

Genetic diversity studies in chickpea (*Cicer arietinum*) germplasm

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ABSTRACT

Genetic diversity among 495 accessions of chickpea collected from different agro-ecological zones of India was assessed for several qualitative and quantitative traits. These accessions were grown in the augmented design with 3 intermittent checks 'BG 256', 'K 850' and 'L 550' after every fifteenth row. Wide range of variability was observed for both qualitative and quantitative traits. The number of primary branches, 100 seed weight (g) and days to maturity showed moderate to high heritability and genetic advance. This suggests that the selection based on these traits would be useful in improving the grain yield. The diversity index indicated ample genetic variation for seed yield per plant in the present set of materials. Therefore, a direct selection based on seed yield and component traits may be practiced to select better genotypes, which could be utilized for development of superior high yielding varieties.

Key words: Chickpea, Cluster analysis, Genotypic correlation, Germplasm, Phenotypic correlation, Principal component analysis

The genus *Cicer* comprises 34 wild perennial, 8 annual wild and one annual cultivated species. Chickpea (*Cicer arietinum*) is the third most important food legumes in the world in terms of area (11.55 million hectares) and production (10.46 million tons) (FAOSTAT 2010). India is the largest producer of chickpea in the world sharing about 72% of area and production (FAOSTAT 2010). In India, chickpea is cultivated in an area of 8.75 million ha with the production of 8.25 million tons (Singh 2011) with productivity of 945 kg/ha. In India, productivity is low as compared to other chickpea producing countries like Mexico (1809 kg/ha), Australia (1268 kg/ha), Ethiopia (1265 kg/ha). Hence, utilization of diverse germplasm is the key to develop high yielding varieties to bridge this yield gap.

In chickpea, there are some reports available on the evaluation of genetic stock in agro-morphological traits (Singh *et al.* 1990, Farshadfar and Farshadfar 2008, Ali *et al.* 2010, Wadikar *et al.* 2010). These reports suggest that pod/plant, 100 seed weight (g), plant height and number of primary branches/plant are the major yield contributing traits (Singh *et al.* 1990). To strengthen ongoing breeding program, study of the available natural genetic variation is of immense importance. It is imperative to collect and evaluate germplasm

precisely to draw valid conclusions. Generally, morphological variations are used to partition the available germplasm into small groups/clusters based on similarity and dissimilarity coefficients (Anderson 1984). Genetic variation present in germplasm arrays and the genetic relationships among genotypes are very useful for efficient conservation as well as utilization of genetic resources in breeding programme. Evaluation of germplasm increases the efficiency to make desirable selection whereas diversity index provides the information regarding the extent of variation present in the population. Therefore, an experiment was conducted to assess genetic variation, trait association and significant contribution of each trait towards yield and also their contribution towards genetic diversity.

MATERIALS AND METHODS

The experimental material comprising 495 accessions collected from different agro-ecological regions were grown in an augmented design (Federer 1956) with 3 intermittent checks 'BG 256', 'K 850' and 'L 550' after every fifteenth row during Rabi 2006-07 at New Research Farm of Indian Institute of Pulses Research, Kanpur-208 024 (26.28°N and 80.21°E), India. Sowing was done in the second week of November. Each accession was grown in paired row of four meters length and inter- and intra- row distance were kept at 30 cm and 10 cm, respectively. Observations were recorded following the standard descriptors of chickpea on nine qualitative traits *viz.*, seedling vigor, plant pigmentation, stem colour, growth habit, flower color, pod size, seed size, seed color, testa texture and six quantitative traits *viz.* days to 50% flowering, days to maturity, no. of primary branches/plant, number of seeds/pod, 100 seed weight (g) and seed yield/plant (g) (Table 1).

Statistical analysis: Data analysis was done for both qualitative and quantitative traits. Frequency distribution was done for each qualitative trait using raw mean data. Analysis of variance (ANOVA) for augmented design for all attributes (qualitative and quantitative) was done using statistical software, Windostat ver. 8.0 (Khetan 2009). Genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), broad sense heritability (h^2) and genetic advance over mean in percent was computed for quantitative traits. Phenotypic and genotypic correlation coefficients were estimated using raw and adjusted mean data, respectively.

Table 1. Details of descriptors used for major characters of chickpea

S. N.	Trait	Descriptors & stage of observation/scoring
1	Seedling vigour	: Vigorous (1-3); Intermediate (4-6); Non-vigorous (7-9) Visual score at 45 days after sowing
2	Plant pigmentation	: No pigmentation (1); Medium pigmentation (2); Strong pigmentation (3) Visual score on stem and leaves at the pod filling stage
3	Stem colour	: Green (1); Pale Green (2); Purple (3) Visual score at 90 days after sowing
4	Days to 50% flowering	: Number of days from sowing to the day on which 50% plants end of flowering of an accession
5	Growth habit	: Erect (0-15 ⁰) (1); Semi-erect (16-25 ⁰) (3); Prostrate (flat on the ground) (5) Visual Score on the angle of the branches from the vertical axis at the pod filling stage
6	Flower colour	: Blue (1); Dark Pink (2); White (3); Others (4) Colour of ventral surface of the vexillum of the freshly opened flower
7	Pod size	: Small (1-3); Medium (4-6); Large (7-9) Visual score at the complete maturation of pods
8	Days to maturity	: Number of days from sowing to the stage when over 90% of pods have matured and turned yellow
9	Primary branches	: Profuse (1-3); Intermediate (4-6); Shy (7-9) Visual score on the number of branching at the maturity
10	Number of seed/pod	: Average of 10 pods from five competitive plants recorded at the time of harvest
11	Seed size	: Large (1); Medium (3); Small (5) Visual score after harvesting and seed drying (about 6% moisture)
12	100 seed weight	: Average weight of three random samples of 100 seed each (Moisture about 6%)
13	Seed colour	: Light brown (4); Dark brown (5); Black (6); Green (7); Others (8) Visual score on dry mature seeds within three month from the harvest
14	Testa texture	: Rough (1); Smooth (2); Tuberculated (3) Visual score on dry mature seeds
15	Seed yield/plant (g)	: Mean yield of five representative plants in a plot. Plant stand should at least be 60%

Based on adjusted mean data Euclidean distance (diversity index) was calculated using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) ver. 2.1 (Rohlf, 2000). Dendrogram and cluster groupings using unweighted pair group with arithmetic averaging (UPGMA) clustering algorithm was done based on Euclidean distance matrix through SAHN option of NTSYSpc ver. 2.2. The variance co-variance matrix was further used to calculate the Eigen values for obtaining principal components. Tocher method was used to produce percent contribution of each trait towards genetic diversity.

RESULTS AND DISCUSSIONS

The Analysis of Variance (ANOVA) for quantitative characters showed that the accessions were significantly different. This indicated that sufficient genetic diversity was present among the accessions.

The frequency distribution of qualitative traits for 495 accessions is represented in Table 2. Three hundred and sixty accessions (72.7%) were vigorous at early stage, 275 accessions (55.6%) were strongly pigmented, 232 accessions (46.7%) had pale green stem colour, 434 accessions (87.7%) showed semi-erect type expression, 425 accessions (85.9%) had dark pink coloured flowers, 484 accessions (97.8%) had small pods, 461 (93.1%) had small sized seed, about more than

two-third accessions (67.6%) showed light or dark brown seed colour and testa texture was found rough for 333 accessions (67.3%). Only one line, namely, 'ICC 5262' had large pod as well as large seed size. This indicated that the population dominated for specific class for many of the traits; simultaneously variation for other classes under different descriptors was also present showing the presence of adequate variation.

Based on ANOVA (Table 3), it was found that seed yield per plant had the highest GCV (33.66) followed by 100-seed weight (18.06) and days to 50% flowering (10.98). The simultaneous GCV and PCV values for each trait showed that number of seed per pod was least affected by environment having the highest heritability of 0.99 but had low genetic advance indicating presence of non-additive gene action. High heritability coupled with high to moderate genetic advance was found for days to 50% flowering and days to maturity which suggests that ample opportunity exists in germplasm collection for improvement of this trait. Due to high difference between PCV and GCV values for seed yield per plant, heritability was recorded to be low (0.32). Presence of high genetic advance (86.18%) indicated that seed yield per plant is governed by additive genes and selection may be more effective in such cases as earlier reported by Kumar and Krishna (1998).

Table 2. Frequency distribution for different morphological characters (qualitative) of chickpea

S. N.	Plant Descriptors	Range in expression	No. of germplasm lines (% of total population)	Few example genotypes
1	Seedling vigour	Vigorous	360 (72.7)	ICC 4907, ICC 5262
		Intermediate	95 (19.2)	ICC 5106, ICC 5695
		Non-vigorous	40 (8.1)	ICC 4212, ICC 4709
2	Plant pigmentation	No pigmentation	64 (12.9)	ICC 5993, ICC 7462
		Medium pigmentation	156 (31.5)	ICC 5465, ICC 7423
		Strong pigmentation	275 (55.6)	ICC 681, ICC 1777
3	Stem colour	Green	71 (14.3)	ICC 740, ICC 884
		Pale Green	232 (46.9)	ICC 472, ICC 836
		Purple	192 (38.8)	ICC 551, ICC 725
4	Growth habit	Erect	15 (3.0)	ICC 1781, ICC 5262
		Semi-erect	434 (87.7)	ICC 267, ICC 413
		Prostrate	46 (9.3)	ICC 422, ICC 430
5	Flower colour	Blue	11 (2.2)	ICC 5926, ICC 5932
		Dark Pink	425 (85.9)	ICC 567, ICC 676
		White	31 (6.3)	ICC 832, ICC 866
		Others	28 (5.7)	ICC 4907, ICC 5262
6	Pod size	Small	484 (97.8)	ICC 414, ICC 5410
		Medium	10 (2.0)	ICC 5485, ICC 5932
		Large	1 (0.2)	ICC 5262
7	Seed size	Large	1 (0.2)	ICC 5262
		Medium	33 (6.7)	ICC 554, ICC 822
		Small	461 (93.1)	ICC 543, ICC 771
8	Seed colour	Light brown	127 (25.7)	ICC 498, ICC 773
		Dark brown	158 (31.9)	ICC 809, ICC 878
		Black	34 (6.9)	ICC 4212, ICC 4772
		Green	12 (2.4)	ICC 5216, ICC 5231
		Others	164 (33.1)	ICC 1743, ICC 1755
9	Testa texture	Rough	333 (67.3)	ICC 865, ICC 870
		Smooth	136 (27.5)	ICC 773, ICC 866
		Tuberculated	26 (5.3)	ICC 567, ICC 676

Table 3. Estimates of variability parameters for various quantitative traits

S. N.	Character Name	Mean	Minimum	Maximum	Standard Deviation	R ²	CV*	GCV	PCV	h ²	Genetic Advance	Genetic Advance as % of mean
1	Days to 50% flowering	83.19	45.00	128.00	11.36	0.987	7.77	10.98	13.46	0.67	15.34	17.44
			ICC 7423	ICC 5454								
2	Days to maturity	152.53	144.00	163.00	9.26	0.990	1.01	1.75	2.02	0.75	4.79	3.13
			ICC 1777	ICC 6083								
3	Primary branches (cm)	3.17	3.00	5.00	0.37	0.959	14.53	6.48	13.42	0.23	0.20	4.75
			ICC 676	ICC 4542								
4	Number of seed/pod	1.01	1.00	2.00	0.06	1.000	0.00003	6.18	6.18	0.99	0.13	12.73
			ICC 676	ICC 554								
5	100 seed weight (g)	14.86	3.40	33.30	4.03	0.960	30.73	18.06	26.66	0.46	3.76	21.60
			ICC 1486	ICC 7406								
6	Seed yield/plant (g)	218.14	7.60	651.30	129.83	0.975	48.97	33.66	59.07	0.32	86.18	39.51
			ICC 1892	ICC 4961								

CV: Co-efficient of variation; GCV: Genotypic co-efficient of variation; PCV: Phenotypic co-efficient of variation

Association among traits: Phenotypic and genotypic correlation coefficients among six quantitative characters viz., days to 50% flowering, days to maturity, primary branches, number of seeds/pod, 100 seed weight (g) and seed yield/plant (g) were computed and presented in Table 4. It was observed that primary branches had highly significant positive genotypic correlation with 100 seed weight (g) (0.336) and

seed yield/plant (0.330). Similar results were also reported by Khorgade *et al.* (1995) in limited germplasm set. Days to maturity showed significant positive genotypic association with 100 seed weight (g) (0.241), primary branches (0.167) and seed yield per plant (0.161). Seed yield per plant was found to have significant positive genotypic association with 100-seed weight (0.184), similar to the findings of Farshadfar and

Farshadfar (2008). On the other hand, days to 50% flowering showed significant negative genotypic association with primary branches (-0.157) and 100 seed weight (g) (-0.199). Such results were also reported earlier by Singh *et al.* (1990). This had a high indirect effect on seed yield per plant. The values of phenotypic correlations were in accordance with genotypic correlations, which reflect the due contribution of genotype in the expression of different traits. The information generated will help in understanding relationship between traits in studied population which subsequently may help in selection process for important traits in breeding programme.

Cluster analysis: The Euclidian distance based dissimilarity coefficient was calculated for each pair of accessions. The maximum distance was observed between accessions 'ICC 4709' and 'ICC 4969' (857.39 unit) whereas minimum between 'ICC 429' and 'ICC 2148' (2.52 unit). Total of thirteen clusters were formed. Maximum number of 86 accessions were present in cluster 3, followed by cluster 2 and cluster 1 having 84 and 81 accessions, respectively. However, minimum of only 6 accessions formed the cluster 12 (Table 5).

The minimum inter-cluster distance was 11.63 units between cluster 1 and 2 and the maximum inter-cluster distance was between clusters 9 and 12 (810.30 unit). The minimum intra-cluster distance was found in cluster 8 (21.87 unit) having 21 accessions, whereas the maximum intra-cluster distance was in cluster 11 (43.67 unit) having 8 accessions (Table 6).

For the ease of presentation, original dendrogram produced for 495 accessions was redrawn using 52 accessions. Those 52 accessions were drawn from each of 13 clusters through Probability Proportional to Size Simple Random Sampling (PPS SRS) technique so as to obtain a true representation of mother dendrogram (Fig 1).

Principal Component Analysis: Before performing PCA analysis, the contribution of each trait towards genetic diversity was worked out using Tocher method. It was observed that seed yield per plant contributed 93.63 percent followed by days to 50 % flowering (4.92 %), days to maturity (0.68 %) and 100 seed weight (0.58 %). Further, for PCA analysis the variance-covariance matrix was used to transform all the attributes into a single index of similarity in the form of principal components, which represented 15 eigen values for 15 eigen vectors. Only 4 eigen vectors were described as they accounted for more than 99% variation present in complete 495 set of accessions (Table 7). The first principal component had maximum contribution by seed yield per plant (-145.19). The second principal component was determined by days to 50 % flowering (11.43). Whereas, 100 seed weight (g) (4.43) determined the third component and days to maturity (3.0) contributed for fourth principal component. It was seen that the result of PCA was in accordance with the result of Tocher method where all the four major principal components represented as per the trait which contributed the maximum to genetic diversity. Two- dimensional PCA of the whole set was

Table 4. Phenotypic^a and genotypic correlation among different quantitative characters

S.N.	Character Name	Days to 50% flowering	Days to maturity	Primary Branches	Number of seeds /pod	100 seed weight (g)	Seed yield/plant (g)
1	Days to 50% flowering	1.00	0.15	-0.16**	0.00	-0.20**	-0.07
2	Days to maturity	0.06	1.00	0.17**	0.02	0.24 **	0.16 **
3	Primary branches	0.03	0.07	1.00	-0.03	0.34**	0.33**
4	Number of seeds/pod	-0.01	0.01	-0.02	1.00	-0.01	-0.07
5	100 seed weight (g)	-0.09*	-0.00	0.18**	0.01	1.00	0.18 **
6	Seed yield/plant (g)	-0.04	0.01	0.17**	-0.05	0.02	1.00

^aPhenotypic correlation: lower diagonal; Genotypic correlation: above diagonal; *,**: Significant at P=0.05 & 0.01, respectively

Table 5. Distribution of genotypes in different clusters

Cluster	No. of genotypes	Name of few genotypes
1	81	ICC 406, ICC 575, ICC 866, ICC 1738, ICC 1789, ICC 4802
2	84	ICC 1331, ICC 1608, ICC 1755, ICC 1921, ICC 6138, ICC 7414
3	86	ICC 554, ICC 862, ICC 1304, ICC 1646, ICC 2834, ICC 4507
4	49	ICC 809, ICC 1483, ICC 4098, ICC 4637, ICC 4920, ICC 5386
5	75	ICC 832, ICC 5188, ICC 5324, ICC 5549, ICC 5641, ICC 7388
6	8	ICC 1705, ICC 4951, ICC 5326, ICC 5498, ICC 5932, ICC 6813
7	49	ICC 484, ICC 819, ICC 851, ICC 1327, ICC 1566, ICC 1739
8	21	ICC 479, ICC 1826, ICC 1892, ICC 2140, ICC 2208, ICC 4670
9	8	ICC 681, ICC 771, ICC 1885, ICC 4569, ICC 5543, ICC 6079
10	15	ICC 1831, ICC 1874, ICC 4688, ICC 5261, ICC 5398, ICC 5427
11	8	ICC 445, ICC 1586, ICC 4806, ICC 5485, ICC 6111, ICC 7447
12	6	ICC 1802, ICC 1824, ICC 5424, ICC 6123, ICC 7401, ICC 7434
13	8	ICC 1508, ICC 1697, ICC 2150, ICC 4934, ICC 6008, ICC 7427

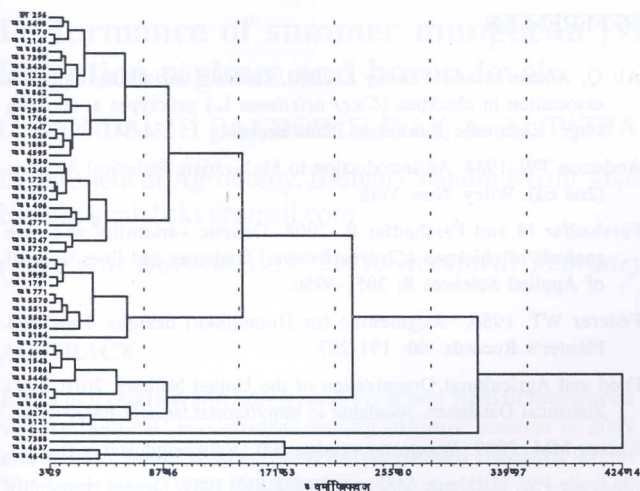


Fig 1. Dendrogram (based on dissimilarity distance) showing 52 genotypes which was drawn from each of 13 original clusters using Probability Proportional to Size (PPS) Simple Random Sampling (SRS) technique

Table 7. Eigen vector of 15 variables for the first 4 principal components

Variables	% contribution*	Principal components			
		1	2	3	4
Seedling vigour	0.06	0.63	0.26	0.08	0.06
Plant pigmentation	0.00	-0.05	0.03	-0.20	-0.06
Stem colour	0.08	-0.04	0.05	-0.15	0.02
Days to 50% flowering	4.92	0.79	11.98	0.38	-0.10
Growth habit	0.00	-0.05	-0.01	-0.03	-0.01
Flower colour	0.00	0.10	-0.05	0.07	0.03
Pod size	0.00	0.12	-0.10	0.15	0.03
Days to maturity	0.68	-0.54	0.07	1.39	3.00
Primary branches	0.00	-0.18	-0.08	0.15	0.00
Number of seed/pod	0.00	0.00	0.00	0.00	0.00
Seed size	0.00	-0.12	0.10	-0.26	-0.05
100 seed weight (g)	0.58	-0.86	-1.02	4.43	-0.94
Seed colour	0.05	-0.18	-0.07	0.44	-0.05
Testa texture	0.00	-0.03	-0.03	0.20	0.00
Seed yield/plant (g)	93.63	-145.19	0.07	-0.03	-0.01

*Computed using Tocher's method

Table 6. Inter and intra cluster distances

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10	Cluster 11	Cluster 12	Cluster 13
Cluster 1	29.30	11.63	70.66	198.55	194.08	196.76	354.17	348.81	359.54	360.43	765.59	795.99	781.68
Cluster 2		27.04	63.50	191.40	186.92	189.61	347.02	341.65	352.38	353.28	758.43	788.84	774.53
Cluster 3			29.43	198.55	203.92	197.66	353.28	347.91	357.75	356.86	765.59	795.99	781.68
Cluster 4				29.64	60.82	189.61	364.01	358.64	370.27	369.38	776.32	806.73	792.42
Cluster 5					31.79	158.30	331.81	355.07	364.91	364.01	772.74	803.15	788.84
Cluster 6						40.95	355.07	349.70	359.54	358.64	767.37	797.78	783.47
Cluster 7							35.21	23.25	138.63	137.73	775.42	805.83	791.52
Cluster 8								21.87	133.26	132.37	775.42	805.83	791.52
Cluster 9									29.08	63.50	779.90	810.30	795.99
Cluster 10										24.56	779.00	809.41	795.10
Cluster 11											43.67	205.71	191.40
Cluster 12												22.92	46.51
Cluster 13													30.42

Diagonals: Intra cluster distances; Above diagonals: Inter cluster distances

also sketched to know the behavior of the population set based on the traits studied (Fig. 2).

Seed yield is highly complex trait, thus indirect selection based on major component trait may increase the efficiency of breeder. In the present study, primary branches, 100 seed weight (g) and days to maturity which showed high to moderate heritability and desirable genetic advance had high positive

effect on yield. Therefore, selection based on these traits would be fruitful. The study of diversity index reflects that in present set of germplasm, ample genetic variation exists for seed yield per plant, therefore a direct selection based on seed yield can be done to develop high yielding varieties. However, after further evaluation, some accessions may be used as parents in the crossing programme to create the better genetic recombinants for improving yield *per se*.

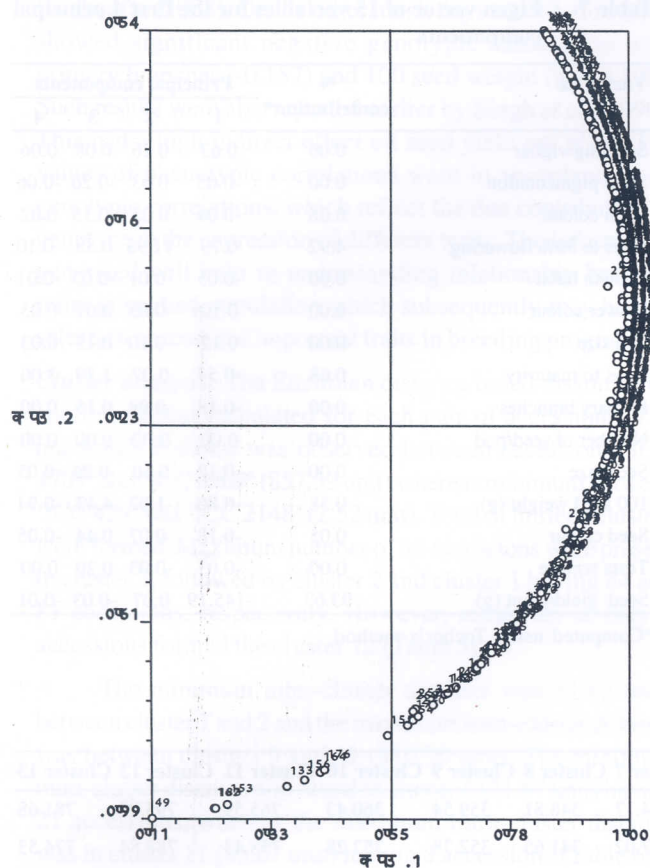


Fig 2. 2-dimensional PCA diagram

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