Multivariate Analysis for Grain Yield and Nutritional Quality Traits of Green Seeded Chickpea (*Cicer arietinum* L.) Genotypes derived from *Desi* and *Kabuli* (GKB-10 × MNK-1): Initial Efforts for Green Seeded Chickpea Bio-fortification

Honnappa M.[1]*, Mannur D.M.[1], Padmashree R.[1], Umesh Babu B.S.[1], Muttappa H.[1], and Archana K.A.[2]
[1]Department of Genetics and Plant Breeding, UAS Raichur (Karnataka), India.
[2]Department of Genetics and Plant Breeding, UAS Dhaward (Karnataka), India.

(Corresponding author: Honnappa M.*)

ABSTRACT: Chickpea is one of the most important nutritious grain legumes crop in the world with plant based protein and mineral elements. There is limited information available on micro and macro nutrients in chickpea. Therefore, an effort was made to evaluate as set of 90 F_6 green seeded chickpea genotypes derived from the cross between milky white *Kabuli* genotype with *Desi* green seeded genotypes (MNK-1 and GKB-10) for nutrients such as Protein (Pr), Iron (Fe), Zinc (Zn), Calcium (Ca), Boron (Bo), Cupper (Cu), Potassium (K), Magnesium (Mg), Manganese (Mn) which are analyzed by ICP-MS technique, and grain yield per plant using multivariate analysis. Results from ANOVA revealed that mean MSS due to test genotypes *vs* checks exhibited highly significant difference for all the nutrient parameters studied except for grain yield per plant, genetic variability revealed existence of High GCV, PCV and heritability (h^2) coupled with high GAM for the entire nutrient parameters studied and grain yield per plant except for the protein content. The correlation analysis revealed as high positive inter correlation among the nutrient parameters, grain yield per plant however shown positive non-significant association for Pr, Zn, Bo, Cu and Mn, while Fe, Ca and K nutrients revealed negative non-significant association. The PC analysis with three component *viz*., PC1, PC2 and PC3 showed 53.96%, 11.03% and 10.40% of variations among the studied parameters respectively, with total cumulative variance of 73.39%, the diversity analysis grouped the genotypes 3 major cluster, cluster III is being major group consisting of 32 green seeded genotypes fallowed by Cluster II and cluster I, the average inter and intra cluster distance was also calculated for these nutrient parameters, the genotypes belongs to the cluster I found to be higher side for all the nutrient content studied, so these promising lines may serve as a genetic resource for improving the nutritional qualities and giving an opportunity for bio-fortification to combat micronutrient malnutrition.

Keywords: Green seeded chickpea, Multivariate analysis, *Desi* and *Kabuli* type, Principle component analysis.

INTRODUCTION

India's green revolution was successful and significantly enhanced food grain crops' production and productivity by introducing high yielding varieties (HYVs) during 1960 to 1970 era, as a result, the majority of people attained self-sustain in food crops. India as a developing nation it is now imperative to concentrate on quality of food crops and dietary diversification as opposed to merely high yield per unit area. Half of the global population reported to have deficiency in nutritional content in food grain crop including Zn and Fe. "Bio-fortification" in simple term nutritional enrichment of mostly accessed foods purpose is to enhance the micronutrient content of many staple food crops using best conventional breeding practices or modern biotechnology (Nestel *et al*., 2006). During pre-green revolution period the poverty was the main problem, but now-a-days it has shifted to micronutrient malnutrition known as "hidden hunger" and poor nutritional content in food crops results in night blindness, xerophthalmia, Iron deficiency leads to anemia, Zn deficiency can cause

fetal abnormalities, protein deficiency can cause atrophy and immune system weakness with high risk of blood coagulation (Solanki and Devi 2020) etc., Food and nutritional security are still far from being achieved, while hidden hunger remains the most prevalent challenge in many regions of the world (FAO, 2021), however, the bio-fortification of staple crops like rice and wheat has shown significant achievement to solve the malnutrition issues, in the same way bio-fortification of major pulse crops (chickpea, green gram and red gram or pigeon pea) helps to improve the balanced dietary food as currently only 11% of India's protein needs are met by pulses (Mohanty and Satyasai 2015; Sharif *et al*., 2018). Zn is the world's most critical micronutrient deficient, about 50% of the world's agricultural soil and Indian soils are deficient, nearly 2 billion people suffered and 1.5 million children die in each year (Li *et al*., 2007; Chakmak *et al*., 2010; WHO, 2009), its rising to 63% by 2025, currently more than 820 million people still suffer from chronic undernourishment and malnutrition (FAO, 2020). Zn fortification in staple food crops including pulses could

save the lives around 48,000 Indian children per year (WHO, 2015).

To combat undernourishment and malnutrition, the development of nutrient-dense and bio-fortified breeding lines could provide a sufficient range of food products necessary for a balanced diet available for poor households at affordable prices. There are opportunities to improve the sustainability of the food systems through the development of high nutritional quality and nutrient dense breeding lines (Amri *et al.*, 2019). Food legume crops could play a key role in improving food and nutritional security and building sustainable production systems due to their multiple agricultural and nutritional benefits. Pulses are the major source of protein provide two times as much as the major cereals and among all cultivated crops and important constituent of Indian diet after cereals; provide 20% of protein and fiber, vital source of vitamins, minerals and micronutrients such as Fe, Zn, Mg, Mn, Ca, Cu and Bo (Ereifez *et al.*, 2001; Wang *et al.*, 2010; Jukanti *et al.*, 2012) and contains potentially health-beneficial phytochemicals such as flavonoids, phenolic acides and carotenoids (Wood and Grusak 2007). Chick pea (*Cicer arietinum*) is an important pulse crop, steady source of human nutrition and it is an important component of production systems that are resilient to climate change. There are mainly two types of chickpea produced, *i.e., Desi* and *Kabuli*. The *Desi*-type chickpea contributes to around 80% and the *Kabuli* type around 20% of total chickpea production. Asia and Africa contribute around 80% of world production and chickpea is considered as 'poor man's meat' as it one of the less expensive sources of protein and occupy a unique place in India because of its multiple uses (Siddique *et al.*, 2000; Mohanty and Satyasai 2015). Globally chickpea is grown on 149.66 lakh ha area, with the total production of 162.25 lakh tonnes and average productivity of 1252 Kg/ha during 2017-18 (FAOSTAT, 2019), while, India contribute 70% of the total world chickpea production and is grown on 112 lakh ha area, with the total production of 116.20 lakh tonnes and average productivity of 1036 Kg/ha during 2020-21(Indiastat, 2021-22). India is the largest producer of chickpea followed by Australia, Myanmar and Ethiopia (FAO STAT, 2019). Among the states Madhya Pradesh ranks first in production has contributed 34% of the total chickpea area and 41% of the total chickpea production in the country.

Since, cereals in combination with pulses will makes a major dietary food for billions of the people and targeting of chickpeas could some extent alleviate the malnutrition problem, In order to create bio-fortified varieties, studies on mining for protein and micro-nutrients levels is essential which helps to ascertain the existing variability, genetic and non-genetic causes for the variation in the breeding population. Significant genetic variation for seed size, iron, zinc and protein content was reported in chickpea (Thavarajah *et al.*, 2012; Farida *et al.*, 2022). Beside proteins, chickpea is also an important source of more than 15 micronutrients such as Fe, Zn, Ca, Cu, Mg, Mn, Bo and K *etc* (Singh and Pratap 2016; Farida *et al.*, 2022). Recently, genetic

bio-fortification and selection for high nutritional quality were considered as one of the main objectives of chickpea breeding programs at both national and international (CGIAR) levels. So the development of superior high yielding lines with high nutritional value becomes central for any breeding program. Even though limited coherent studies on levels of micronutrient and protein content are available in Indian sub-continent conditions, accessibility of such information can pave the way to design a sound breeding strategy for enhancing the nutrient load and bio-fortification chickpea. Thus, the present study is taken up with the objective to determine the protein and micronutrient levels in green seeded chickpea genotypes with the help of multivariate analysis to know existing genetic variability, potential correlation between the nutritional quality traits and diversity (D^2), further to identify the suitable genotypes with tailored traits for micronutrient enrichments by crop improvement.

MATERIAL AND METHODS

Plant material and soil status. An experimental material consist 90 F_6 lines of green seeded chickpea genotypes here after named as RGK (Raichur Green *Kabuli*) lines derived from the cross between GKB-10 × MNK-1. GKB-10 is characterized with small *Desi* type seed with green seeded colored, while MNK-1 is *Kabuli* type seed with milky white colored. As a *Kabuli* types are more preferred in cooking dishes and eating it table purpose, here the crosses were made to have *Kabuli* genotypes with green seeded types to substitute's green peas as a cooking and table purpose. The progenies of green seeded chickpea genotypes exhibited high variability in terms seed size, colour (Plate 1A and 1B), wilt resistant lines further were classified into five different groups such as Raichur Green *Kabuli* (RGK lines = 33), Wilt resistance Raichur Green *Kabuli* (WRGK lines = 12), High seed size Raichur Green *Kabuli* (HRGK lines = 10), Small seed size Raichur Green *Kabuli* or Chinoli types (SRGK lines = 6), Raichur Green *Kabuli* selection (RGKS lines = 29). The green seeded genotypes were grown along with the checks such as, KAK-2, JG-11, GKB-10 and MNK-1 in Augmented RBD design with the spacing of 30 cm ×10 cm at ARS Kalaburagi, UAS Raichur during *Rabi*-2020. Kalaburgi as it is situated in North Eastern dry zone (zone-2) of Karnataka in Deccan Plateau consisting black soil with parent basalt rock having P^H 8.4, soil nutrient status indicated that soils are medium in available nitrogen and phosphorus and high in potassium, the micronutrients iron and zinc are below critical level where as copper, manganese and magnesium status is above critical level.

Macro and micro nutrient analysis. Major macro and micronutrient content *viz.*, Fe, Zn, Ca, Cu, Mg, Mn, Bo and K were determined in the genotypes according to standard method developed by Pequerul *et al.* (1993). For each genotype, 0.25g of whole meal flour was placed in individual digestion tubes. A volume of 7 ml of 70% nitric acid (HNO_3) was added and left to stand overnight. Each tube was then placed in a digestion

block at 90°C for 1 h before adding 3 ml of 30% hydrogen peroxide (H_2O_2); the tubes were replaced again in the digestion block at 90°C until the complete digestion (colorless solution). The obtained extracts were filtered using Whatman papers (Grade 595) and then diluted to 1:10 with 6 M hydrochloric acid (HCl). The concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS) technique (Plate 2) (**Instrument make:** Perkin Elmer and Model name: Nexion 350X) at Pesticide residue and food quality analysis laboratory at UAS Raichur. The total contents of macro elements (K, Ca and Mg) and micronutrients (Fe, Zn, Mn, Cu and Bo) in ppm were recorded.

Plate 1 (A) showing phenotypic growth view in the field of green seeded genotypes (F_6population).

Plate 1 (B) showing sample population of green seeded genotypes (F_6population) with parents having contrasting features in seed size and colour.

Plate 2. Inductively coupled plasma mass spectrometry (ICP-MS) technique (**Instrument make:** Perkin Elmer and Model name: Nexion 350X).

Protein analysis. The protein content was estimated by analyzing the nitrogen in seed samples using a single digests were used to determine nitrogen using sodium hydroxide by Kjeldahl distillation method (Kjeldahl, 1883) and the total nitrogen content in powdered seeds was multiplied by a factor 6.25 to arrive at seed protein content in percentage (Jones, 1931).

Statistical analysis. To determine significant difference among all the genotypes ANOVA, genetic variation and diversity (D^2), correlation and principal component analysis (PCA) were carried out by using R studio (*version 3.5.2*) augmented RCBD package developed by Aravind, *et al.* (2019) (https://aravind-j.github.io/augmented RCBD/.). Clustering of genotypes into similar groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances using SAS *version 9.2* software (SAS Institute, 1996, Cary, NC).

RESULTS AND DISCUSSION

ANOVA and Descriptive statistics. The estimated data of seed protein and micro nutrient content for Fe, Zn, Ca, Cu, Mg, Mn, Bo and K of 96 green seeded chickpea genotypes including checks varieties were subjected to analysis of variance and it revealed (Table 1) that mean sum of squares (MSS) due to test genotypes *vs* checks shown highly significant difference for all the nutrient content studied except for grain yield per plant, similarly MSS due to test genotypes reported highly significant difference for all the nutrient parameters including grain yield per plant. The findings of the study confirm the presence of significant difference for the studied micronutrients (Fe, Zn, Ca, Cu, Mg, Mn, Bo and K) in the experimental material and offers scope for further investigations. In literature, Jayalaksmi (2018) Valenciano *et al.* (2011); Kahraman *et al.* (2017); Thavarajah (2012), studied and reported significant difference among the Fe, Zn, Cu, Ca, K and Protein etc. Descriptive statistics for protein and nutrient content revealed considerable diversity, protein content ranged from 12.52 to 24.42 ppm with mean of 19.21ppm, Fe content ranged from 3.12 to 94.86 ppm with mean of 38.17 ppm, Zn was ranged from 10.15 to 55.88 ppm with mean value 16.95, Ca was ranged from 28.91 to 611.37 ppm, wide range and high variance value were observed for Fe, Zn, Ca, K and Mg similarly Neugschwandtner *et al.* (2015); Farida *et al.* (2022) reported higher concentration with wide variation for the some elements like K, Ca, Mg, Cu and Mn because the quantities of minerals in cultivated plants are influenced by numerous complex factors including genotypes, soil, environmental condition and nutrient interactions (Simic *et al.*, 2009) however, little variability was observed for Protein, Bo, Cu, Mn and grain yield per plant (Table 2). The min, max and mean data revealed extent of variation for protein and nutrient content present in the breeding population in the form of frequency distribution as shown in (Fig. 1) indicating scope for the breeder to operate selection for trait improvement. Measure of statistical analysis such as skewness and kurtosis so called descriptive statistics helps to understand the

location and variability of the data set as a relative mean performance and nature of distribution of traits. Skewness which measure symmetry or degree of distortion, the in the present study the skewness values were ranged from -0.26 for protein to 3.13 for Bo, the nutrient parameters such as protein, Fe, Ca, K, Mg and Mn revealed the presence of symmetric skewness, results were similar as reported by Kanavi*et al.*, 2020, Zn, Bo and grain yield per plant exhibited highly skewed data while, Cu shown moderately distribution of skewed data (Table 2). As with skewness, if the value of kurtosis is too big or too small, there is concern about the normality of the distribution. Kurtosis is about the distribution of tails whether it is peakedness or flatness. It is actually the measure of outliers present in the distribution. Lower kurtosis values in a data set are an indicator that data has light tails or lack of outliers. Positive kurtosis indicates a relatively peaked distribution. Negative kurtosis indicates a relatively flat distribution. The kurtosis values were peakedness, and it was ranged from 1.91 for Mg to 15.68 for Bo it shown higher kurtosis values indicating heavy tailed data set and exhibited leptokutic similar reports were revealed by Mariyammal *et al.* (2019); Sumathi *et al.* (2018).

Variability analysis. The value of phenotypic coefficient of variation (PCV) were higher than the genotypic coefficient of variation (GCV) for all the nutrient content studied indicating the high influence of environmental factors (Table 3). However low difference were also observed between PCV and GCV for Fe, Zn, Bo, Cu, K, Mg, Mn and grain yield per plant which revealed that these parameters were comparatively less influenced by the environments. The value of PCV and GCV were found to be higher for protein and Ca indicating the presence of wide variation which allows operating selection or key to initiate planned breeding programs by breeder for designer trait, similar finding observed by Mohammadi (2015). The GCV and PCV were higher for all the studied nutrient parameters along with grain yield (Fe, Zn, Ca, Bo, Cu, K, Mg, Mn) except for protein which reported low to medium GCV and PCV as shown by graphical representation (Fig. 2a). The results were in similar with the Kumar *et al.* (2019); (Jayalakshmi, 2018). The coefficient of variation indicated only the extent of variation present for different nutrient content and it did not indicate the heritable portion of the variation present in the population or breeding lines; genotypic coefficient of variation along with obtained heritable portion of variance gives better idea of genetic gain through phenotypic selection so it is essential to know heritability estimates for different nutrient contents as suggested by Burton, 1952. In practical the characters having high heritability estimates are less influenced by environment and they are under the influence of more number of fixable factors. In the present study heritability (broad sense) was recorded higher for all the nutrient parameters studied and the Mg and K concentration topped among them followed by Mn, Bo, Fe, Zn, Cu, grain yield, Ca and protein as shown by graphical representation (Fig. 2b). Johnson *et al.*

(1955); Dwevedi and Lal (2009), suggested that the heritability estimates along with genetic advance is more useful than the heritability alone in predicting the resultant effect of selection. In the present study, high heritability coupled with high genetic advance were observed for all the traits studied this could be due to the additive gene action and selection pressure could effectively excreted on these traits for population improvement or advancement of population for subsequent generation to bring stabilized lines. While protein content revealed high heritability with medium genetic advance indicating that non-additive gene action could be in control of this trait; hence selection would be effective at later stage of generation as shown by graphical representation (Fig. 2c). These findings are in confirmation with the finding of the authors Bueckert *et al.* (2011); Ray *et al.* (2014); Sali *et al.* (2016); Jayalakshmi (2018).

Correlation analysis. Correlation for protein and nutrient along with grain yield per plant for advanced chickpea breeding lines were shown in (Table 4 and Fig. 3), it revealed that none of the nutrient parameters have shown significant correlation with grain yield per plant, in the same way none of the nutrient parameters were shown significant association with protein content. The study shown high inters correlation among the nutrient contents like., Zn, Ca, Bo, Cu, K, Mg and Mn. Fe shown highest correlation with Mg (0.845**) fallowed by K (0.844**), Mn (0.830**), Cu (0.772**), Ca (0.677**), Zn (0.509**) and Bo (0.279**). Zn has shown highest positive correlation with Cu (0.437**) fallowed by Ca (0.431**), K (0.401**), Mg (0.381**), Mn (0.365**) and Bo (0.217*). Ca has shown highest positive significant correlation with Mg (0.744**), followed by Mn (0.685**), K (0.676**), Cu (0.639**) and Bo (0.524**). Similarly Bo with Mg (0.333**), K (0.298**), Mn (0.294**); Cu revealed maximum correlation with Mn (0.860**), K (0.859**) and Mg (0.854**); K with Mg (0.964**) and Mn (0.952**); Mg with Mn (0.956**). So from the correlation analysis overall it revealed that highest positive correlation was observed by Ca with Mg fallowed by Mg with Mn; Mn with K; Mn with Cu; Cu with Mg shown, these results are in line with report of Vandemark *et al.* (2018) in lentil, Gibson and Mullen (2001) in soyabean, Ma *et al.*, 2017 in peas, While, protein exhibited negative correlation with Fe, Ca, Cu, K, Mg and Mn, the positive associations were observed with Zn and Bo and grain yield. In literature the similar reports were revealed by (Jayalakshmi, 2018; Farida *et al.*, 2022) as among the *Desi* types (*Desi* brown, *Desi* green and *Desi*balck) *Desi* brown and green types are associated with higher mineral nutrient content. Negative correlation were observed for grain yield per plant with Fe, Ca, K and Mg, however it also revealed positive correlation with proteins, Zn, Bo, Cu and Mn (Table 4 and Fig. 3), negative correlation between the grain yield and mineral concentration might due to the dilution effect, whereby the whole-plant uptake or internal pool of certain minerals may be limited and unable to meet the genetic potential of a higher seed mineral load. Hence, breeding for increased uptake of some minerals may be needed to sustain higher mineral concentrations in higher-yielding lines (Ma *et al.*, 2017; Sankaran and Grusak 2014; Kaur *et al.*, 2019) in chickpea.

Principal component analysis (PCA). PCA analysis was carried out for protein and nutritional content in order to describe and to gain the better understand the source of genetic variation among green seeded chickpea genotypes. The three component *viz.*, PC1, PC2 and PC3 showed 53.96%, 11.03% and 10.40% of variations among the studied parameters respectively, as shown with the help of pie chart in Fig. 4a. The first three main PCAs are extracted from the complicated ten PCA components, the total cumulative variance of these first three principal components (PC1, PC2 and PC3) account for 73.39% of the total variation as shown in the graph of cumulative percent variance in Fig. 4b. In literature similar results were reported for PCA analysis of rice germplasm lines under high temperature stress by Mahendran *et al.* (2015); Farida *et al.* (2022) reported PCA for morphological traits along with nutritional traits in chickpea germplasm accessions. Mahmood *et al.* (2018); Ghafoor *et al.* (2003); Talebi *et al.* (2013); Upadhyaya *et al.* (2007) reported contribution of more than two PCs in variability for different agronomic and nutrient traits.

The eigenvectors decreased significantly from PC1 (5.39) to PC3 (1.04), it indicates that decrease in the eigenvalues after PC3, the remaining principal components did not described much variation, thus only the first three PCs were considered which explaining much of the variation for the studied population. Results revealed by rotated component matrix showed that the PC1 which accounts for the maximum variability (53.96%) and highly loaded with parameter such as K (0.988), Mg (0.154), Ca (0.028), Fe (0.004), Mn (0.002), Zn (0.001), Bo (0.001), Cu (0.00) and grain yield per plant (0.00), contributed in positive direction whereas, only the Pr contributed in negative direction as shown in Table 5 and Fig. 5B in variable PCA plot, it clearly indicated that the variation in PC1 is majorly contributed by Pottasium (K), Magnesium (Mg) and Calcium (Ca) nutrient parameters. PC2 accounted 11.03% of the total variation and loaded with nutrient parameters *viz.*, Mg (0.869), Ca (0.472), Fe (0.016), Zn (0.007), Mn (0.005), Bo (0.003), Cu (0.001) and Pr (0.00), while, the nutrient K and grain yield per plant contributed in negative direction (Table 5). PC3 had the positive contribution from the characters like, Ca (0.880), K (0.048), Zn (0.036), Fe (0.015), Bo (0.012), Cu (0.002), Pr (0.00) and grain yield per plant (0.00). Thus the prominent nutrient parameters combining together in different principal components and contributing towards the explaining the variability and have tendency to remain together, so these nutrient parameters may be kept into consideration and may be utilized in breeding program for improving nutrient parameters in green seeded chickpea genotypes.

The PCA-Biplot analysis revealed that the PC1 consisting of the 15 genotypes contributing high level of variability for nutrient parameters among the 94 genotypes studied namely 33: RGK-28-15, 17: RGK-12-15, 91: RGK-29-15-29, 20: RGK-15-15, 85: RGK-

23-15-23, 26: RGK-21-15, 32: RGK-27-15, 51: WRGK-42-15, 74: RGK-5-15-5, 54: RGK-45-15, 13: RGK-8-15, 68: RGK-2-15-2, 21: RGK-16-15, 47: WRGK-38-15, 16: RGK-11-15 (Fig 5A).Several reporters said that first two PCs were the most important in reflecting the variation pattern among genotypes and the traits highly associated with these should be used in differentiating the breeding lines. Neugschwandtner *et al.* (2015) reported higher concentrations for some elements *i.e.*, K, Ca, Mg, Cu, Mn, while the observed Zn concentration was similar. Quantities of minerals in cultivated plants are influenced by numerous complex factors including genotype, soil, environmental conditions and nutrition interactions. The scree plot of the PCA showed in Fig. 6 explained both percent explained variation and eigenvalues associated with each principle component. PC1 showed 53.96% variability with the eigenvalue of 5.39 and the eigenvalues are gradually declined from PC1 to PC3. Elbow type with semi curve line is obtained after PC3 tended to straight with minute difference observed in each PC and it is clearly showed that the maximum variation was observed in PC1. So the principal components having more than one eigenvalue that showed more variation which act as key factor for selection of diverse breeding lines. The principle component with <1 Eigenvalue should be eliminated due to their minimum contribution towards variability. In literature similar reports were mentioned by Hammed *et al.* (2007); Chowdhury *et al.* (2002).

Diversity analysis. The genotypes were grouped into major three clusters based on the protein and nutrient content of green seeded chickpea genotypes by using Ward's hierarchical algorithm based on squared Euclidean distances in SAS *version 9.2*, software as shown in the Fig. 7. Cluster III had maximum number of genotypes fallowed by cluster II and cluster I as shown in the Table 6, distribution pattern of all the genotypes into three clusters showed the presence of considerable genetic diversity among the genotypes for most of the nutrient parameters studied. The average inter and intra cluster distance was also calculated for these nutrient parameters with three cluster as given in (Table 7 and Fig. 8). The cluster I has revealed highest inter cluster distance cluster II (6.32) fallowed by cluster III and cluster I, similarly the cluster II has shown highest inter cluster distance with cluster III fallowed by cluster II. The greater the distance between two clusters, wider is the expected genetic diversity between them. The cluster mean for all the nutrient parameters along with grain yield per plant has shown in Table 8. The maximum value for grain yield per plant was recorded in cluster III (12.69) g, followed by cluster I and II, cluster II showed higher mean values for the nutrient parameters namely, Fe, Zn, Ca, Bo, Cu, K, Mg and Mn, while cluster III has shown higher mean values for protein and grain yield per plant, the results were in accordance with Sharifi *et al.* (2018); Arora *et al.* (2018). The cluster means will be useful for hybridization of selected genotypes from divergent clusters and it will be essential for combining all the targeted traits.

Table 1: Analysis of variance for protein and nutrients content along with grain yield per plant of green seeded chickpea genotypes.

Source	df	Pr	Fe	Zn	Ca	Bo	Cu	K	Mg	Mn	GY
Treatment	93	3.88 *	483.07 **	165.87 **	23564.06 *	32.63 **	6.27 **	16781873.51 **	438597.76 **	59.62 **	14.08 **
Check	3	0.57 ns	5014.78 **	26.08 *	15582.56 ns	35.78 **	0.48 ns	700154.07 *	20543.53 **	1.21 ns	22.52 **
Test vs. Check	1	11.24 *	244.50 **	236.04 **	39694.54 *	13.81 **	89.80 **	279691106.35 **	8192239.46 **	971.41 **	1.43 ns
Test genotypes	89	3.91 *	333.00 **	169.79 **	23651.86 *	32.74 **	5.53 **	14369917.62 **	365569.91 **	51.34 **	13.94 **
Blocks	2	0.07 ns	19.61 ns	4.97 ns	4777.81 ns	0.99 ns	0.63 ns	63456.15 ns	1523.35 ns	4.24 *	0.46 ns
Residuals	6	0.96	6.46	5.44	5527.35	0.54	0.30	95308.19	1072.66	0.58	0.78
A Test Treatment *vs* a Control Treatment	CD	3.09	8.03	7.37	234.86	2.32	1.72	975.23	103.46	2.41	2.78
Control Treatment Means		1.95	5.08	4.66	148.54	1.47	1.09	616.79	65.43	1.52	1.76
Two Test Treatments (Different Blocks)		3.78	9.84	9.02	287.64	2.85	2.10	1194.41	126.71	2.95	3.41
Two Test Treatments (Same Block)		3.38	8.80	8.07	257.27	2.55	1.88	1068.31	113.34	2.64	3.05
	CV (%)	5.07	6.72	14.07	29.07	15.48	10.81	3.16	2.16	4.35	8.06

Note: Pr: Protien (ppm); Fe: Iron (ppm); Zn: Zinc (ppm); Ca: Calcium (ppm); Bo: Boron (ppm); Cu: Cupper (ppm); K: Potash (ppm); Mg: Magnesium (ppm); Mn: Manganese (ppm); GY: Grain yield per plant (g)**;***df*: Degrees of freedom, CV (%): Coefficient of variation in percent, CD: critical difference
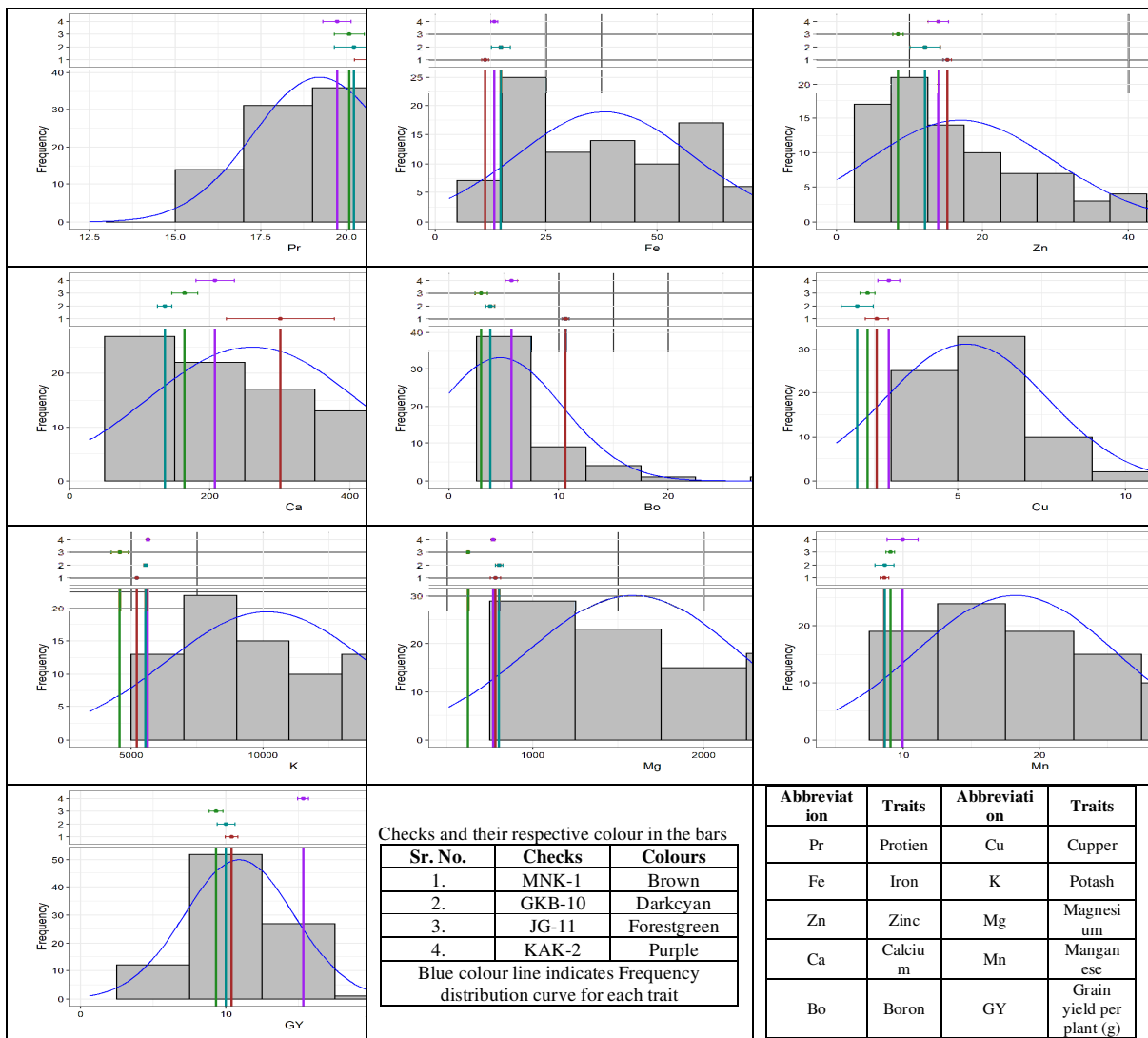
Fig. 1. Graphical representation of frequency distribution for protein and nutrients content along with grain yield per plant of green seeded chickpea genotype.

Table 2: Descriptive statistics for protein and nutrients content along with grain yield per plant of green seeded chickpea genotypes.

Traits	Std. Error	Std. Deviation	Min	Max	Mean	Skewness	'Sk' Types	Kurtosis	'K' Tpyes
Pr	0.20	1.93	12.52	24.42	19.21	-0.26 ns	Symetrically Skewed	3.79 ns	Leptokurtic
Fe	2.04	19.83	3.12	94.86	38.17	0.42 ns	Symetrically Skewed	2.40 ns	Leptokurtic
Zn	1.32	12.76	10.15	55.88	16.95	1.07 **	Highly Skewed	3.55 ns	Leptokurtic
Ca	15.47	150.03	28.91	611.37	260.38	0.42 ns	Symetrically Skewed	2.06 **	Leptokurtic
Bo	0.58	5.67	5.21	37.12	4.68	3.13 **	Highly Skewed	15.68 **	Leptokurtic
Cu	0.25	2.40	1.37	13.54	5.24	0.98 **	Moderately Skewed	4.27 *	Leptokurtic
K	398.90	3867.48	3441.86	18924.40	10159.70	0.25 ns	Symetrically Skewed	1.98 **	Leptokurtic
Mg	64.09	621.34	509.49	2892.03	1585.79	0.21 ns	Symetrically Skewed	1.91 **	Leptokurtic
Mn	0.76	7.36	5.07	35.05	18.23	0.31 ns	Symetrically Skewed	2.12 **	Leptokurtic
GY	0.39	3.74	0.65	28.73	10.91	1.14 **	Highly Skewed	7.44 **	Leptokurtic

Note 1: If the skewness is between -0.5 and 0.5, the data are fairly symmetrical: If the skewness is between -1 and – 0.5 or between 0.5 and 1, the data are moderately skewed: If the skewness is less than -1 or greater than 1, the data are highly skewed. If kurtosis is close to 0 the data are normally distributed: If kurtosis is less than 0 the data is platikurtic: If kurtosis is less than 0 the data is leptokurtic

Note 2: Pr: Protien (ppm); Fe: Iron (ppm); Zn: Zinc (ppm); Ca: Calcium (ppm); Bo: Boron (ppm); Cu: Cupper (ppm); K: Potash (ppm); Mg: Magnesium (ppm); Mn: Manganese (ppm); GY: Grain yield per plant (g)

Table 3: Genetic variability studies for protein and nutrients content along with grain yield per plant of green seeded chickpea genotypes.

Traits	GCV	GCV category	PCV	PCV category	h²BS	hBS category	GAM	GAM category
Pr	8.94	Low	10.29	Medium	75.52	High	16.03	Medium
Fe	47.34	High	47.80	High	98.06	High	96.71	High
Zn	75.65	High	76.89	High	96.8	High	153.55	High
Ca	51.70	High	59.06	High	76.63	High	93.37	High
Bo	121.21	High	122.23	High	98.35	High	247.99	High
Cu	43.62	High	44.83	High	94.66	High	87.55	High
K	37.19	High	37.31	High	99.34	High	76.46	High
Mg	38.07	High	38.13	High	99.71	High	78.43	High
Mn	39.09	High	39.31	High	98.87	High	80.18	High
GY	33.25	High	34.22	High	94.43	High	66.66	High

Note: Pr: Protien (ppm); Fe: Iron (ppm); Zn: Zinc (ppm); Ca: Calcium (ppm); Bo: Boron (ppm); Cu: Cupper (ppm); K: Potash (ppm); Mg: Magnesium (ppm); Mn: Manganese (ppm); GY: Grain yield per plant (g); GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation; h²BS: Heritability in broad sense; GAM: Genetic advance percent mean
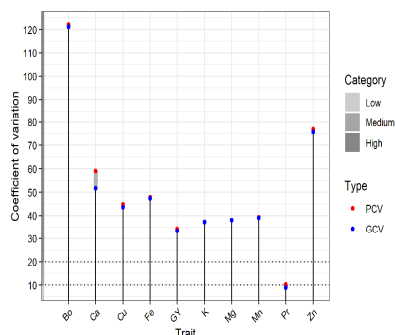

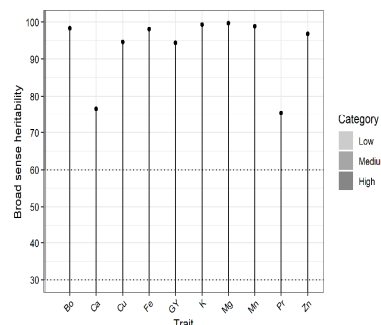
Fig. 2a. Genotypic and phenotypic variation
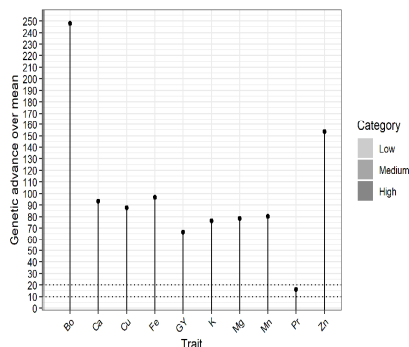

Fig. 2b. Broad sense heritability

Range for phenotypic and genotypic coefficient of variation and GAM

Range of variance	Category
< 10%	Low
10 to 20%	Moderate
> 20%	High

As suggested by Allard (1960); Sivasubramanian and Madhavamenon (1973) and Johnson *et al.* (1955)

Classification of heritability

Range of heritability	Category
0 to 30%	Low
30 to 60%	Moderate
> 60%	High

As suggested by Johnson *et al.* (1955)

Fig. 2c. Variation for Genetic advance percent mean

Fig. 2d. Classification of GCV, PCV h^2 and GAM.

Fig. 2 (a, b and c). Graphical representation of phenotypic and genotypic coefficient of variation, heritability and genetic advance percent mean.

Table 4: Correlation for protein and nutrients content along with grain yield per plant of green seeded chickpea genotypes.

| Traits	Pr	Fe	Zn	Ca	Bo	Cu	K	Mg	Mn	GY
Pr	1	-0.066 ns	0.054 ns	-0.054 ns	0.102 ns	-0.116 ns	-0.109 ns	-0.089 ns	-0.127 ns	0.091 ns
Fe		1	0.509**	0.677**	0.279**	0.772**	0.844**	0.845**	0.830**	-0.057 ns
Zn			1	0.431**	0.217*	0.437**	0.401**	0.381**	0.365**	0.007 ns
Ca				1	0.524**	0.639**	0.676**	0.744**	0.685**	-0.052 ns
Bo					1	0.144	0.298**	0.333**	0.294**	0.008 ns
Cu						1	0.859**	0.854**	0.860**	0.069 ns
K							1	0.964**	0.952**	-0.009 ns
Mg								1	0.956**	-0.060 ns
Mn									1	0.011 ns
GY										1
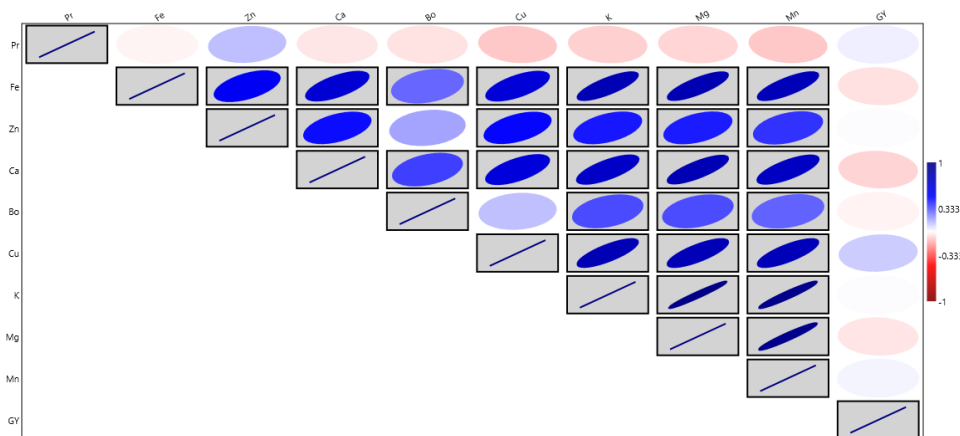
Pr: Protien (ppm); Fe: Iron (ppm); Zn: Zinc (ppm); Ca: Calcium (ppm); Bo: Boron (ppm); Cu: Cupper (ppm); K: Potash (ppm); Mg: Magnesium (ppm); Mn: Manganese (ppm); GY: Grain yield per plant (g)

Significance level 0.05 (*): 0.204; 0.01 (**): 0.267; ns: Non-significant

Fig. 3. Graphical representation of correlation for protein and nutrients content along with grain yield per plant of green seeded chickpea genotypes.

Table 5: Eigen values, Percent variance, cumulative proportion and component loading of protein and nutrient parameters in green seeded chickpea genotypes.

Parameters	PC1	PC2	PC3
Pr	-4.50E-05	0.000	0.000
Fe	0.004	0.016	0.015
Zn	0.001	0.007	0.036
Ca	0.028	0.472	**0.880**
Bo	0.001	0.003	0.012
Cu	0.000	0.001	0.002
K	**0.988**	-0.149	0.048
Mg	0.154	**0.869**	-0.470
Mn	0.002	0.005	-0.002
GY	0.00	-5.222E-03	0.002
Eigen value	**5.39**	**1.10**	**1.04**
Proportion of variance	**53.96**	**11.03**	**10.40**
Cumulative proportion	**53.96**	**64.99**	**75.40**
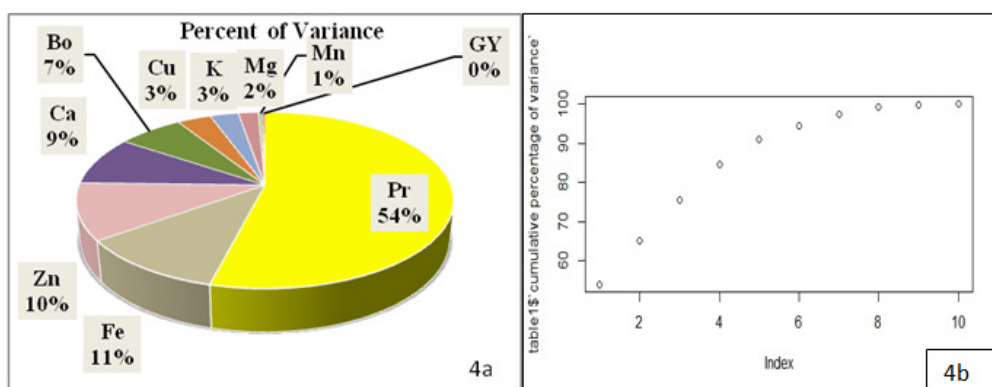
Note: Pr: Protien (ppm); Fe: Iron (ppm); Zn: Zinc (ppm); Ca: Calcium (ppm); Bo: Boron (ppm); Cu: Cupper (ppm); K: Potash (ppm); Mg: Magnesium (ppm); Mn: Manganese (ppm); GY: Grain yield per plant (g)

Fig.4a: Pie chart showing % variance for protein and nutrient **Fig.4b:** Graph of Cumulative % variance.

Fig. 4. variation showing pie chart and graph of cumulative percent variance for nutrients parameters in green seeded chickpea genotypes.
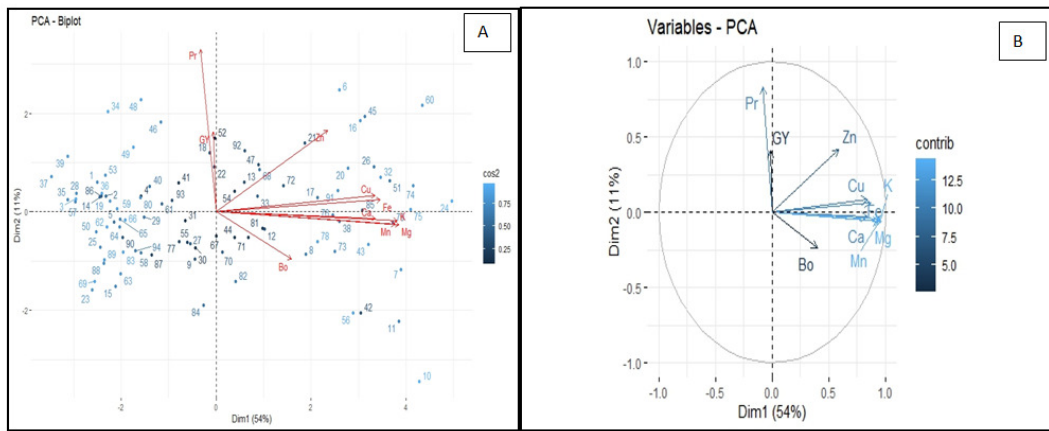
Note: PCA-Biplot PC1 genotypes: 33: RGK-28-15, 17: RGK-12-15, 91: RGK-29-15-29, 20: RGK-15-15, 85: RGK-23-15-23, 26: RGK-21-15, 32: RGK-27-15, 51: WRGK-42-15, 74: RGK-5-15-5, 54: RGK-45-15, 13: RGK-8-15, 68: RGK-2-15-2, 21: RGK-16-15, 47: WRGK-38-15, 16: RGK-11-15

Fig. 5. 2D PCA- Biplot and Variable- PCA showing the distribution of protein and nutrients contenton PC1 and PC2 of green seeded chickpea genotypes on PCA axes.
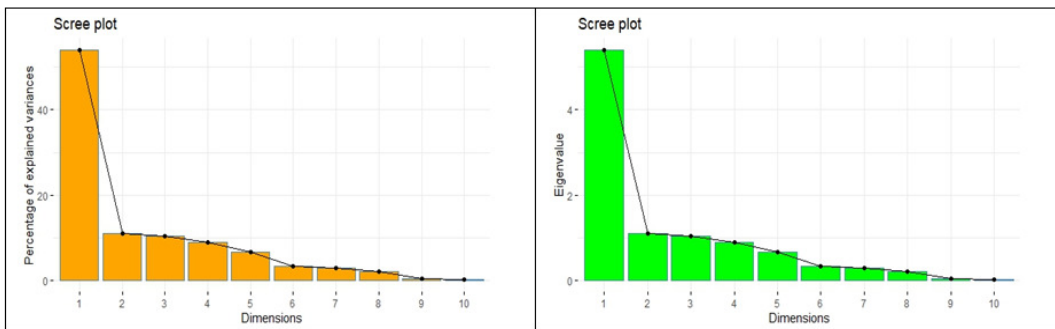
Note: 1 to 10 number = component numbers

Fig. 6. Scree plot showing percentage of explained variance and Eigenvalues in response to number of components for the estimated variables of green seeded chickpea genotypes.
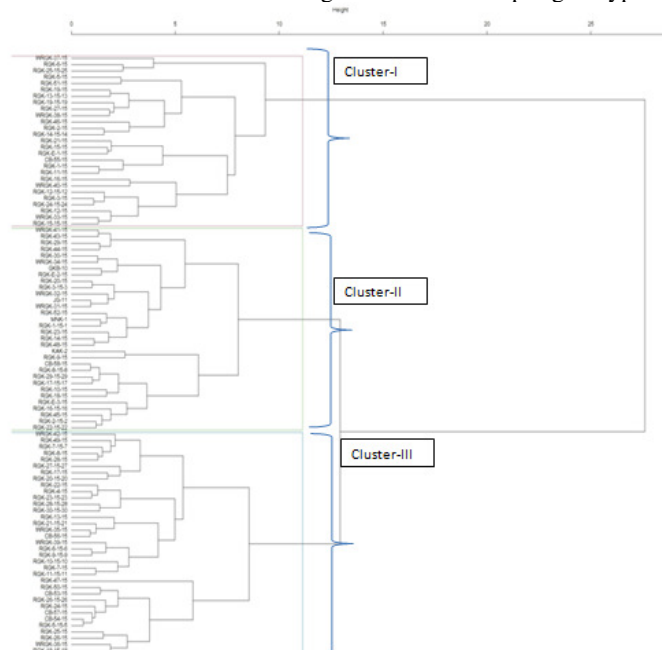

Fig. 7. Clustering of green seeded genotypes relation was inferred by using Ward's hierarchical algorithm based on squared Euclidean distances in SAS *version 9.2* software.

Table 6: Genetic diversity for protein and nutrients content of green seeded chickpea genotypes.

Number of cluster	Number of genotypes	Genotypes
Cluster I	27	WRGK-37-15, RGK-6-15, RGK-25-15-25, RGK-5-15, RGK-51-15, RGK-19-15, RGK-13-15-13, RGK-19-15-19, RGK-27-15, WRGK-38-15, RGK-46-15, RGK-2-15, RGK-14-15-14, RGK-21-15, RGK-15-15, RGK-E-1-15, CB-55-15, RGK-1-15, RGK-11-15, RGK-16-15, WRGK-40-15, RGK-12-15-12, RGK-3-15, RGK-24-15-24, RGK-12-15, WRGK-33-15, RGK-15-15-15
Cluster II	32	WRGK-41-15, RGK-43-15, RGK-29-15, RGK-44-15, RGK-30-15, WRGK-34-15, GKB-10, RGK-E-2-15, RGK-20-15, RGK-3-15-3, WRGK-32-15, JG-11, WRGK-31-15, RGK-52-15, MNK-1, RGK-1-15-1, RGK-23-15, RGK-14-15, RGK-48-15, KAK-2, RGK-9-15, CB-58-15, RGK-8-15-8, RGK-29-15-29, RGK-17-15-17, RGK-10-15, RGK-18-15, RGK-E-3-15, RGK-16-15-16, RGK-45-15, RGK-2-15-2, RGK-22-15-22
Cluster III	35	WRGK-42-15, RGK-49-15, RGK-7-15-7, RGK-8-15, RGK-28-15, RGK-27-15-27, RGK-17-15, RGK-20-15-20, RGK-22-15, RGK-4-15, RGK-23-15-23, RGK-28-15-28, RGK-30-15-30, RGK-13-15, RGK-21-15-21, WRGK-35-15, CB-56-15, WRGK-39-15, RGK-6-15-6, RGK-9-15-9, RGK-10-15-10, RGK-7-15, RGK-11-15-11, RGK-47-15, RGK-50-15, CB-53-15, RGK-26-15-26, RGK-24-15, CB-57-15, CB-54-15, RGK-5-15-5, RGK-25-15, RGK-26-15, WRGK-36-15, RGK-18-15-18

Table 7: Average inter and intra cluster distance (By Tocher's method) for protein and nutrients content of green seeded chickpea genotypes.

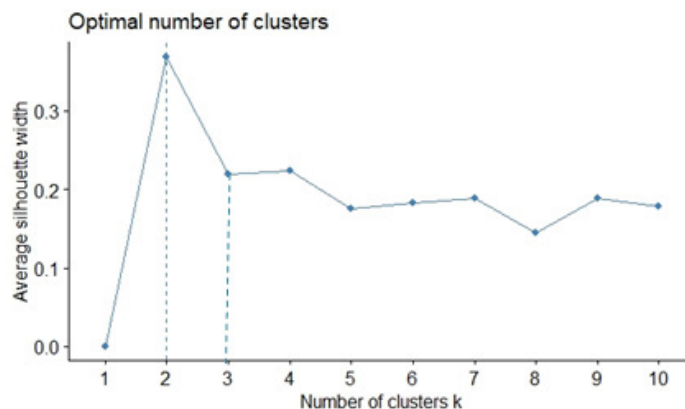| Cluster	I	II	III
I	2.71	6.32	3.45
II		3.70	4.19
III			2.72

Fig. 8. Average inter and intra cluster distance (By Tocher's method) for protein and nutrients content of green seeded chickpea genotypes.

Table 8: Cluster means for protein and nutrients content of green seeded chickpea genotypes.

Cluster	Pr (%)	Fe (ppm)	Zn (ppm)	Ca (ppm)	Bo (ppm)	Cu (ppm)	K (ppm)	Mg (ppm)	Mn (ppm)	GY (g)
I	19.13	26.68	11.46	166.67	2.92	3.97	7640.15	1172.24	13.53	11.07
II	19.33	56.68	25.75	411.34	7.46	7.28	14219.03	2252.06	25.79	10.64
III	21.42	33.95	20.61	211.62	6.59	4.15	1635.64	1564.69	20.61	12.69

CONCLUSIONS

The present study that used multivariate techniques to appraise the measure of genetic variation in micro and macro nutrient parameters with grain yield components of 90 green seeded chickpea genotypes with *Kabuli* types was a first step in achievement an understanding into the genetic diversity of *Desi* green seed with *Kabuli* milky white parental genotypes, which is an important step towards an efficient utilization of genetic resources of chickpea breeding. Results from the work revealed existence of sufficient amount of variability giving an opportunity for exploring the genetic diversity along with strong association between the micro and macro nutrients studied. The classifying of genotypes would be of practical value to chickpea breeders to identify the genotype with desired trait for utilization in breeding program for genetic

improvement. The cluster analysis classified the chickpea genotypes into three separate clusters, exhibiting that hybridization of genotypes across clusters could lead to increase in heterosis in cross progenies. The results of present study can be used in breeding strategies for future hybridization programs for nutrition improvement and to combat micronutrient malnutrition by generating bio-fortified green *Kabuli* chickpea genotypes which can be used as a nutrient dense seeds to mitigate many human health problems associated with protein and micronutrient deficiency.

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