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Estimating Genetic Diversity in Chickpea (*Cicer arietinum* L.) Lines: Cluster Analysis and Trait Impact

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was carried out in *rabi* 2023-24, with the objectives to study about genetic diversity in sixty elite lines of chickpea. The observations recorded for eleven morphological traits were subjected to estimation of genetic diversity using the Mahalanobis D^2 statistics and the clustering of genotypes was performed using Tocher's method. The results of analysis revealed that sixty genotypes were grouped into ten different clusters. Having 27 genotypes in total, cluster I was the largest, followed by cluster II with 7 and cluster III having 6 genotypes. The cluster X was solitary. Highest inter-cluster distance was obtained between cluster VIII and X (295.30) while

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highest intra-cluster distance was obtained for cluster IX (103.60). Lowest inter-cluster distance was obtained between cluster II and X (74.17) while lowest intra-cluster distance was obtained for cluster X (0). The characters which majority contributed towards the diversity in these genotypes was seed yield per plant (16.7%) followed by days to maturity (16%), biological yield (12.9%), days to 50% flowering (11.4%) and Number of primary branches (10.1%).

Keywords: Chickpea; genetic diversity; cluster analysis; D^2 statistics; tocher's method.

1. INTRODUCTION

Chickpea, [*Cicer arietinum* (L.)] is a cool season, self-pollinated, annual grain legume belonging to the family Leguminoseae, sub-family Papilionaceae and tribe *Cicereae*. The name *Cicer* is of Latin origin, derived from the Greek word 'kikus' meaning force or strength [1]. The word *arietinum* is also Latin. Commonly known as Egyptian pea, Garbanzo bean, Bengal gram, Gram, and *Chana* [2]**,** chickpea is considered one of the earliest domesticated grain legumes in the old world [3,4]. Wild *C. reticulatum* is interfertile, but it is regarded as the wild progenitor of chickpea. Global chickpea production has reached approximately 15.87 million tons covering an area of 16.01 million hectare in 2022 [5]. India is a leading producer of chickpea in world with a production of 13.75 million tons from an acreage of 10.91 million hectare having productivity of 12.6 q/ha [6]. Chickpea is classified into two broad types, *Desi* and *Kabuli* [7]**.** It is an important source of protein for millions of people in the developing countries, particularly in South Asia where people are largely vegetarian in food habit and depend solely on pulses for their protein requirements [8]. In addition to having high protein content (20 - 22%), chickpea is rich in dietary fiber, major micronutrients such as iron (4.6 - 6.7 mg /100g) and zinc (3.7 - 7.4 mg /100g) [9]. It is consumed fresh as green vegetable, fried, roasted or boiled. Its tender leaves are eaten as vegetables [10]. *Dal* (split chickpea without seed coat) and flour is extensively used as besan in India. The effectiveness of the breeding programme would largely rely on the level of genetic variability available for important economic traits [11,12]. Information on the nature and magnitude of genetic divergence would assist the breeder to select genetically divergent parents in a hybridization programme to obtain desirable heterotic segregants [13,14]. Utilizing genetic diversity effectively in crops aid in selecting appropriate parents for hybridization and achieving breeding goals [15]. The more the genetic diversity, the better are the prospects of developing new superior cultivars. Mahalanobis (1936) introduced the concept of D^2 statistics to measure the divergence between two populations. It provides results on the basis of the magnitude of divergence and is independent of the sample size. The genetic diversity studies provide baseline data that may be utilized to choose parental lines and plan a breeding programme [16]. D^2 statistical analysis is a powerful tool in quantifying the degree of divergence among the population. In view of the above facts, the present investigation was undertaken to study the genetic diversity and clustering pattern among sixty chickpea genotypes using Mahalanobis D^2 statistics and the clustering of genotypes was performed using Tocher's method [17].

2. MATERIALS AND METHODS

The present investigation was carried out at N. E. B. Crop Research Centre, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, with the objectives to estimate genetic diversity among sixty elite lines of chickpea, sown in a Randomized Complete Block Design with three replications in *rabi* 2023-24. All the recommended practices were followed to raise a healthy crop. Observations were recorded on five randomly selected competitive plants from each genotype in each replication for eleven morphological characters namely days to 50 per cent flowering, days to maturity, plant height (cm), number of primary branches, number of secondary branches, number of pods per plant, number of seeds per pod, biological yield (g), seed yield (g), hundred seed weight (g), harvest index. The data for days to 50 per cent flowering and days to maturity was recorded on whole plot basis. The genetic diversity amongst the genotypes was calculated using the Mahalanobis D^2statistics [18]**.** The clusters were prepared by following Tocher's method as suggested by Rao [17]. The percent contribution of characters towards genetic divergence was estimated according to Singh and Choudhary

[19]. The statistical analysis was done using the R software package.

3. RESULTS AND DISCUSSION

Utilizing genetic diversity is crucial for selecting parents for hybridization programs. It evaluates the diversification and determines how each component character contributes towards overall diversity. The clusters are accordingly formed. The differentiation forces are assessed on two levels: inter-cluster distances and intra-cluster distances. The technique reliably estimates divergence and allows for the evaluation of multiple germplasm lines simultaneously for genetic diversity.

3.1 Cluster Composition

Utilizing the D^2 values from Mahalanobis and Tocher's analysis the sixty genotypes were arranged in ten clusters revealing significant genetic variation among the experimental material used in the study (Table 1). The analysis revealed that cluster number I was composed of the maximum number of genotypes (27) followed by cluster II (7 genotypes), cluster III (6 genotypes), cluster IV (5 genotypes), cluster V (4 genotypes), cluster VII and IX having 3 genotypes each and cluster VI and VIII having 2 genotypes each. The cluster X was solitary having only one genotype. Grouping of almost half of the genotypes in Cluster I point towards limited genetic divergence among them which can be potentially attributed to a shared genetic background from their common ancestral population. This uniformity could also result from unidirectional selection pressures favoring specific traits or linked traits, leading to the convergence of phenotypes into a singular cluster. Similar results on cluster analysis analogous to the present investigation were also obtained by [20,21,22,23] while working with different experimental material in chickpea.

3.2 Average Intra and Inter Cluster Distances

Table 2 offers a comprehensive overview of the computed intra-cluster and inter-cluster distances encompassing all possible combinations of the ten clusters. The highest intra-cluster distance was observed for the cluster IX (103.60) followed by cluster IV (75.71), cluster III (69.08), cluster VIII (62.66) cluster V (59.17), cluster VII (55.16), cluster I (50.73), cluster VI (44.94) and Cluster II (43.02). Notably, cluster X exhibited no intra-cluster distance, comprising only one genotype. The maximum intra-cluster distance values signify the presence of genetic diversity among genotypes grouped within those clusters, suggesting substantial potential for gene exchange. Highest inter cluster distance was observed between the cluster number VIII and X (295.30) followed by cluster III and VI (254.46) and cluster III and IV (248.22) exhibiting the fact that the crossing between the genotypes from both the group may result in high heterotic combinations there by producing a superior recombinant for a breeding objective. These results also align with the previous studies from [20,21,24,25].

Table 1. Composition of different genotypes into various cluster

Cluster Number	No. of Genotypes	Genotypes Included
Cluster I	27	GNG 469, GNG 1958, NN185, HC5, PG9, PG34, PG39, PG158, PG 170, PG 172, PG256, PG 258, PG 260, PG268, PG270, PG271, PG276, PG281, PG 282, PG 285, PG 286, PG 289, PG 290, PG 296, PG298, PG 303, PG 308
Cluster II	7	GNG 1581, CSG8962, PG114, PG 35, PG 44, PG45, PG55
Cluster III	6	ICC17095, PG 311, PG 317, PG 319, PG 328, PG 329
Cluster IV	5	H208, PG5, PG255, PG 265, PG 266
Cluster V	4	GL10006, GLK 2812, PG279, PG301
Cluster VI	2	PG 37, GNG2171
Cluster VII	3	P13273, NN1836, CSJ 515
Cluster VIII	2	PG6, PG8
Cluster IX	3	BGM547, DCP 92-3, Phule G-517,
Cluster X	1	PG 46

Table 2. Inter and intra cluster distances

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Cluster I	**50.73**	85.38	158.38	122.61	104.72	83.44	177.83	159.66	98.76	115.49
Cluster II		**43.02**	189.47	204.47	168.66	81.12	233.12	230.23	111.24	**74.17**
Cluster III			**69.08**	248.22	207.82	254.46	180.94	144.14	113.47	224.38
Cluster IV				**75.71**	169.62	160.74	206.14	112.85	230.39	215.06
Cluster V					**59.17**	111.40	112.12	218.69	161.19	148.91
Cluster VI						**44.94**	188.62	231.74	136.58	114.71
Cluster VII							**55.16**	147.79	203.68	238.76
Cluster VIII								**62.66**	204.05	**295.30**
Cluster IX									**103.60**	154.50
Cluster X										**0.00**

Table 3. Cluster mean values for various traits

	D50F	DM	PH	PB	SB	P/P	S/P	BY	SY	HSW	HI
Cluster I	82.69	142.60	55.33	2.22	3.55	20.82	1.81	19.29	6.47	19.74	33.88
Cluster II	78.18	145.73	**38.11**	**1.93**	3.42	21.46	1.79	16.27	6.22	19.26	38.30
Cluster III	70.11	**125.83**	47.89	2.55	3.83	17.96	1.88	23.04	5.94	20.53	26.33
Cluster IV	84.92	140.17	49.67	2.53	3.74	**25.60**	2.19	27.68	9.11	17.99	33.13
Cluster V	73.33	150.67	**61.83**	2.23	3.60	19.33	2.01	23.51	7.74	26.08	33.40
Cluster VI	78.67	**151.65**	43.16	2.16	**3.33**	**15.03**	1.62	**14.34**	5.56	**29.31**	**38.70**
Cluster VII	78.92	139.80	51.71	2.85	3.57	23.76	**2.25**	**28.18**	**10.61**	23.08	38.36
Cluster VIII	81.94	148.35	52.73	**2.93**	**4.27**	20.40	1.74	26.43	6.02	19.11	**23.56**
Cluster IX	**85.67**	129.67	58.43	2.46	3.76	20.88	**1.37**	15.68	**4.04**	**17.36**	25.89
Cluster X	**65.67**	145.33	58.96	2.25	3.60	16.75	1.53	15.37	4.66	20.11	30.70

3.3 Cluster Mean for Various Characters

To illustrate the clustering pattern among chickpea genotypes, the average performance of the clusters was calculated (Table 3). Cluster IX exhibited the maximum cluster mean value for days to 50% flowering (85.67) while it exhibited minimum mean cluster values for seed per pod (1.37), seed yield per plant (4.04), seed per pod (1.37), and hundred seed weight (17.36). Cluster VI exhibited maximum cluster mean values for Days to maturity (151.65), hundred seed weight (29.31) and harvest index (38.70) while, it exhibited minimum cluster mean values for secondary branches (3.33), pod per plant (15.03) and biological yield (14.34). Cluster V exhibited maximum mean values for plant height (61.83). Cluster VIII exhibited maximum mean values for primary branches (2.93) and secondary branches (4.27) while it had minimum mean value for harvest index (23.56). Cluster IV exhibited maximum mean value for pod per plant (25.60). Cluster VII exhibited maximum mean values for seed per pod (2.25), biological yield (28.18) and seed yield per plant (10.61). Cluster X exhibited minimum mean values for Days to 50% flowering (65.67) while cluster III exhibited minimum mean for days to maturity (125.83). Cluster II showed minimum mean values for plant height (38.11) and primary branches (1.93).

3.4 Contribution of Characters Towards Diversity

The contribution of a trait in total divergence forms the basis of the selection and choice of parent in a breeding programme [26,27]. The number of times each of the 11 traits appeared at the first rank along with its respective percent contribution to diversity is presented in Table 4 and in Fig. 1 for visual representation. It is evident from the result that the character Days to maturity contributed maximum (22.21%) towards the genetic divergence in the given sixty lines while the character secondary branches recorded minimum contribution (1.12%) to the genetic divergence value. Other characters which showed high values of contribution towards the genetic divergence were seed yield (14.92%), Days to 50% flowering (13.95%), biological yield (13.73%) plant height (13.05%) & primary branches (10.85%). the characters which contributed comparatively low towards the diversity were pod per plant (3.95%), seed/pod (2.49%), hundred seed weight (2.54%) and Harvest Index (1.19%). It is also evident from the results that in present investigation the trait number of secondary branches, seed per pod, and harvest index had very less approximately 1-2% contribution towards the total diversity divulge the fact that these traits might have been fixed in the experimental population taken here for study, indicating that all the genotypes are having non-significant differences for the given characters resulting in no genetic variation for the traits or the traits might be highly correlated with other traits and the variation for these traits has already been captured by some other traits or the gene controlling these traits has multiple effects and the variation for these traits is not contributing to the overall diversity Similar studies on maximum contribution of character towards genetic divergence was performed by [28] for 100 seed weight and number of pods, [29] for 100 seed weight, number of pods per plant and days to 50% flowering [20] for 100-seed weight and pods per plant, [30] for days to 50% flowering and 100-seed weight.

Table 4. Contribution of Various Characters Towards Diversity

S.No	Source	Times ranked first	% contribution
1	Days to 50% flowering	247	13.95
2	Days to maturity	413	**22.21**
3	Plant Height	231	13.05
4	Primary Branches	192	10.85
5	Secondary Branches	18	**1.12**
6	Pod / Plant	70	3.95
7	Seed /pod	44	2.49
8	Biological yield	243	13.73
9	Seed yield	264	14.92
10	Hundred seed weight	45	2.54
11	Harvest Index	21	1.19

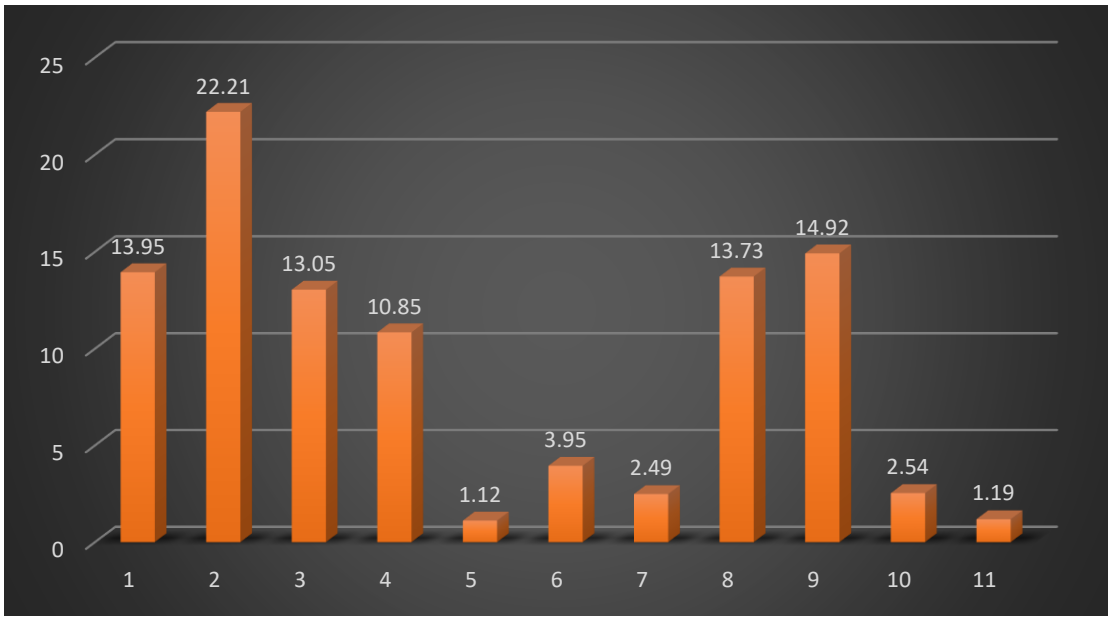

Fig. 1. Graphical representation of contribution (%) of quantitative traits towards genetic divergence

4. CONCLUSION

D^2 analysis is an important technique which not only represents the percentage contribution of traits but also describes the diversity present in the breeding lines by grouping them into diverse clusters. In present study the sixty diverse lines of chickpea were grouped into ten clusters which pointed towards the presence of ample amount of diversity in these lines. It was also evident that the genotypes originated in different eco-geographical regions grouped together in the same clusters indicating that there is no relationship between geographical and genetic diversity. The characters which have a direct relation with the seed yield are harvest index, pod per plant and hundred seed weight. The inter-cluster distance was found to be maximum among the cluster VIII and X suggested that the genotypes in these cluster will serve as a good parent for crossing programme in breeding objective and can lead to better heterotic combinations and superior recombinants. Cluster IV exhibited highest mean value for pod per plant while high mean value for harvest index and hundred seed weight was shown by cluster VI.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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