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Assessment of genetic diversity for different quantitative traits in chickpea (*Cicer arietinum* L.)

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Abstract
Thirty-five chickpea genotypes were tested with an objective to estimate the magnitude of genetic diversity present among them using Mahalanobis D^2 statistics. The experiment was conducted during *Rabi* 2020-21 at Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences. The magnitude of genetic diversity was studied using data from 13 quantitative traits. Analysis of Variance confirmed the presence of significant variation among the genotypes. The 35 genotypes were divided into seven clusters based on their D^2 values. Out of seven clusters, cluster Ⅰ was the largest with 19 genotypes followed by cluster Ⅱ with 10 genotypes and cluster Ⅳ containing two genotypes. The clusters Ⅲ, Ⅴ, Ⅵ and Ⅶ are mono-genotypic with one genotype each. Highest intra cluster distance was observed in cluster Ⅰ (54.37) which has maximum number of genotypes followed by cluster Ⅱ (52.47) and cluster Ⅳ (26.49). Maximal inter-cluster distance was observed between clusters Ⅱ and Ⅵ (180.36) followed by clusters Ⅳ and Ⅶ (146.15). The genotypes under clusters Ⅱ and Ⅵ, clusters Ⅳ and Ⅶ can be used as parents in future hybridization programmes and the offspring produced is highly heterotic and contains high variability.

Keywords: Chickpea, D^2 statistics, Genetic diversity, Inter-cluster distance, Intra-cluster distance

1. Introduction
Chickpea (*Cicer arietinum* L.) is an annual legume crop in the Leguminosae family and the Papilionaceae subfamily. It is a self-pollinated crop with a genome size of 738Mbp and diploid chromosome number (2n = 2x = 16). (Varshney *et al*. 2013) [12]. Among the pulse crops chickpea is the third most important crop. Worldwide, the area under chickpea cultivation is about 137.18 lakh ha. while the production is about 146.46 lakh tons. The worldwide productivity of chickpea is about 1038.4 Kg/ha (Source: FAOSTAT 2019). India is the leading producer of chickpea in the world with an area of production about 96.9 lakh ha. the production of the country is about 110.78 lakh tonnes with the productivity about 1142 kg/ha. the area under cultivation of chickpea in Uttar Pradesh is about 6.21 lakh ha while the overall chickpea production of the state is about 8.51 lakh tonnes with the productivity about 1371 Kg/ha (Source: Directorate of Economics and Statistics, Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, GOI, 2019-20).
Two distinct cultivated types of chickpeas are *desi* and *kabuli* types. The *desi* types are characterized with small sized seeds, pink color flowers, presence of anthocyanin pigmentation in the stems and colored and thick seed coats. While the *kabuli* types are with white or beige colored seeds and with thin seed coats (Moreno and Cubero, 1978) [4].
The protein content in chickpea is about 16.7% to 30.6% and 12.6% to 29.0% in *desi* and *kabuli* types, respectively. Carbohydrate concentration in chickpea seeds is about 51-65% in *desi* types and 54-71% in *Kabuli* types. Total lipid content ranges from 2.9%- 7.4% and 3.4% to 8.8% in *desi* and *kabuli* types respectively. Apart from the above-mentioned components, chickpea also has some minerals like Calcium, Phosphorus, potassium, Magnesium etc. useful for development of bones. Some useful vitamins like vitamin C, Vitamin K and vitamin A are also present in chickpea. (J.A. Wood & M.A. Grusak 2007) [10].
Utilization of genetically diverse genotypes by plant breeders as parents in hybridization would result in highly heterotic hybrids and also high degree of variability in segregating generations (Jeena and Arora 2002) [2]. The hybridization involving the genetically diverse parents also results in suitable genetic recombination which can be useful for improvement in yield. Mahalanobis's D^2 analysis is a powerful tool in quantifying the degree of variability at the genotype stage. The effectiveness of multivariate analysis has significantly been

emphasized (Murty and Arunachalam, 1966) [5]. It helps to study the magnitude of genetic divergence present in the germplasm. The present analysis was conducted with the goal to discover the extent of genetic divergence in 35 chickpea genotypes and to perceive the divergent determine for subsequent hybridization programmes.

2. Materials and Methods

Thirty-five genotypes including one check (Pusa-362) were evaluated during *Rabi* 2020-21 at Field experimentation centre, Department of Genetics and Plant Breeding, SHUATS, Prayagraj. Randomized Block Design (RBD) with three replications was implemented as the experimental design and the approximate spacing about 30 cm x 10 cm was maintained. The data were recorded for 13 quantitative traits *viz.*, days to 50% flowering, days to 50% pod formation, plant height (cm), number of primary branches, number of secondary branches, days to maturity, Seed count per plant, pod count per plant, seed count per pod, biological yield per plant (g), 100 seed weight (g), harvest index (%) and seed yield per plant (g). The data were analysed according to Panse and Sukhatme's methodology (ANOVA) (1985) to test if there is any significant variation among the genotypes with respect to the traits. The data were evaluated with the help of D^2 (Mahalanobis 1936) [3], as advocated by Rao (1952) [13]. The genotypes were grouped together into seven clusters based on the computed D^2 values and the inter and intra cluster distances were calculated.

3. Results and Discussion

The presence of genetic variability among the population is an imperative factor for the selection and it is essential to confirm the degree of variability before implementing a breeding programme as the success is determined by the variability existing in the species for the specific traits. In the present study analysis of variance revealed the existence of significant variation among thirty-five genotypes for all the traits (Table 1). Later, this study was proceeded further for diversity analysis.

3.1 clustering of genotypes

Thirty-five genotypes were categorized into seven clusters by comparing the D^2 values among the genotypes (Table 2). Tocher's approach (Rao, 1958) was used to organize the genotypes into seven diverse clusters. The grouping was performed on the assumption that genotypes belonging to the same cluster had substantially lower D^2 values than genotypes belonging to other clusters. Among the seven clusters, cluster Ⅰ has the highest percentage (54.28%) of genotypes i.e., 19 genotypes followed by cluster Ⅱ which contains 10 genotypes and cluster Ⅳ with two genotypes. Clusters Ⅲ, Ⅴ, Ⅵ & Ⅶ are solitary with only one genotype each which indicates that the genotypes belonging to these clusters are genetically more divergent than that of genotypes belonging to other clusters. Whereas the genotypes in cluster Ⅰ have narrow genetic divergence, means they are genetically uniform in comparison with the genotypes under different cluster. This may be due to the similarities in the base population from which they have been developed (Thakur *et al* 2018) [8].

Similarly, 36 genotypes were grouped into seven clusters by Agrawal *et al.* (2018) [1] and 40 genotypes were divided into seven clusters by Jakhar *et al*. (2016) [14]. The pattern of grouping the genotypes into clusters demonstrated that the genetic diversity is not associated by geographical diversity. The main cause of genetic diversity was natural selection, genetic drift and environmental effects.

3.2 Inter and Intra-cluster distances among seven clusters including 35 genotypes

The D^2 values are also used to calculate the intra and inter-cluster distances. The highest inter-cluster distance (180.36) was found between clusters Ⅱ and Ⅵ, followed by clusters Ⅳ and Ⅶ (146.15), clusters Ⅱ and Ⅳ (135.01) and clusters Ⅳ and Ⅴ (134.25). The hybridization involving the utilization of genotypes as parents would result in high heterotic hybrids and can be used for varietal improvement by implementing suitable breeding methods. Further, the cross involving the genotypes separated by higher genetic distance also produces the segregating generations with high extent of variability. The maximum intra-cluster distance (54.37) was found in cluster Ⅰ as this cluster has the most genotypes, followed by clusters II and IV, which had intra-cluster distances of 52.47 and 26.49, respectively. The remaining clusters Ⅲ, Ⅴ, Ⅵ and Ⅶ were mono-genotypic hence no intra cluster distance was found.

3.3 Cluster mean values of seven clusters

Cluster mean is the mean potential of all the genotypes comprising in that cluster for a particular trait. The greatest cluster mean value for cluster Ⅱ was found for number of secondary branches per plant, pod count per plant, seed count per pod and seed count per plant. The mean potential of genotypes under cluster Ⅳ was maximum for number of primary branches per plant, biological yield per plant and seed yield per plant. Mean values of cluster Ⅴ were found highest for traits like plant height and harvest index, cluster Ⅵ was found to be maximum for 100 seed weight and the cluster Ⅶ was early for the traits like days to 50% flowering, days to 50% pod setting and days to maturity. based on the cluster means, the traits like seed count per plant, seed count per pod, pod count per plant, number of secondary branches per plant, 100 seed weight, number of primary branches per plant, harvest index, seed yield per plant, days to maturity should be given importance while selecting parents from the clusters. The cluster mean values were represented in table no. 4

3.3 Contribution of different traits to divergence

Of the 13 parameters evaluated, the major significance towards divergence was shown by seed yield per plant (23.56%) followed by biological yield per plant (17.82%), number of primary branches per plant (14.79%), number of seeds per plant (11.60%) and 100 seed weight (10.92%). The least contribution to divergence was reported in seed count per pod i.e., 1.01% (Table 5). Mahalanobis's D^2 statistics is also used to assess the contribution of a trait towards total divergence. Based on how often the trait ranked first, it is used to estimate the contribution of that particular trait towards divergence. According to present analysis, the traits seed yield per plant, biological yield per plant, number of primary branches per plant, seed count per plant and 100 seed weight can be used for selection of the parents for future hybridization.

Table 1: Summary of Analysis of Variance

S. No	Traits	Mean sum of squares		
		Replications (d.f=2)	Treatments (d.f=34)	Error (d.f=68)
1	Days to 50% flowering	7.396	63.389 **	23.377
2	Days to 50% pod setting	80.671	372.903 **	37.091
3	Days to maturity	59.274	564.461 **	59.825
4	Plant height	47.178	220.290 **	18.814
5	Number of primary branches per plant	0.067	0.663 **	0.014
6	Number of secondary branches per plant	0.476	5.845 **	0.096
7	Pod count per plant	117.564	952.468 **	23.85
8	Seed count per pod	0.019	0.306 **	0.014
9	Seed count per plant	255.319 *	1975.051 **	51.758
10	Seed weight	17.120	49.078 **	4.666
12	Biological yield per plant	106.330	417.907 **	21.555
13	Harvest index	74.147	163.838 **	16.678
14	Seed yield per plant	19.478	65.103 **	3.951

* &** represents 5% and 1% Level of Significance respectively

Table 2: Clustering of 35 genotypes into various clusters

Cluster	No. of genotypes	Genotypes
I	19	ICC-709, ICC-711, ICC-273, ICC-42, ICC-2, ICC-166, ICC-176, ICC-16921, ICC-86, ICC-92, ICC-764, ICC-255, ICC-75, ICC-45, PUSA-362, ICC-111, ICC-26, ICC-1356, ICC-729
II	10	ICC-1338, ICC-95, ICC-16887, ICC-1360, ICC-16912, ICC-317, ICC-67, ICC-80, ICC,143, ICC-16915
III	1	ICC-299
IV	2	ICC-161, ICC-228
V	1	ICC-701
VI	1	ICC-219
VII	1	ICC-15

Table 3: Intra and Inter cluster distances of 35 genotypes of chickpea

Cluster	I	II	III	IV	V	VI	VII
I	54.37	89.13	80.32	102.16	70.98	88.25	70.24
II		52.47	99.56	135.01	123.11	180.36	115.63
III			0	101.2	64.49	62.67	118.88
IV				26.49	134.25	126.45	146.15
V					0	55.17	66.19
VI						0	104.91
VII							0

Table 4: Cluster mean values of seven clusters for 13 quantitative traits

Cluster	DF 50%	DP 50%	DM	PH	PBPP	SBPP	PCPP	SCPP	SCPPl	SI	BYPP	HI	SYPP
I	88.35	107.83	135.54	68.15	1.95	5.04	63.92	1.45	88.67	21.6	40.94	45.25	18.19
II	88.13	115.07	135.76	66.58	1.95	7.25	77.12	1.75	106.06	17.3	43	40.62	17.14
III	86	143.33	124.8	72.13	2.26	5.53	50.33	1.6	54.53	27.67	37.6	44.04	16.53
IV	85.33	105.5	154.33	79.42	3.43	5.27	59.97	1.73	99.07	23	44.77	43.06	18.93
V	84.33	103.67	154.33	80.8	1.47	4.33	31.27	1.67	47.8	23	25.8	58.59	15.2
VI	89	113	129	77.75	2	3.67	27.8	1.2	39.13	28	41.2	31.94	13.15
VII	84	102	121	52.73	1.6	3.2	33.93	1.47	62.66	12	11.47	44.81	5.13

DF: Days to 50% flowering, DP: Days to 50% pod setting, DM: Days to Maturity, PH: Plant height, PBPP: Primary Branches per Plant, SBPP: Secondary Branches per Plant, PCPP: Pod count per Plant, SCPP: Seed count Per Pod, SCPPl: Seed count per Plant, SI: Seed Index, BYPP: Biological Yield Per Plant, HI: Harvest Index, SYPP: Seed Yield Per Plant.

Table 5: Contribution of 13 quantitative traits towards Genetic Divergence

Serial number	Traits	Contribution %	Times ranked 1st
1	Days to 50% flowering	7.00	42
2	Days to 50% pod setting	1.85	11
3	Days to maturity	1.34	8
4	Plant height (cm)	1.68	10
5	Number of primary branches per plant	14.79	88
6	Number of secondary branches per plant	2.18	13
7	Pod count per plant	8.24	49
8	Seed count per pod	1.01	6
9	Seed count per plant	11.60	69
10	100 Seed weight (g)	10.92	65
11	Biological yield per plant (g)	17.82	106

12	Harvest index (%)	5.21	31
13	Seed yield per plant (g)	23.36	139

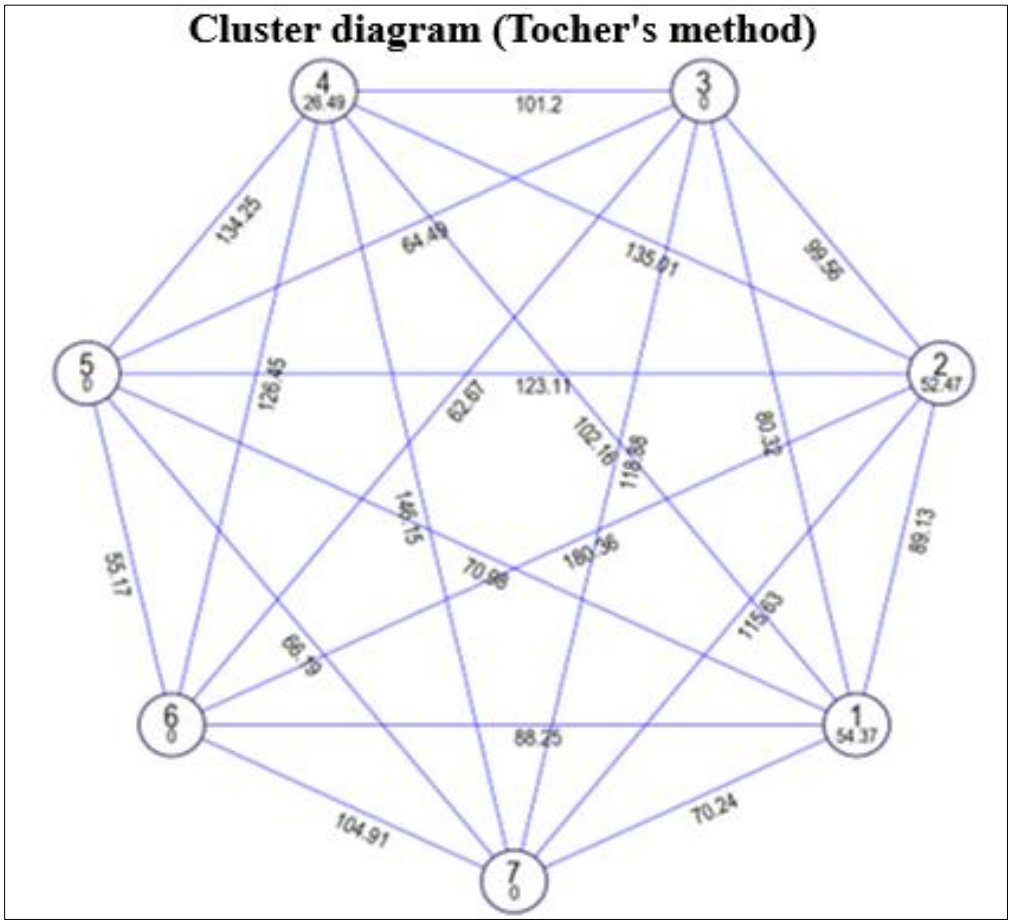
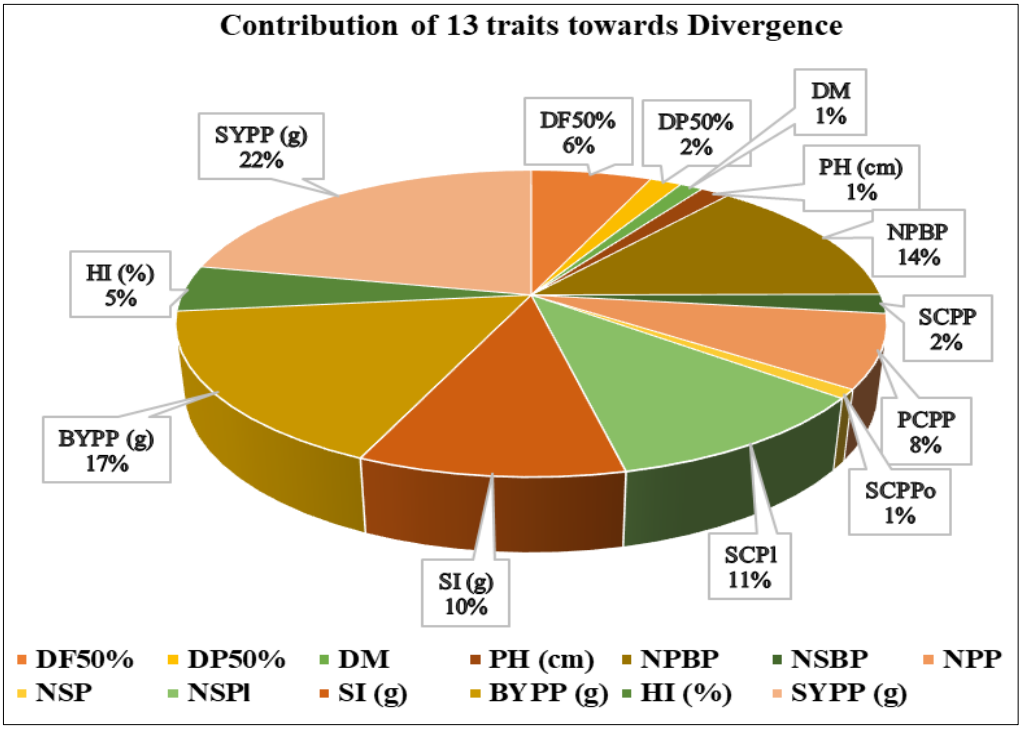


Fig 1: Cluster diagram showing intra and inter-cluster distances



DF: Days to 50% flowering, DP: Days to 50% pod setting, DM: Days to Maturity, PH: Plant height, PBPP: Primary Branches per Plant, SBPP: Secondary Branches per Plant, PCPP: Pod count per Plant, SCPP: Seed count Per Pod, SCPPl: Seed count per Plant, SI: Seed Index, BYPP: Biological Yield Per Plant, HI: Harvest Index, SYPP: Seed Yield Per Plant.

Fig 2: Diagram representing the contribution of thirteen traits towards divergence

4. Conclusion

All the genotypes are subsequently categorised into seven clusters in the present analysis and the maximum genotypes were present under cluster Ⅰ (19), which recorded highest intra cluster distance and maximum distance between the clusters was found between clusters Ⅱ and Ⅵ (180.36) followed by clusters Ⅳ and Ⅶ (146.15). according to the cluster means cluster Ⅳ was highest for seed yield per plant and cluster Ⅱ was highest for number of secondary branches, pod count per plant, seed count per pod and seed count per plant. Maximum contribution towards divergence was shown by seed yield per plant (23.36%) followed by biological yield (17.82%). As a result, it is possible to establish that the genotypes in the cluster Ⅱ and cluster Ⅵ were found to be highly divergent and they can be selected as parents in the hybridization programmes to create a diverse array of variation in segregating generations as well as to produce transgressive segregants and also results in the accumulation of the desirable genes into a variety.

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