133	0.035	-0.152	-0.223[a]	-0.202[a]	-0.159	1.000

a = significant at 0.05 probability levels (2-tailed); DTF = Days to 50% flowering; DTM = Days to maturity; PH = Plant height (cm); NPB = No. of primary branches; NSB = No. of secondary branches; NPP = No. of pods/plant; HSW = Hundred seed weight (g); PYG = Plant yield(g) Correlation coefficient significance testing at 0.05 probability level without Z transformation test is justified.

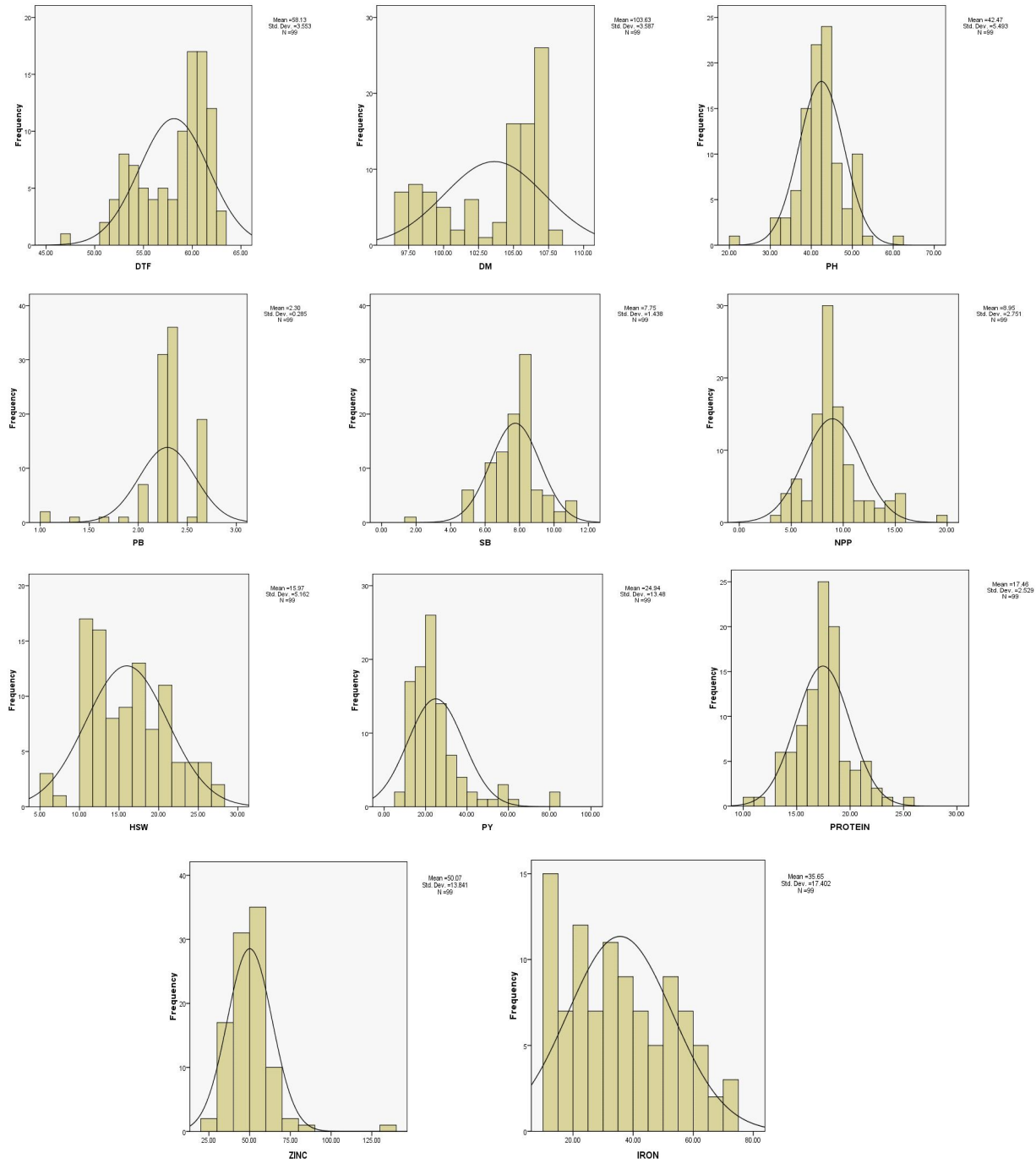

Fig 1: Histogram of eleven yield and yield attributing traits in chickpea germplasm.

that blue flowered plants with smaller seeds had higher protein content compared to pink flowered plants with larger seeds. They suggested linkages between genes for flower colour, protein and seed weight. Existence of linkage between flower colour and seed size was also reported by Atta *et al.* (2003).

Zinc and Iron (mg/kg)

High variability in iron concentration was found as compared to zinc. Iron content ranged from 10 to 73.65 mg/kg whereas, zinc ranged from 21.70 to 136.00 mg/kg with the mean of 35.65 and 50.07 mg/kg, respectively. Vijay recorded very high zinc (136 mg/kg) followed by RGH-7 (81.90) and ICC269712 (79.90). RGH52 and RGH11 recorded high iron concentrations of 73.65 and 72.89 mg/kg, respectively. Iron concentration tended to be less affected by market class, although small-seeded chickpea contained more Fe than large seeded. RGH53 hadhigh values for both protein and zinc, with an average of 21.86% and 73.00 ppm, respectively, but it showed low iron concentration (20.77 ppm).

Association analysis

Results revealed that days to flowering showed very strong relation with days to maturity. Plant height recorded significant association with primary branches, secondary branches; pods/plant, hundred seed weight and plot yield (Table 2). However, negative association was recorded between plant height and protein. Primary branches possessed strong association with secondary branches and pods per plant; secondary branches recorded significant relation with pods per plant and hundred seed weight. Plant yield represented strong association with pods per plant and hundred seed weight. A positive correlation was seen between hundred seed weight and pods / plant. Except plant height, no other trait gave any relationship with protein. As far as iron and zinc is concerned, none of the trait showed any kind of significant relationship with zinc, whereas, for iron concentration, negative significant trend was seen with days to flowering, maturity, plot yield and protein. Primary branch indicated that hike in this trait will increase the iron. Negative but non-significant trend was seen between seed weight and protein content. A negative relationship between seed size and protein content implies that as seed increases in size there is an increased amount of starch deposition altering the starch / protein ratio (Bahl *et al.* 1979). Protein content has been found to be negatively correlated in chickpea (Frimpong *et al.* 2009; Gangola *et al.* 2012). Similar to this study, protein content was found to be negatively correlated with seed size in pigeonpea. However, breeding lines combining high protein content with medium seed size were successfully developed. The existence of a negative correlation between protein content and seed size and significant associations of protein content with flower color, seed coat color and seed shape suggests that development of chickpea cultivars with high protein content and desired seed traits (size, shape and color) would require large segregating populations and the selection of desired recombinants. Blue flower color, grey seed coat color and pea seed shape of high protein line show pleiotropic effects

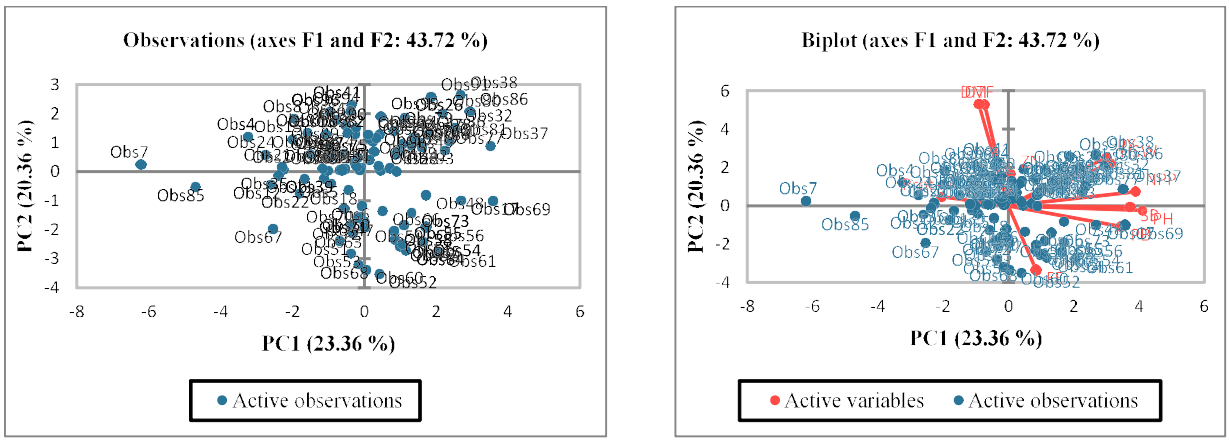

Fig 2: Principal coordinates between PC1 and PC2 based on active observations and variables.

Table 3: Eigen vectors and eigen values for the six principal components in chickpea genotypes.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	2.56	2.24	1.32	1.04	0.90	0.75
Percent variance	23.35	20.36	12.01	9.50	8.24	6.83
Cumulative variance	23.35	43.71	55.73	65.23	73.48	80.31
Descriptor			**Factor loadings**			
	PC1	PC2	PC3	PC4	PC5	PC6
DTF	0.016	**0.752**	0.165	0.006	0.005	0.003
Days to maturity	0.025	**0.759**	0.146	0.008	0.004	0.002
Plant height (cm)	**0.525**	0.001	0.048	0.030	0.001	0.014
Primary branches	**0.365**	0.031	0.071	0.127	0.144	0.014
Secondary branches	**0.433**	0.000	0.156	0.047	0.006	0.043
Number of pods/plant	**0.469**	0.014	0.015	0.000	0.052	0.228
Hundred seed weight (g)	0.303	0.130	0.036	0.040	0.001	**0.370**
Plant yield (g)	**0.280**	0.168	0.203	0.001	0.029	0.022
Protein (%)	0.131	0.006	0.015	**0.706**	0.003	0.046
Zinc (mg/kg)	0.000	0.073	0.243	0.028	**0.582**	0.009
Iron (mg/kg)	0.022	**0.305**	0.224	0.053	0.080	0.000

Significant loadings are marked in bold.

of gene (s) and these traits were also associated with seed size and protein content. Thus, it would be important to search for other high protein lines in the germplasm and use diverse sources of high protein content in breeding programs for development of high protein chickpea cultivars with desired seed traits (size, shape and color).

Principal component and cluster analysis

Relationships among the different traits were evaluated by principal component analysis (PCA). The first six principalcomponents provided a reasonable summary of the data and explained 80.19% of the total variation. In the chickpea genotypes, PC1 explained variation in plant height, primary branches, secondary branches, number of pods/ plant and grain yield per plant, PC2 in DTF and maturity and iron concentration, PC4 in protein %, PC5 and PC6 for zinc and 100-seed weight, respectively. PC3, however, did not report variation for any of the trait (Table 3 and Fig 2).

In cluster analysis, 99 genotypes were subjected to cluster analysis using Agglomeration method: Un-weighted pair group average based on dissimilarity estimated by Euclidean distance (Table 4 and Fig 3). Results indicated that the entire material was grouped into ten clusters. 33 genotypes were categorized into cluster I followed by Cluster III (28), cluster V (23) and cluster II and IV with 6 and 4 genotypes, respectively. Rest of the five clusters had one entry each. The inter and intra cluster distances were also worked out to understand the relationship of the genotypes within and between the clusters based on genetic distances (Table 5). Inference can be made that maximum intra cluster distance was observed in cluster V, I and III. All these three clusters also beard more number of genotypes. The inter cluster distance was found high in clusters VI and IX followed

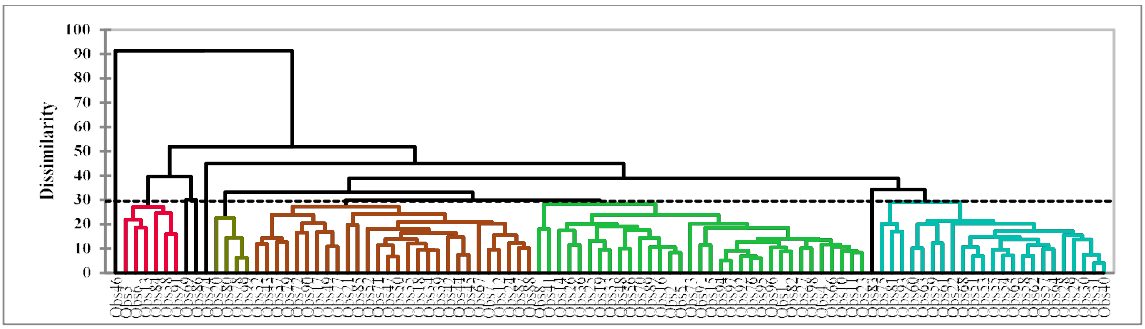

Fig 3: Dendrogram of ninety nine genotypes based on UPGMA-Euclidean distances.

Table 4: Cluster analysis presenting ten clusters along with genotypes based on inter and intra cluster distances.

Clusters	No. of genotypes	I	II	III	IV	V	VI	VII	VIII	IX	X
I	33	**26.24**	32.01	21.31	26.28	37.95	81.44	65.92	42.16	54.17	59.88
II	6		**19.09**	44.06	32.97	53.02	80.29	41.92	58.23	67.17	31.42
III	28			**24.57**	29.43	25.09	97.39	66.33	45.21	34.60	65.98
IV	4				**16.21**	49.42	101.86	59.15	65.33	53.33	50.15
V	23					**26.51**	93.47	65.08	35.71	31.39	70.91
VI	1						**0.00**	105.71	65.19	121.29	99.98
VII	1							**0.00**	82.19	70.89	30.08
VIII	1								**0.00**	63.56	82.10
IX	1									**0.00**	79.05
X	1										**0.00**

Table 5: Cluster means of eleven yield and nutritional traits.

Cluster	DTF	DM	PH	PB	SB	NPP	HSW	PY	Protein	ZN	FE
1	58.72	104.51	41.33	2.23	7.70	8.91	17.09	23.54	18.05	55.75	22.44
2	59.50	104.00	44.76	2.33	6.96	9.10	16.16	55.00	17.13	60.08	21.02
3	58.53	103.85	40.20	2.27	7.77	9.03	14.48	18.94	18.17	40.73	36.54
4	60.50	106.25	47.05	2.25	**8.40**	10.15	22.25	35.50	14.53	35.15	15.81
5	56.00	101.52	44.63	2.37	7.85	8.59	14.60	20.08	16.23	49.55	59.23
6	56.00	101.00	47.20	2.40	**8.40**	7.00	**24.00**	31.50	16.46	**136.00**	27.04
7	**52.00**	**97.00**	43.80	2.40	8.20	**14.20**	19.00	**83.00**	13.38	46.40	46.14
8	62.00	107.00	43.40	**2.60**	7.60	9.20	10.00	11.50	19.96	81.90	52.00
9	61.00	107.00	36.80	2.40	7.80	4.40	20.00	22.00	**21.02**	21.70	**63.72**
10	60.00	106.00	**60.00**	2.40	7.20	11.40	17.00	80.00	18.91	50.20	25.38

by VI and VII and IV and VI. Thus, for chickpea improvement program the genotypes from these clusters based on inter cluster distances can be taken as donors. For DTF, ICC12365 flowered early (cluster I), two genotypes, one each from cluster II (RG 2016-50) and VII (RG 2016-117) recorded early and late maturity. High means of plant height were shown by RG 2003-28 from cluster X. Three genotypes (RG 2016-50; RG 2016-29 and RGH7) from clusters (II, V and VII) showed high cluster means for primary branches. One entry each from cluster IV (RGH 35) for secondary branches, VII (RG 2016-117) for number of pod/plant, IV (RGH 85) for hundred seed weight, VII (RG 2016-117) for plant yield, IX (RGH 58) for protein percent, VI (Vijay) for zinc and IX (RGH 58) for high iron.Genetic diversity among the parents is a prerequisitefor ensuring the chances of selecting improved segregants for various characters (Dwevedi *et al*., 2009). Success of hybridization in highly self-pollinated crops like chickpea mainly depends on the genetic diversity of the parents. Cluster analysis was found to be a potential tool in quantifying the degree of divergence in germplasm collections of crop plants.

CONCLUSION

Billions of people worldwide are facing the major global health problems related to micronutrient malnutrition, may be due to low dietary intake of the micronutrients especially Zn and Fe. Plant breeding is considered to be the most economical and sustainable way to deal with aforesaid problem it is a useful way to reach people living in remote tribal areas that have limited access to fortified foods. Considering the importance of chickpea crop in the dietary element of majority of the people, it is desirable to understand the existing genetic variability pattern of yield, protein, seed-Fe and Zn concentrations leading to their bio-fortification through genetic enhancement.

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