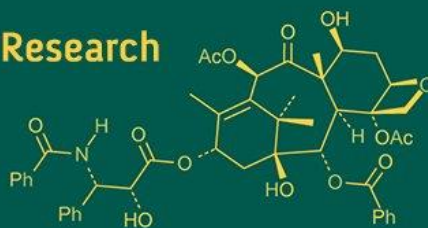
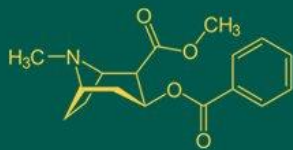


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Exploring variability, mean performance and genetic diversity for seed protein content in chickpea (*Cicer arietinum* L.)

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Abstract

Chickpea is an abundant source of seed protein content among all pulse crops. However, the current protein content in chickpea genotypes range between 20 to 22% in the varieties which is not sufficient to meet the protein demand of burgeoning population. Ascertaining genetic diversity in chickpea genotypes for the trait is a prerequisite for improvement of seed protein content higher than the existing content. The present study focused on exploring variability, mean performance and genetic diversity for seed protein content in chickpea. Augmented block design was deployed to evaluate 156 chickpea genotypes including landraces, advanced breeding lines, cultivars and a variety BG 3062. Seed protein content was estimated in two technical replicates using Kjeldahl method. Highly significant variation for seed protein content was observed with the range from 15.21% to 32.43%. The extremely close value of phenotypic coefficient of variance (8.21) and genotypic coefficient of variance (7.41), indicated the minimal impact of environment on seed protein content. High heritability (90.28%) and high genetic advance as percent of mean (22.60%) for seed protein content indicated the predominance of additive gene action and suggested simple selection is effective in the genetic improvement of the trait. A total of 93 genotypes recorded high seed protein content over the high seed protein content check ICC 4639 (23.09%), whereas 12 genotypes demonstrated low seed protein content than the lower seed protein content check KAK 2 (18.49%), indicating that these are the potential donors and recipients in the breeding programs aimed at the development of protein abundant chickpea varieties. Genetic diversity analysis divided the 156 genotypes into three distinct clusters advocating that strategic selection of contrasting genotypes for seed protein content can fulfil the development of protein abundant chickpea varieties higher than existing varieties.

Keywords: Chickpea, heritability, genetic advance, genetic diversity, seed protein content (SPC)

Introduction

Chickpea (*Cicer arietinum* L.) with a chromosome number $2n = 2x = 16$ belongs to the family Fabaceae. It is the third most important food legume, which is grown in more than 52 countries worldwide in the area of 14.09 million hectares with a production of 16.53 million tonnes and a productivity of 1171.7 kg/ha (FAOSTAT, 2023) [2]. The cultivated chickpea is categorised into two distinct types i.e. Desi and Kabuli type. Desi type is characterized by mostly pink flowers, angular shaped, brown-coloured and small seeds, whereas Kabuli type is with white flowers, owl's head shaped, beige-coloured and large seeds. Its seeds are rich source of seed protein content (SPC) which range from 20-22% (Pundir *et al.*, 1988; Jadhav *et al.*, 2015) [13, 6]. It is consumed as plant-based protein (PBP) along with animal-based protein (ABP). Regular consumption of PBP has been shown to prolong longevity (Jukanti *et al.*, 2012) [10]. Moreover, it also contributes to farming systems in ways like improving soil fertility through nitrogen fixation by Rhizobium (Singh *et al.*, 2022) [14].

Considering the importance of seed protein content in nutritional security, improving its content in chickpea seeds is imperative. The world's population is expected to reach 9.7 billion by 2050 (The Lancet 2022) [12]. The increasing population growth will create more protein demand. So, breeding and selection for high protein chickpea varieties is urgent need to meet growing population. To achieve this goal, it is necessary to understand the existing variability among the diverse chickpea genotypes and identification of high and low seed

protein content genotypes. Heritability estimates together with genetic advance are more important than the heritability alone to predict the resultant effect of selecting the best genotypes. High genetic advance coupled with high heritability estimates offers most effective condition for selection (Singh *et al.*, 2014) ^[15]. The variability of seed protein content in the currently available chickpea cultivars 20 to 25% (Pundir *et al.*, 1988; Jadhav *et al.*, 2015) ^[13, 6].

Selection of most desirable and genetically diverse parents for attempting successful breeding programs requires the information of mean performance and divergence existing in the available material. These better performing genetically diverge genotypes most probably bring out heterotic effects and transgressive segregants when utilized in artificial hybridization for the crop improvement programs (Geethanjali *et al.*, 2018) ^[5]. In the recent years, efforts were made to evaluate the genetic diversity of seed protein contents in chickpea (Aliu *et al.*, 2016; Jakhar *et al.*, 2016; Gediya *et al.*, 2018; Tsehay *et al.*, 2020) ^[1, 7, 4, 17]. There are limited studies on evaluation of genotypes including diversity in terms of type of chickpea *i.e.* desi and kabuli, biological origin and combination of germplasm accessions and improved lines together in a single experiment. Therefore an attempt was made to evaluate the desi and kabuli types of chickpea genotypes to identify the suitable parents to be selected for use in breeding programmes for the improvement of seed protein content higher than in existing varieties.

Materials and Methods

The present study was carried out with 156 genotypes which includes 80 desi and 76 kabuli type of chickpea genotypes from diverse geographical origin along with six checks *viz.*, JG 11, GCP 105, KAK2, ICC 8151, HABRU and ICC 4639. Experiment was conducted by following Augmented Block Design with two replicates of five blocks each at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India 2022-23. Each block contains 30 genotypes and six checks. Each genotype was sown in single row with 4m length with a spacing of 30 cm between rows and 10 cm between the plants. Physiologically matured pods were harvested and manually threshed to obtain the seeds. Careful attention was given during harvesting and threshing to avoid contamination with other genotypes.

A weighted 80g seed from each genotype was cleaned and milled into flour using a CM 290 Cemotech™ laboratory grinder (FOSS, Hillerod, Denmark). The flour samples were thoroughly dried at 60 °C for overnight in an oven and were subsequently cooled to room temperature before performing chemical analysis. The total nitrogen content for each sample was estimated by Kjeldahl method in two technical replicates adhering to the standard AOAC protocol (Official methods 925.10 and 2001.11). The total protein content of sample was then computed by multiplying the nitrogen content (N%) by a protein conversion factor of 6.25 (Protein = N% × 6.25). Mean for the two technical replicates was calculated for seed protein content and used for data analysis. Mean performance of the genotypes was analysed based on the mean values of SPC. The analysis of variance, descriptive statistics, genetic parameters were generated using the mean data of the SPC in R Studio. Hierarchical cluster analysis was performed in R Studio using wards D² method. The adapted seed protein content scale categorised

the genotypes with SPC values ≤ 24.69% as low, those with values between 24.69 and 26.35% as medium and with ≥ 26.35% as high SPC genotypes (Kaur *et al.*, 2019) ^[11].

Results and Discussion

All sources of variance were found statistically highly significant indicating that there is an ample scope for the exploitation of variability in seed protein content by selection (Table 1). The high value of treatments (eliminating blocks) *i.e.* 7.55** representing the highly significant difference in seed protein content among the treatments. Such kind of mean squares for SPC were observed earlier by Jayalakshmi *et al.* (2019) ^[9]. The very high value of test vs check *i.e.* 239.66** revealed significant difference in SPC between test and check varieties. The very low value of the residuals *i.e.* 0.80 indicated that the unexplained variation was small, experiment is well controlled, and the treatments and blocks accounted for the most of the differences in SPC.

The seed protein content in the selected chickpea genotypes ranged from 15.21 to 32.43% with a mean of 23.56%. The low value of standard deviation *i.e.* 2.88 indicating that the protein values are close to mean and small value of standard error (SE) represented that sample mean is more accurate. Similar kind of SE for SPC was observed by Gaur *et al.* (2016) ^[3]. The negative skewness value *i.e.* -0.03 indicating that tail of the data distribution is longer on the left side. The kurtosis value 3.24 indicated leptokurtic distribution of the data suggesting that seed protein content data has heavier tails and sharper peak suggesting that trait is under control of relatively few segregating genes. Contrary, the normal distribution of SPC was observed by Vijayalakshmi *et al.* (2001) ^[17]. The frequency distribution of the SPC was depicted in Figure 1 and descriptive statistics were tabulated in Table 2.

Lower difference between phenotypic variance (PV) and genotypic variance (GV) was found (PV = 8.21 and GV = 7.41), indicating that higher influence of genetic makeup on SPC than environmental effect. The moderate and close values of phenotypic co-efficient with genotypic co-efficient confirms that genetic factors are the major source of variation in SPC. The high heritability of 90.28% for SPC indicated that the trait was highly inherited with least influencing environment. The value of genetic advance with 5.33 suggested the significant potential for improvement of SPC through breeding. The high genetic advance as percent of mean with value 22.60% indicated that mean SPC of the selected population could be 22.60% higher than the original population mean SPC (23.56%). Similar reports of high heritability coupled with high genetic advance as percent of mean was reported by Jayalakshmi *et al.* (2019) ^[9]. The high heritability and genetic advance as percent of mean indicates additive gene action in the inheritance of SPC and effective breeding programs could improve the SPC in chickpea. Moderate genotypic variability coupled with high heritability and genetic advance as percent of mean for SPC was reported earlier by Tefera *et al.* (2021) ^[16] and Jayalakshmi *et al.* (2021) ^[8]. The genetic parameters of the selected chickpea genotypes for seed protein content was presented in Table. 3.

The seed protein content in the evaluated genotypes ranged from 15.21% to 32.43% with a mean of 23.56%. Similar kind of SPC variability was observed 14.15% to 23.08% by Tefera *et al.* (2021) ^[16] and 16% to 32.7% by Jayalakshmi *et*

al. (2021) [8]. Among the studied genotypes JAKI 9218 exhibited low SPC, while ICC 8752 showed highest SPC. A total of 82 genotypes exhibited high protein content compared to the general mean and 93 having high protein content over the high protein content check ICC 4639 (23.09%) and 12 demonstrated low SPC than the low SPC check KAK 2 (18.49%). These extreme genotypes could be exploited for the development of protein rich chickpea varieties.

The hierarchical clustering analysis (HCA) was performed based on the wards D² method to characterize the genetic divergence among the selected chickpea genotypes. From the HCA analysis, the total genotypes were divided into three clusters (Table 4) among which cluster I exhibited highest number (115) of genotypes, followed by cluster II (21) and cluster III (20). Cluster means for seed protein content ranged from 18.18 in Cluster III to 28.32% in Cluster II. In the earlier reports cluster means were less than the lowest cluster mean of the current study (Tsehaye *et al.*, 2020) [17]. Intra cluster distance was ranged from 0.52 to 0.57 and inter cluster distance was ranged from 1.57 and 3.40. Maximum inter cluster distance was observed between Cluster II and III indicated the presence of substantial

genetic divergence among the clustered genotypes.

The distribution of genotypes into different cluster groups based on biological origin are represented in Figure 2. The studied genotypes represents nine different biological sources with Cluster I consisted of six sources (Landrace, Traditional Cultivar/Landrace, Unknown, Breeding Material, Cultivar and Advanced Cultivar), Cluster II limited to two (Landrace and Traditional Cultivar/Landrace) and Cluster III the most diverse containing seven sources (Landrace, Traditional Cultivar/Landrace, Unknown, Cultivar, Advanced/Improved Cultivar, Introgression Line and a Variety BG 3062).

Out of 156 genotypes, 102 genotypes were classified as low SPC category, 33 genotypes as medium SPC group and 21 genotypes as high SPC class. The genotypes from the extreme category are the potential genotypes for the development of protein rich chickpea varieties. Similarly Tsehaye *et al.* (2020) [17] reported IE-16-109/2, ICC 14778, ICC 510, DZ-2012-CK-0253 and ICC 5135 and Jayalakshmi *et al.* (2021) [8] reported ICC 14402, ICC 1398, ICC 9942, ICC 6874, ICC 14831 and ICC 7441 as promising genotypes for breeding protein rich chickpea.

Table 1: ANOVA for Augmented Block Design for seed protein content

S. No	Source of variation	Degree of freedom	Mean squares
1	Block (ignoring Treatments)	4	115.23**
2	Treatment (eliminating Blocks)	155	7.55**
3	Test	149	8.21**
4	Check	5	32.95**
5	Test vs Check	1	239.66**
6	Test and Test vs Check	150	6.70**
7	Treatment (ignoring Blocks)	155	10.50**
8	Block (eliminating Treatments)	4	0.97
9	Residuals	20	0.80

*, ** is significant at p = 0.05 and p = 0.01 respectively

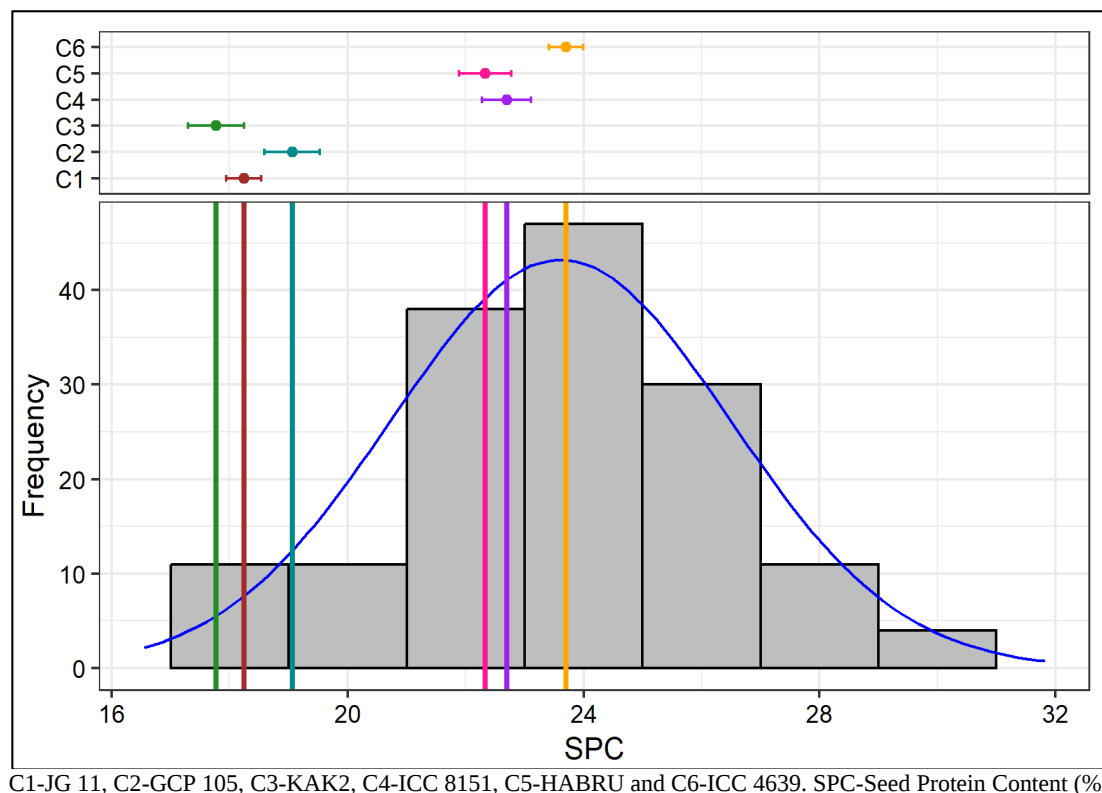


Fig 1: Frequency distribution of chickpea genotypes and checks for seed protein content

Table 2: Descriptive statistics of chickpea genotypes for seed protein content

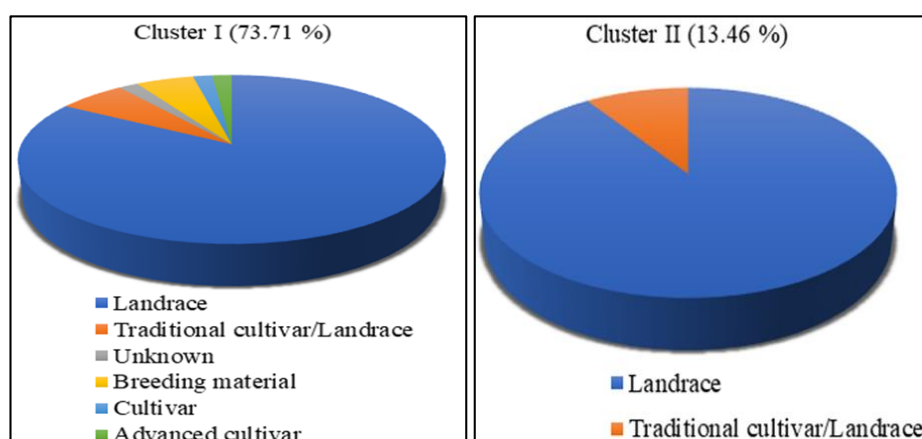
S. No	Descriptive statistic	Value
1	Minimum	15.21
2	Maximum	32.43
3	Range	17.22
4	Mean	23.56
5	Standard Error	0.23
6	Standard Deviation	2.88
7	Skewness	-0.03
8	Kurtosis	3.24
9	Coefficient of variance (%)	3.85

Table 3: Genetic parameters of chickpea genotypes for seed protein content

S. No	Genetic Parameter	Value
1	Phenotypic variance	8.21
2	Genotypic variance	7.41
3	Environmental variance	0.80
4	Phenotypic co-efficient of variance (%)	12.13
5	Genotypic co-efficient of variance (%)	11.53
6	Environmental co-efficient of variance (%)	3.78
7	Heritability (%)	90.28
8	Genetic advance	5.33
9	Genetic advance as percent of mean	22.60

Table 4: Grouping of chickpea genotypes into clusters based on wards D² method and cluster wise range of seed protein content

S. No	Cluster	No. of genotypes	Genotypes	Range of SPC (%)
1	I	115	ICC867, ICC1882, ICC1915, ICC1923, ICC3892, ICC4853, ICC4872, ICC5383, ICC5845, ICC5878, ICC5879, ICC6306, ICC6874, ICC7184, ICC7413, ICC8350, ICC9137, ICC9586, ICC9636, ICC9643, ICC9712, ICC9848, ICC9872, ICC10018, ICC10673, ICC10685, ICC10939, ICC11121, ICC11198, ICC11279, ICC11303, ICC11378, ICC11664, ICC11879, ICC12037, ICC12851, ICC12947, ICC13219, ICC13357, ICC14778, ICC14831, ICC15406, ICC15510, ICC15518, ICC15762, ICC15785, ICC15888, ICC16374, ICC20259, ICC18839, ICC19100, NBeG119, ICC67, ICC1164, ICC1230, ICC2482, ICC3410, ICC3421, ICC4363, ICC4639, ICC5337, ICC5613, ICC5639, ICC6571, ICC6579, ICC6816, ICC7255, ICC7272, ICC7308, ICC7315, ICC7571, ICC8058, ICC8151, ICC8261, ICC8318, ICC8384, ICC8740, ICC8855, ICC10341, ICC10393, ICC10399, ICC10466, ICC11498, ICC11764, ICC12324, ICC12328, ICC13187, ICC13283, ICC13461, ICC13599, ICC13628, ICC13764, ICC14199, ICC14402, ICC15333, ICC15435, ICC15697, ICC16654, ICC16796, ICC18679, ICC18720, ICC18858, ICC18912, ICC19095, ICC20265, HABRU, ICC4814, ICC3776, ICC12824, ICC15614, ICC5434, ICC13892, ICC2277, ICC7323 and ICC10885	20.59 to 26.27
2	II	21	ICC1180, ICC2679, ICC3946, ICC4841, ICC6877, ICC7668, ICC8195, ICC8752, ICC9402, ICC11284, ICC11584, ICC13816, ICC15802, ICC20261, ICC18828, ICC2593, ICC6263, ICC10755, ICC9418, ICC9434 and ICC12492	26.73 to 32.43
3	III	20	ICC4958, ICC20262, ICC18836, KWR108, KAK2, ICCV96029×EGILL_065, ICCV96029×SAVUR_063, ICC13077, ICC18847, ICC20263, GCP105, JG11, JG62, ICCV96836, ICCV10, JAKI9218, ICCV96029×BARI3_106D, ICCV96029×CUDIA_152, ICCV96029×KESEN_075 and BG3062	15.21 to 20.12



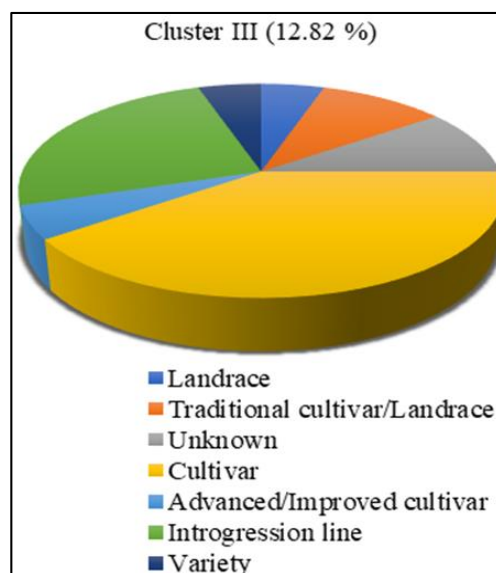


Fig 2: Cluster wise pie chart of chickpea genotypes based on the biological origin

Conclusion

The study demonstrated that the existence of significant genetic diversity for seed protein content in chickpea. High heritability and genetic advance for SPC indicated the genetic control of additive gene effects in expression of SPC, hence offers scope for trait improvement by simple selection. Cluster analysis confirmed the presence of divergent genotypes, suggesting the potential role of the genotypes from Cluster II and III in the development of protein enriched chickpea varieties by crop improvement programmes.

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