

Evaluation of Genetic Diversity Through D^2 Statistic in Chickpea (*Cicer arietinum* L.)

ABSTRACT

The present investigation was conducted at the Agriculture Research Farm, Department of Genetics and Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India. The objective of the investigation was to assess the genetic diversity among 71 different chickpea genotypes in relation to their yield and its attributing traits. The experimental design employed was a complete randomized block design with two replications. A comprehensive set of observations was made on twelve distinct yield accrediting traits from five randomly selected plants within each genotype. Based on D^2 Statistics analysis, the 71 chickpea genotypes were classified into 26 distinct clusters. Conspicuously, the cluster with the highest numbers of genotypes was designated as cluster 1. A remarkable finding emerged from the analysis of intra-cluster distances, with cluster 16 displaying the greatest distance within its constituents. The evaluation of inter-cluster distances revealed significant dissimilarity between clusters 22 and 26, suggesting presence of considerable genetic variation between these clusters. Conversely, the inter-cluster distance was minimal between clusters 2 and 4, indicating a closer genetic relationship between genotypes ICCV 201109 and SAGL- 162387. In terms of the genetic diversity analysis, it became evident that the yield related traits exerting the most substantial influence on the overall genetic divergence among the 71 chickpea genotypes were biological yield per plant, 100-seed weight, and the numbers of pods per plant. In contrast, the numbers of effective pods per plant contributed minimally to the overall genetic divergence. Based on the findings from both inter-cluster distances and individual performance assessments (*per se* performance), two specific genotypes viz., ICCV 201207 and SAGL 22-121, were short out as promising for inclusion in a hybridization programme. These findings contribute to a deeper understanding of chickpea genetic variability and lay the groundwork for further breeding programmes aimed to enhance chickpea crop productivity.

Key words: Chickpea, D^2 statistics, Diversity, Genotype, Clusters, Yield

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.), commonly known as garbanzo bean or Bengal gram, is an ancient and globally significant leguminous crop cultivated for its nutritious seeds [1-2]. Belonging to the Fabaceae family, chickpea holds historical and cultural importance, being cultivated and consumed across various regions for millennia [3-4]. It is a self-pollinated crop [5] and known as King of pulses. Chickpea is a cool season legume with a genome size of ~738 mb [6] and originated in south eastern Turkey and Syria [7]. Two primary categories of chickpea germplasm are recognized: "Kabuli" and "Desi." Kabuli chickpea (*Cicer arietinum* var. *macrosperma*) is characterized by its sizeable creamy-colored seeds, accompanied by white flowers, and lacks anthocyanin pigmentation. In contrast, Desi chickpea (*Cicer arietinum* var. *microsperma*) is distinguished by its smaller seeds of diverse colors, accompanied by purplish flowers, and contains anthocyanin pigments [8]. The diminished productivity of chickpea is attributed to a combination of different biotic and abiotic factors, as substantiated by recent research [9-20].

The consumption of chickpeas enjoys popularity across numerous global regions, primarily attributed to its remarkable nutritional excellence. Within the chickpea seed matrix, a harmonious composition of essential components prevails, encompassing 50–58% carbohydrates, 15–22% protein, 7–8% moisture, 3.8–10.20% fat, and <1% micronutrients [21-22, 11]. The average protein content found in chickpeas is approximately 18%, with kabuli chickpeas containing around 18.4% ranging from 16.2% to 22.4%, and desi chickpeas contain about 18.2% in range of 15.6% to 21.4%. This protein content surpasses that of lentils and field peas [23]. As a significant member of the legume family, chickpea holds a pivotal position among pulses. Notably devoid of cholesterol, it boasts remarkable nutritional value, being rich in dietary fiber (DF), vitamins, and minerals. Although its methionine content ranges between 1.3% to 1.6% and cysteine content containing sulfur is around 2.5% to 3.0%, these sulfur-containing amino acids are typically found in relatively lower amounts. However, when consumed alongside cereals deficient in lysine amino acid, chickpea's nutritional profile becomes complementary, ensuring a balanced diet [24].

Assessing genetic diversity through the measurement of genetic distance proves to be an invaluable tool in crop breeding [25-41] including chickpea [4-5], aiding in the selection of suitable parent plants to facilitate novel genetic recombination and enhance grain yield [42]. The presence of genetic diversity stands as a pivotal factor in the formulation of effective and fruitful breeding initiatives [23]. Employing cluster analysis serves as a suitable approach to delineate familial relationships and genetic affinities among genotypes, enabling the quantification of genetic distance between them. D^2 statistic, a variant of generalized distance, pioneered by Mahalanobis [43], offers a means to evaluate genetic diversity among genotypes. The prosperity of any crop enhancement programme is intricately linked to the genetic diversity within the existing germplasm pool [44-45, 25-41]. Subsequently, a quantitative assessment of the genetic diversity present within a population assists plant breeders in the meticulous selection of desirable parental candidates for breeding undertakings. In the present investigation, the extent of genetic diversity present in a set of seventy-one chickpea genotypes for various yield attributing traits were evaluate by means of D^2 statistic for further enhancing understanding of the species for genetic enhancement.

2. MATERIALS AND METHOD

EXPERIMENTAL MATERIAL

In this investigation, Mahalanobis (D^2) statistics was utilized to analyze genetic diversity present among 71 distinct chickpea genotypes.

EXPERIMENTAL SITE

The experiment was conducted at the Agriculture Research Farm, Department of Genetics and Plant Breeding, College of Agriculture, RVSKVV, Gwalior, Madhya Pradesh, India.

EXPERIMENTAL DESIGN AND MANAGEMENT

The genotypes were evaluated in randomized block design with two replications during the *Rabi* seasons of 2021 and 2022. The observations were recorded, with a pooled data of both the seasons and further analyzed. Each genotype was planted across four rows, each spanning a length of 3 meters. The spatial arrangement maintained a row-to-row distance of 30 units and a plant-to-plant distance of 15 cm. The entire cultivation process adhered to recommended agronomic practices.

DATA COLLECTION

The recorded data encompassed twelve distinct morpho-physiological attributes, including days to 50 % flowering, days to maturity, plant height, numbers of primary and secondary branches per plant, numbers of pods per plant, numbers of effective pods per plant, numbers of seeds per pod, 100-seed weight, harvest index, biological yield per plant and seed yield per plant. To ensure comprehensive observations, a random selection of five plants was made from each replication of every genotype, serving as the foundation for data collection across all measured traits.

STATISTICAL ANALYSIS

The multivariate analysis, specifically employing the D^2 statistics proposed by Mahalanobis [43], has emerged as a robust and indispensable tool for elucidating the extent of differentiation observed among biological populations at the genotypic level [44]. Through this analytical approach, one can effectively assess the relative contributions of distinct components to the overarching divergence phenomenon, spanning both inter and intra-cluster levels. Remarkably, the D^2 statistical analysis, as pioneered by Mahalanobis, was complemented by the construction of a dendrogram, which not only offers a comprehensive visual representation but also provides insights into the intricate genetic relationships inherent within the investigated populations. The Tocher's method [45] was used to form clusters based on the calculated D^2 values. For the rigorous execution of statistical computations and dendrogram construction, the specialized Statistical software WINDOSTAT version 9.2 provided by INDOSTAT Services, Hyderabad, India was harnessed, thereby ensuring a heightened level of precision, robustness, and reliability in the resulting analytical outcomes.

3. RESULTS AND DISCUSSION

3.1 Clustering of Genotypes

D^2 statistical analysis was employed to assess the genetic divergence among 71 genotypes. Consequently, the 71 genotypes were categorized into four main clusters and 22 minor clusters. Among these clusters, cluster 1 was found to be polygenotypic, encompassing 32 genotypes. On the other hand, Clusters 11, 13, and 16 were oligogenotypic, comprising more than two genotypes each. The remaining clusters consisted of only one genotype, rendering them

monogenotypic (Table 1, Fig. 1). Cluster 1 stood out with its significant count of 32 genotypes, indicating a limited genetic divergence among them. This similarity could potentially be attributed to their shared ancestry in the base population from which they evolved. It is worth noting that the convergence of similar phenotypes within a cluster might arise from focused unidirectional selection on specific traits or a cluster of linked traits across different geographical regions.

These factors could encompass a range of influences such as natural and artificial selection pressures, the exchange of breeding materials, genetic drift, and environmental variability. As such, the intricate interplay of these multifaceted forces appears to play a pivotal role in shaping and sustaining the observed genetic diversity landscape. In a congruent investigation, Gupta *et al.* [46] conducted a genetic divergence study encompassing 25 chickpea genotypes, ultimately arranging them into seven clusters through the utilization of Tocher's method. Koinain *et al.* [47] undertook a study involving thirty diverse chickpea genotypes, classifying them into eight distinct clusters, Rajkumar *et al.* [48] partitioned a collection of 100 distinct chickpea genotypes into sixteen clusters.

3.2 Intra and Inter Cluster Divergence D^2 Values

When examining a range of important agronomic traits like yield potential, disease resistance, growth duration, and seed quality, the Euclidean distance method is frequently employed. This method facilitates the identification of hierarchical patterns within the analyzed accessions. By grouping these accessions based on specific traits, it enables the identification of promising pairs for crossbreeding purposes in the context of chickpea studies [4-5]. This approach serves to assess both the likeness and distinctiveness among different accessions, while also quantifying the extent of a particular trait's expression. Past research, exemplified by studies conducted by Syed *et al.* [49] and Malik *et al.* [50], has demonstrated the effectiveness of this method in characterizing and evaluating chickpea accessions.

The average D^2 values for both intra and inter-cluster distances, along with the nearest clusters based on D^2 values are presented in Table 2 and Fig. 2. Intra-cluster distances spanned from zero to 30.50. Cluster 16 exhibited the highest intra-cluster distance at 30.50, followed by cluster 13 (25.02), cluster 11 (22.82), and cluster 1 (19.7). Other clusters had an intra-cluster distance of 0.00 due to their single-genotype composition. Furthermore, inter-cluster distances

ranged between 6.59 to 186.56. The most substantial inter-cluster distance was observed between cluster 22 and cluster 26 (199.44), succeeded by cluster 24 and 26 (186.56), cluster 16 and cluster 19 (153.40), and cluster 16 and cluster 20 (153.37). Conversely, the smallest inter-cluster distances were observed between cluster 2 and cluster 4 (6.59), cluster 3 and cluster 6 (7.54), and cluster 2 and cluster 5 (8.71). These extensive inter-cluster distances signify that the genotypes within these clusters exhibit a wide array of genetic diversity. They hold promising potential for utilization in chickpea improvement through hybridization programmes. To enhance the likelihood of generating favorable recombinants in segregating generations, crossing between genetically diverse genotypes from clusters with substantial inter-cluster distances would be advantageous. These are the similar work done by Janghel *et al.* [51], non-hierarchical Euclidean cluster analysis categorized 60 chickpea genotypes into seven distinct clusters. Katkani *et al.* [51] identified nine clusters among forty-two desi chickpea lines through divergence analysis. Moreover, Mihoariya *et al.* [4] grouped eighty-three chickpea genotypes into twelve clusters after evaluating the nature and extent of genetic divergence.

3.3 Cluster Mean Values

The cluster mean values for diverse traits have been presented in Table 3 revealing significant differences among the studied traits. The data highlights specific clusters with distinct trait averages. Cluster 25 displays the highest mean for days to 50% flowering (61.17), whilst cluster 19 exhibits the lowest (49.50). Cluster 2 recorded the maximum mean for days to maturity (121.50), contrasting with the lowest mean in cluster 17 (112.17). In terms of plant height, cluster 21 demonstrates the highest mean (75.67cm), whereas cluster 19 reflects the minimum (42.99cm). The numbers of primary branches per plant is maximized in cluster 24 (4.87), whereas cluster 10 displays the least (2.55). For the numbers of secondary branches per plant, cluster 18 contributed the highest mean (10.88), while cluster 10 showing the lowest (6.37). Regarding pod-related traits, cluster 25 comprehends the highest mean for the numbers of pods per plant (55.50), while cluster 19 presents the lowest (32.17). Similarly, cluster 25 demonstrates the maximum mean for the numbers of effective pods per plant (52.17), whereas cluster 19 showing the minimum (29.17). Cluster 9 displays the highest mean for the numbers of seeds per pod (1.73), while clusters 24 consisted the lowest (1.18). For 100-seed weight, cluster 12 exhibits the highest mean (29.77g), contrasting with the lowest mean in cluster 6 (17.41g). Harvest index is maximized in cluster 18 (68.57), whereas cluster 6 presents the lowest (40.59). In terms of

yield, cluster 26 had the highest mean for biological yield per plant (51.07g), In contrast, cluster 23 indicates the lowest value (29.14g). On the other hand, cluster 13 exhibits the highest mean for seed yield per plant (25.59), while cluster 22 reveals the lowest (13.82).

Likewise, Gediya *et al.* [52] also conducted a study focusing on 13 distinct characters within the realm of chickpea. The observed cluster mean values exhibited a broad spectrum of averages across the clusters for various traits. Notably, cluster 15 showed the highest mean values for numbers of pods (136.00) and seeds per plant (201.87), while registering the lowest mean value for the first pod height (33.87 cm). Within cluster 12 the highest mean values were recorded for seed yield per plant (38.77 g) and harvest index (54.89%). Cluster 10 displayed the highest cluster mean value for 100-seed weight (42.34 g), whereas cluster 16 exhibited the highest mean average for plant height (105.87 cm). Conversely, cluster 14 exhibited the lowest mean worths for days to 50 percent flowering (44.00 days) and days to maturity (107.33 days), while simultaneously showcasing the highest mean value for numbers of seeds per pod (1.93). Cluster 1 displayed the highest mean value for numbers of primary branches per plant (3.13), whereas cluster 6 exhibited the highest mean value for numbers of secondary branches per plant (12.70). In the study conducted by Reddy *et al.* [54], cluster 2 exhibited the highest mean values for a range of traits, including the numbers of secondary branches per plant, pod count per plant, seed count per pod, and seed count per plant. Cluster 4, conversely, displayed the highest mean potential for the numbers of primary branches per plant, biological yield per plant, and seed yield per plant. Furthermore, the mean values for cluster 5 were most pronounced in traits like plant height and harvest index. Cluster 6 demonstrated its highest mean values in the context of 100 - seed weight, whilst cluster 7 showed an early disposition for traits such as days to 50% flowering, days to 50% pod setting, and days to maturity.

3.4 Contribution of Individual Characters Towards Genetic Divergence

The percentage contributions to genetic divergence attributed to various characters are presented in (Table 4, Fig. 3). Among these, the character biological yield per plant exhibited the most substantial contribution at 38.07%, followed by 100-seed weight (28.01%), numbers of pods per plant (14.57 %), plant height (6.4%), seed yield per plant (6.24 %), harvest index (2.37%), numbers of primary branches per plant (1.77%), days to 50% flowering (0.76 %), numbers of

seeds per pod (0.64%), days to maturity (0.56 %), numbers of secondary branches per plant (0.52%) and numbers of effective pods per plant (0.08%).

Consistent with earlier studies, Pahre *et al.* [55], Kuldeep *et al.*[56], and Thakur *et al.* [57] reported similar trends wherein 100-seed weight, numbers of pods per plant, and days to 50% flowering emerged as the primary contributors to the observed genetic diversity. This alignment underscores the significance of these specific traits in driving the divergence documented within the chickpea population.

4. CONCLUSION

In this ongoing divergence analysis, assessing the proportional influence of each trait on genetic divergence offers valuable insights that can aid plant breeders in discerning and selecting superior genotypes from the existing germplasm pool. These chosen genotypes hold the potential to serve as optimal parent candidates in forthcoming crop enhancement initiatives. Concurrently, the present investigation unveils a significant level of diversity within the examined assemblage of 71 chickpea genotypes. This diversity is particularly driven by the traits of biological yield per plant, 100-seed weight, and the numbers of pods per plant. Among the clusters, cluster I stands out as the most populous, encompassing 32 genotypes. Furthermore, the analysis underscores a substantial inter-cluster disparity, prominently observed between clusters 22 and 26. The genotypes within these distinct clusters hold potential significance as parental candidates for generating transgressive segregants in future breeding endeavors. This elucidates their viability in augmenting the genetic variability of chickpea cultivars.

5. REFERENCES

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Table 1. The distribution of 71 chickpea genotypes into different clusters based on D² statistic.

Clusters	No. of genotypes	Genotypes
Cluster 1	32	ICCV 20116, ICCV 20115, ICCV 201206, ICCV 201117, RVG 202, SAGL 22-116, SAGL 22-117, SAGL 22-118, SAGL- 152237, SAGL- 152278, SAGL- 152227, SAGL- 162364, SAGL- 152356, SAGL- 153226, SAGL- 152222, SAGL- 152223, SAGL- 152234, SAGL- 152329, SAGL- 162376, SAGL- 162377, RVSSG 84, JG 315, RVSSG 92, RVSSG 74, JG 130, RVSSG 83, ICC 4958, RVSSG 71, RVSSG 52, SAGL- 161025, SAGL- 163007, JG 62
Cluster 2	1	ICCV 201109
Cluster 3	1	SAGL- 161024
Cluster 4	1	SAGL- 162387
Cluster 5	1	SAGL- 162299
Cluster 6	1	RVSSG 68
Cluster 7	1	JG 6
Cluster 8	1	SAGL- 163006
Cluster 9	1	ICCV 201112
Cluster 10	1	ICCV 201214
Cluster 11	6	ICCV 201210, SAGL- 152339, SAGL- 152258, SAGL- 152318, SAGL- 152344, SAGL- 162381
Cluster 12	1	SAGL- 152250
Cluster 13	7	SAGL 22-122, SAGL 22-124, SAGL 22-119, SAGL 22-120, JAKI 9218, RVG 204, SAGL- 152324
Cluster 14	1	ICCV 201104
Cluster 15	1	SAGL- 152336
Cluster 16	4	SAGL- 152330, SAGL- 152238, SAGL- 152405, SAGL-152327
Cluster 17	1	ICCV 201205
Cluster 18	1	SAGL- 152231
Cluster 19	1	SAGL 22-110
Cluster 20	1	H12-55
Cluster 21	1	ICCV 201211
Cluster 22	1	ICCV 201207
Cluster 23	1	SAGL- 152337
Cluster 24	1	Pant Gram-5
Cluster 25	1	SAGL 22-123
Cluster 26	1	SAGL 22-121

Table 3 Cluster mean performance for 12 characters of 71 chickpea genotypes

	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches per plant	Secondary branches per plant	Pods per plant	Effective pods	Seed per pod	100 seed weight (g)	Harvest index (%)	Biological yield per plant (g)	Seed yield per plant (g)
Cluster 1	55.82	115.19	53.48	3.63	8.91	39.49	34.96	1.43	24.16	52.17	35.73	18.56
Cluster 2	53.17	121.33	62.31	4.22	10.65	47.83	42.50	1.40	24.88	59.43	32.38	19.19
Cluster 3	56.50	118.17	53.04	3.68	7.80	40.00	34.83	1.67	18.45	46.69	39.92	18.56
Cluster 4	53.83	116.17	61.70	4.03	9.98	46.83	41.50	1.20	28.24	59.48	34.31	20.40
Cluster 5	57.67	117.50	55.68	3.75	9.47	50.67	45.83	1.40	27.09	60.98	33.64	20.51
Cluster 6	56.83	113.00	56.46	3.42	7.82	39.00	36.17	1.45	17.41	40.59	42.06	17.06
Cluster 7	54.50	114.33	58.35	4.23	9.51	47.50	41.33	1.47	27.48	46.96	41.06	19.22
Cluster 8	56.67	116.50	52.10	3.55	7.95	41.67	36.33	1.32	29.24	63.35	35.36	22.34
Cluster 9	57.50	117.00	66.57	3.85	8.97	52.00	46.50	1.73	27.54	55.63	32.24	17.91
Cluster 10	54.17	115.83	57.84	2.55	6.37	45.83	41.67	1.60	23.28	57.92	31.72	18.34
Cluster 11	55.17	116.78	56.09	3.76	9.95	45.06	40.11	1.44	31.88	49.54	37.73	18.70
Cluster 12	54.50	115.00	45.06	3.62	9.20	32.83	28.33	1.13	29.77	51.82	40.28	20.85
Cluster 13	56.31	114.05	55.03	3.64	8.38	40.98	36.40	1.49	22.64	56.12	45.58	25.59
Cluster 14	54.17	115.83	70.82	3.42	9.31	40.50	35.33	1.25	22.26	57.27	31.21	17.85
Cluster 15	54.17	114.17	53.50	3.42	6.91	45.33	39.33	1.93	28.58	56.25	30.37	17.10
Cluster 16	58.13	117.50	63.42	3.68	8.93	50.17	44.79	1.21	28.74	59.41	48.48	28.71
Cluster 17	52.17	112.17	68.42	3.42	8.59	36.83	32.50	1.40	25.92	60.11	35.60	21.38
Cluster 18	57.83	112.83	52.23	4.22	10.88	48.67	43.17	1.60	27.86	68.57	31.29	21.41
Cluster 19	49.50	115.17	42.99	4.27	10.57	32.17	29.17	1.20	27.24	49.55	34.03	16.82
Cluster 20	53.33	111.83	45.20	3.93	7.83	38.00	35.17	1.42	20.32	60.82	33.75	20.49
Cluster 21	59.17	120.00	75.67	4.25	10.26	48.33	44.00	1.33	26.89	55.17	38.41	21.13
Cluster 22	56.17	117.00	46.11	4.42	9.11	33.50	31.50	1.25	24.28	46.52	29.70	13.82
Cluster 23	57.67	117.17	39.04	4.02	9.24	38.17	33.33	1.40	25.60	63.64	29.14	18.52
Cluster 24	51.83	119.83	48.32	4.87	9.08	52.17	45.17	1.18	21.78	58.07	30.63	17.78

Cluster 25	61.17	118.83	60.90	4.48	9.17	55.50	52.17	1.52	22.27	58.03	39.78	23.08
Cluster 26	57.67	118.83	55.66	3.72	9.60	37.00	33.00	1.30	26.18	45.84	51.07	23.41

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Table 4 Number percent contribution of characters towards divergence in 71 chickpea genotypes

S.No.	Characters	Time ranked first	Percent contribution
1	Days to 50 % flowering	19	0.76
2	Days to maturity	14	0.56
3	Plant height (cm)	159	6.4
4	Number of primary branches per plant	44	1.77
5	Number of secondary branches per plant	13	0.52
6	Number of pods per plant	362	14.57
7	Number of effective pods per plant	2	0.08
8	Number of seeds per pod	16	0.64
9	100 seed weight (g)	696	28.01
10	Harvest Index(g)	59	2.37
11	Biological yield per plant (g)	946	38.07
12	Yield per plant (g)	155	6.25
Total			100

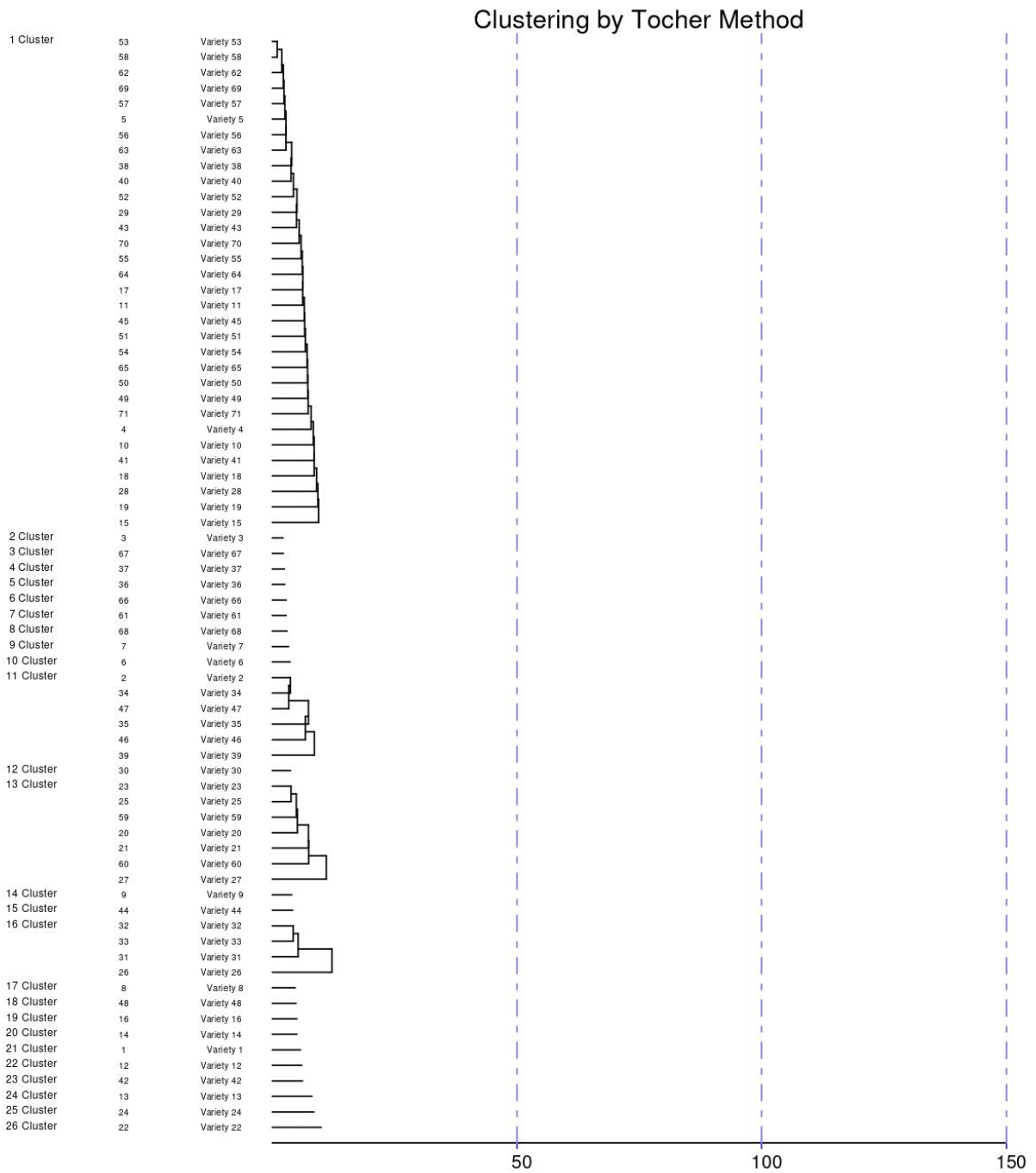


Fig.1 Dendrogram based on Euclidian distance using an unrooted neighbour-joining tree in 71 chickpea genotypes

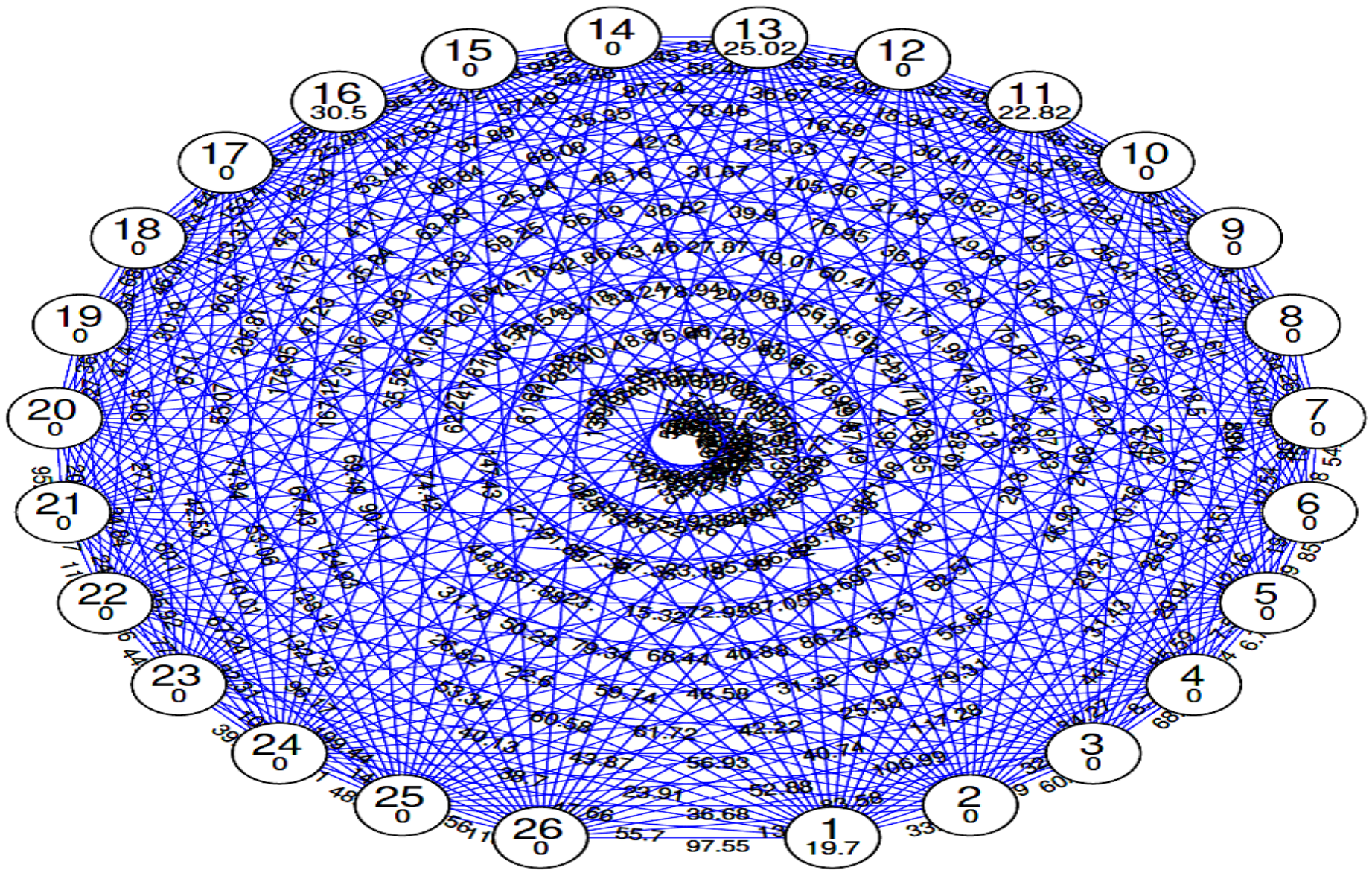


Fig. 2. Cluster diagram depicting intra and inter-cluster distance based on Mahalanobis Euclidean distance

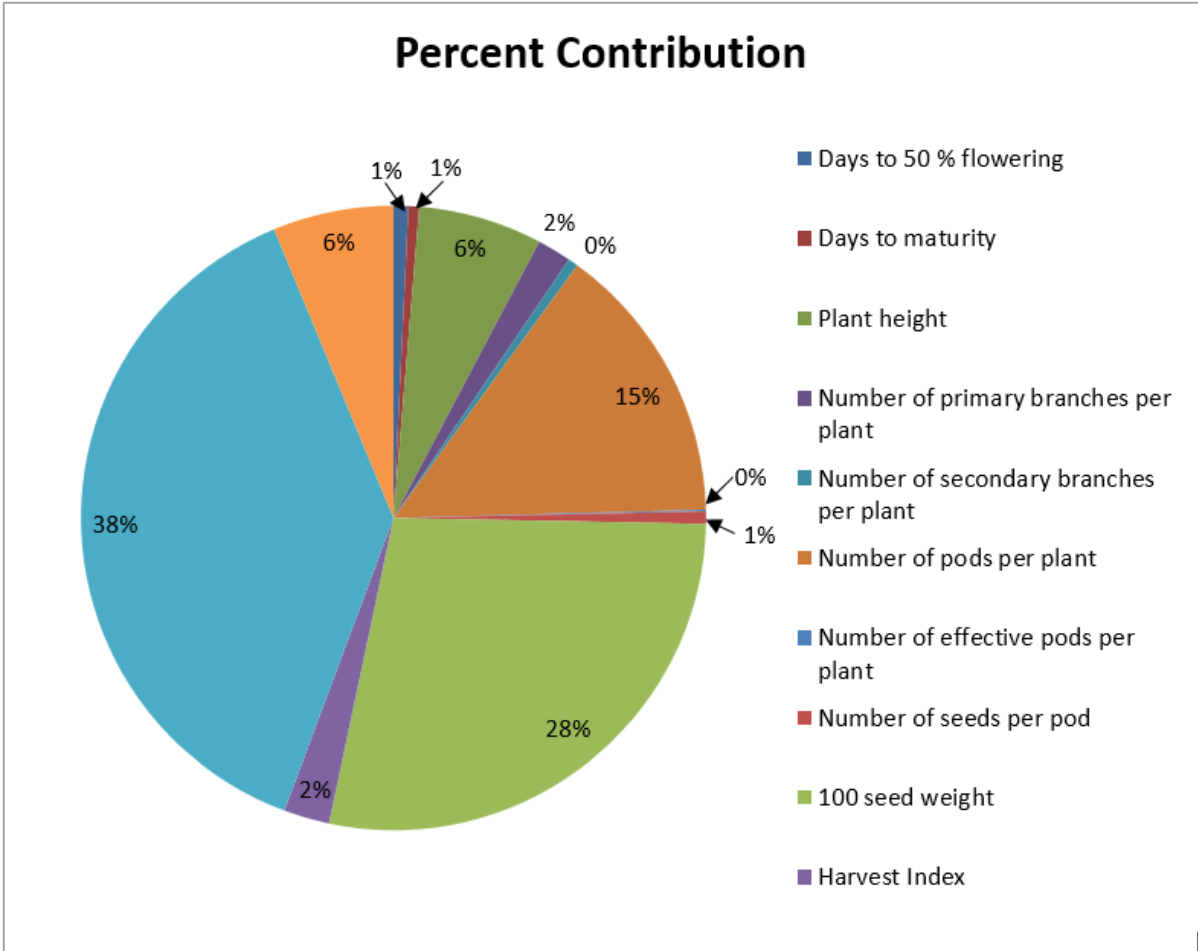


Fig. 3. Percent contribution of characters

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