



# Genetic Divergence for Seed Yield Enhancing and Quality Traits in Pigeonpea [*Cajanus cajan* (L.) Millsp.]

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## ABSTRACT

**Background:** Pigeonpea has remarkable contribution in flourishing the Indian agricultural economy but over the decades, its productivity has remained stagnant. Presence of genetic diversity in breeding population is the key to develop high yielding varieties. Therefore the current study was aimed to explore genetic divergence among pigeonpea genotypes.

**Methods:** A set of 178 pigeonpea germplasm lines were evaluated in randomized block design to assess the genetic divergence and clustering pattern of pigeonpea genotypes. Observations on various yield component traits were recorded. For the estimation of the Fe and Zn content ICP-OES (Perkin Elmer USA) was used. Mahalanobis D<sup>2</sup> analysis was carried out to assess the genetic divergence.

**Result:** Wide range of genetic diversity was revealed among lines for eleven traits studied. Mahalanobis D<sup>2</sup> analysis grouped all the genotypes into eleven non-overlapping clusters. The inter cluster D<sup>2</sup> values indicated that cluster IV and XI (21046.45) were the most diverse and cluster V and cluster VII (60.67) were the less diverse. Out of the eleven characters studied days to maturity, plant height and yield per plot contributed 93.32 per cent of the total divergence. Based on mean performances, cluster XI and cluster II were found to be beneficial for early flowering and early maturity genotypes, cluster V and cluster VII for number of pods per plant and yield per plot, respectively. The highest mean value for grain iron and zinc content was recorded in cluster III rendering simultaneous improvement for these traits. Therefore, trait-wise selection of diverse parents from different clusters would be desirable and beneficial.

**Key words:** Cluster analysis, D<sup>2</sup> statistics, Diversity, Pigeonpea.

## INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an often cross-pollinated perennial member of the family *Fabaceae* with chromosome number  $2n=2x=22$ . It is one of the most accomplished grain legume crop grown mainly for rainfed agriculture in semi-arid tropics of Asia, Africa and Caribbean. Globally pigeonpea covers an area of 7Mha with 6.8MT production and 969 kg ha<sup>-1</sup> productivity (FAOSTAT 2019). It is known to play an important role in food security, balanced diet supplemented with cereals and mitigation of poverty due to wide usage as food, feed, fodder and fuel purpose (Rao *et al.* 2002). It is a good source of protein (about 25%), amino acids and dietary minerals such as calcium, phosphorus, magnesium, iron, sulphur and potassium (Patel *et al.* 2018).

A number of varieties have been developed and area under pigeonpea has also been increased in India but the yield has remained stagnant due to various biotic and abiotic factors and narrow genetic base. For the past six decades, the average yield in the country has remained around 900 kg ha<sup>-1</sup> (FAOSTAT 2017). At global level also, the pigeonpea productivity has remained more or less stagnant around 700-800 kg ha<sup>-1</sup> (Bohra *et al.* 2020). Although, green revolution has expanded the production and reduced the starvation and mal nutrition at a large scale but ultimately caused depletion of micronutrients in soil and plants leading to wide spread micronutrient deficiencies among children and adults Hanumanthappa *et al.* (2018). Among micronutrient deficiencies, the most common and widespread are the iron and zinc deficiency affecting half of the human population

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(WHO 2002, White and Broadley, 2009). This micronutrient deficiency is also known as 'Hidden hunger' that results in poor growth and development of children and even death in acute cases (Stein 2010). Therefore, nutritional breeding is a step forward towards attaining food security to feed the ever increasing world population with nutritionally enriched genotypes to make a halt on hidden hunger.

Presence of genetic variability and diversity in breeding population is the key to develop high yielding varieties. Selection and crossing of genetically diverse parents is very important to get more number of desirable recombinants in segregating generations. Therefore, genetic diversity analysis is the first hand tool to identify divergent genotypes and to utilize such genotypes in crossing program. Divergent parents are likely to produce heterotic effect as well as useful segregants. A method suggest by Mahalanobis (1936) known as "Mahalanobis D<sup>2</sup> statistics" is used to estimate

genetic diversity in the available germplasm. The above mentioned technique calculates the force of differentiation at intra-cluster and inter-cluster levels and thus helps in selection of parents with genetically diverse genetic makeup. It also deciphers the degree of diversification and relative proportion of each component character to the total divergence. Keeping this in view, the present study was carried out to study genetic divergence among different pigeonpea genotypes and their utilization in future crop improvement programme.

## MATERIALS AND METHODS

The present investigation experiment was conducted with 178 genotypes of pigeonpea for assessment of genetic diversity. The genotypes were raised in a randomized block design with two replications at the experimental area of Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during *kharif* seasons of 2018 and 2019. Each genotype was grown in a single row plot of 4 m length with row spacing of 50 cm. Observations on days to 50 per cent flowering, days to maturity, plant height (cm), primary branches per plant, secondary branches per plant, pods per plant, seeds per pod, 100 seed weight (g) and seed yield per plot (g) were recorded in 5 randomly taken plants of each genotype in each replication. For grain iron and zinc content, 5 gm of seeds from each replication were grinded followed by microwave digestion with di-acid mixture (4:1 nitric acid and perchloric acid). After microwave digestion, the final volume was raised to 30 ml, using double distilled water in a fume hood to prevent contamination. This sample was further processed in ICP-OES (Perkin Elmer USA instrument) for the estimation of the Fe and Zn (Kumaravel and Alagusundram, 2014). The Replicated data recorded was analyzed using WINDOWSTAT ver. 8.5 software developed by Indostat services, Hyderabad. Analysis was carried out as per the principles of Mahalanobis (1936) for  $D^2$  statistics to estimate the genetic diversity and clustering of genotypes into different clusters by Tocher's method (Rao 1952), respectively.

## RESULTS AND DISCUSSION

The genotypes were found to be significant based on mean squares for all the eleven characters studied representing the diversity present in the material used for investigation. On the basis of  $D^2$  values, the 178 genotypes of pigeonpea were grouped into eleven clusters depending on their genetic similarities and differences are presented in Table 1. The distribution pattern of different genotypes indicated that cluster I had the highest number of genotypes (148) followed by cluster IV (12) and cluster IX (10). The all other remaining clusters had one genotype in each cluster, advocating the influence of selection pressure in increasing the genetic divergence (Singh *et al.* 2015). Generation of large clusters in general and sole clusters in specific indicated the existence of large amount of diversity between the set of

genotypes. The large number of genotypes fell into same cluster exhibited narrow range of genetic diversity among them. Selection of such genotypes results in unproductive breeding. Furthermore, genotypes in solitary clusters exhibited distinctive characters which made them more divergent. Origin of such clusters may be due to isolation, which restricted the gene flow or rigorous natural or human selection adaptive complexes. Presence of large amount of genetic diversity among the evaluated set of germplasm lines indicated that this can serve as good source for selecting diverse parents for hybridization and will lead to generation of variability and isolation of desirable segregants for yield and other traits of economic importance. Existence of large amount of genetic diversity in pigeonpea material has also been reported earlier by Katiyar *et al.* (2004), Sreelakshmi *et al.* (2011), Rupika and Kannan Bapu (2014) and Reddy *et al.* (2015). It was also reported that genotypes developed in same region are included in different clusters. So, based on this clustering pattern, it can be inferred that there is no correlation between genetic diversity and geographical origin. So, selection of parents only on the basis of geographic diversity alone may not be a useful criterion. Similar findings were reported by Katiyar *et al.* (2004), Gupta *et al.* (2008) and Nag and Sharma (2012).

The intra and inter-cluster distances between eleven clusters were computed and presented in Table 2 and depicted in Fig 1. The highest intra-cluster distance was observed in cluster IX (642.17) followed by cluster I (500.21) and cluster IV (253.89). The intra-cluster distance in other clusters was zero because they had only one genotype. The maximum inter-cluster values (21046.45) were noticed between cluster IV and XI indicated that these clusters were distantly placed from each other and genotypes in these would be highly diverse from each other. The least inter-cluster distance (60.67) was recorded between cluster V and cluster VII signified a close relationship between the genotypes of these clusters. The inter cluster distances were larger than intra cluster distances depicted the wider range of genetic diversity between the genotypes of the clusters. It is always advisable to select genotypes from clusters with maximum inter-cluster distance for further use in crossing programme. By doing this the possibility of isolating good segregants in segregating generations would be more. These results were in agreement with the earlier work reported by Sharma *et al.* (2018), Ramya *et al.* (2018) and Satish *et al.* (2020).

### Contribution of various characters towards genetic divergence

Contribution of various characters towards genetic divergence has been presented in Table 3. The results have revealed that the plant height (cm) (74.68%) had maximum contribution towards genetic divergence followed by days to maturity (11.62%) and yield per plot (7.02%). The other remaining traits had shown negligible or very low contribution towards genetic divergence. This suggests that while performing selection importance should be given to these

**Table 1:** Clustering pattern of 178 germplasm lines of pigeonpea.

| Cluster | No. of genotypes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| I       | 148              | IC245294, AH092, AL1465, AL303, AL311, PBR2, AL1820, PantA251, AH151, AL1455, AL1396, ICP8947, IC245219, AL1471, AL1584, AL1313, PantA252, AL1466, Paras, P2121, PantA163, AL304, ICPL2033, AL1848, AL1533, AL1823, P994, IC245507, ICP9900, AL1430, AH093, IC245524, PantA855, AL1627, AL1730, IC245314, IC245268, AH067, PantA51, IC245245, Pusa991, AH1502, IC245183, H948, IC245315, AL986, H013, PAU881, AL1856, AL1449, IC245523, AL1782, AH1501, MTH103, PantA234, AH1507, AL1592, ICPL2037, IC245716, Pusa84, AL1490, AL1543, IC245533, AL1452, CORG111, Pant234, H0329, CIPB5120, AH0911, P2003, AF370, AL1813, AH1525, Pusa992, ICP9202, PANTA250, IC245525, MN5, P20016, CORG9704, AL1789, H493, H0137, CORG105, AL1538, AL1459, AL1740, AL1486, AL1474, AL2062, AL1487, AH1508, ICPL9308, CORG6012, AL307, AL2131, AL1835, AH1516, H005, AH0612, AH1519, AL2170, AL2133, H6121, H0420, H9332, H0118, PantA174, P226, P2007, AH091, AL1721, H200014, T21, Pant37, ICPL98015, AL1477, AP1371, AL2207, H0328, AL2132, AH0930, AL1554, MN1, AL1476, ICP11250, IC3977, H0512, H0265, AH0941, VLA1, AH069, H0535, TAT108, ICPL920, AH1506, AL1846, AL1525, AL1790, IC245560, H0571, AL2128, AL1760, IC245139, AH063, AL2087, AL1747, AL2025 |
| II      | 1                | PantA37                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| III     | 1                | IC245186                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| IV      | 12               | IC245273, AH061, AL1491, AL1676, IC245497, AL1849, AL1847, AL1801, AL997, AL1853, AL292, AL1508                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| V       | 1                | Sarita                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| VI      | 1                | H057                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| VII     | 1                | AL1781                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| VIII    | 1                | PUSA2001                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| IX      | 10               | AL1594, IC245350, Manak, V10099, AL1759, AL1922, AL882, AL1725, P2002, PADT16                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| X       | 1                | AL2091                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| XI      | 1                | AL2204                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

**Table 2:** Intra (diagonal) and inter-cluster distances ( $D^2$  value) of 178 germplasm lines of pigeonpea.

| Cluster | I      | II      | III     | IV      | V       | VI      | VII     | VIII    | IX      | X        | XI       |
|---------|--------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|
| I       | 500.21 | 1012.84 | 1070.57 | 1527.11 | 994.29  | 1060.42 | 982.71  | 1232.67 | 2407.39 | 6746.47  | 12518.80 |
| II      |        | 0.00    | 224.63  | 301.56  | 2833.04 | 2898.34 | 2706.61 | 3366.64 | 5131.54 | 11378.04 | 18781.96 |
| III     |        |         | 0.00    | 198.51  | 3076.08 | 3203.38 | 3065.88 | 3306.27 | 5394.67 | 11781.73 | 19198.04 |
| IV      |        |         |         | 253.89  | 3816.51 | 3916.26 | 3767.04 | 4230.78 | 6379.82 | 13212.17 | 21046.45 |
| V       |        |         |         |         | 0.00    | 128.01  | 60.67   | 217.90  | 624.60  | 2984.14  | 7132.05  |
| VI      |        |         |         |         |         | 0.00    | 175.67  | 310.47  | 625.71  | 2927.92  | 7147.97  |
| VII     |        |         |         |         |         |         | 0.00    | 382.20  | 684.77  | 3204.26  | 7426.16  |
| VIII    |        |         |         |         |         |         |         | 0.00    | 629.58  | 2699.15  | 6661.52  |
| IX      |        |         |         |         |         |         |         |         | 642.17  | 1723.15  | 4894.30  |
| X       |        |         |         |         |         |         |         |         |         | 0.00     | 966.80   |
| XI      |        |         |         |         |         |         |         |         |         |          | 0.00     |

**Table 3:** Per cent contribution of each character towards genetic divergence in pigeonpea.

| Characters                    | Times ranked first | Contribution (%) |
|-------------------------------|--------------------|------------------|
| Days to 50 per cent flowering | 234                | 1.49             |
| Days to maturity              | 1831               | 11.62            |
| Plant height (cm)             | 11765              | 74.68            |
| Primary branches per plant    | 2                  | 0.01             |
| Secondary branches per plant  | 5                  | 0.03             |
| Pods per plant                | 133                | 0.84             |
| Seeds per pod                 | 34                 | 0.22             |
| 100 seed weight (g)           | 615                | 3.9              |
| Yield per plot (g)            | 1106               | 7.02             |
| Grain iron content (ppm)      | 3                  | 0.02             |
| Grain zinc content (ppm)      | 25                 | 0.16             |

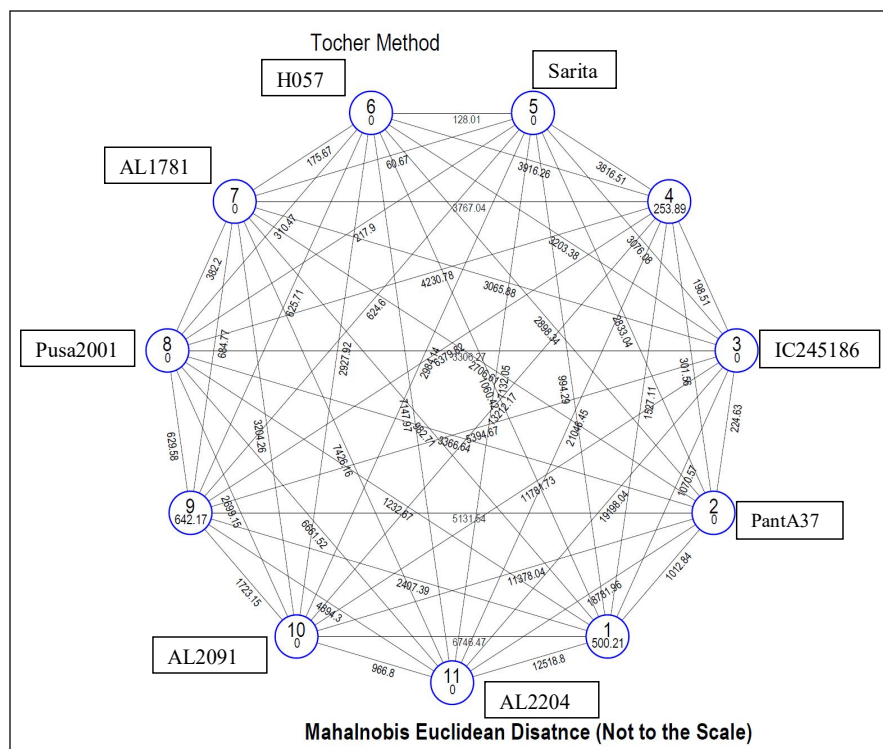


Fig 1: Cluster diagram showing euclidean<sup>2</sup> distance.

Table 4: Mean values of different clusters for various characters in 178 germplasm lines of pigeonpea.

| Characters                    | I      | II     | III    | IV     | V      | VI     | VII    | VIII   | IX     | X      | XI     |
|-------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Days to 50 per cent flowering | 102.46 | 108.50 | 109.50 | 102.04 | 88.50  | 97.00  | 97.00  | 97.00  | 101.90 | 87.00  | 79.00  |
| Days to maturity              | 123.60 | 105.00 | 134.00 | 124.17 | 118.50 | 111.00 | 111.50 | 140.50 | 121.60 | 122.50 | 130.50 |
| Plant height (cm)             | 209.70 | 235.50 | 237.50 | 243.67 | 183.50 | 182.50 | 184.50 | 180.50 | 166.50 | 129.50 | 99.50  |
| Primary branches per plant    | 13.20  | 12.00  | 13.00  | 13.46  | 17.00  | 8.50   | 13.00  | 13.00  | 12.35  | 13.00  | 9.00   |
| Secondary branches per plant  | 18.45  | 17.50  | 16.00  | 19.96  | 19.00  | 11.00  | 18.00  | 21.00  | 17.50  | 22.00  | 11.00  |
| Pods per plant                | 173.42 | 184.50 | 138.00 | 181.00 | 125.00 | 197.00 | 136.00 | 132.50 | 159.10 | 171.50 | 69.50  |
| Seeds per pod                 | 36.33  | 39.00  | 33.50  | 35.00  | 34.50  | 37.50  | 39.00  | 37.50  | 36.25  | 39.00  | 35.00  |
| 100 seed weight (g)           | 7.89   | 8.05   | 8.35   | 8.56   | 7.70   | 8.50   | 6.15   | 8.80   | 7.55   | 8.85   | 7.15   |
| Yield per plot (g)            | 264.25 | 212.50 | 225.00 | 294.25 | 252.50 | 285.00 | 232.50 | 177.50 | 239.25 | 220.00 | 117.50 |
| Grain iron content(ppm)       | 24.42  | 21.00  | 27.95  | 26.06  | 22.58  | 27.09  | 21.31  | 24.36  | 22.44  | 27.61  | 23.36  |
| Grain zinc content (ppm)      | 23.74  | 23.72  | 29.59  | 25.97  | 21.44  | 23.57  | 24.58  | 25.98  | 25.03  | 24.36  | 20.87  |

traits as they have contributed more towards genetic diversity. Similar results were also reported by Rao *et al.* (2013), Pushpavalli *et al.* (2018) and Qutadah *et al.* (2019).

#### Cluster means for different characters

The average cluster mean analysis of eleven quantitative traits revealed high magnitude of variation for all the characters among different clusters (Table 4). Cluster mean analysis indicated that cluster V (88.50), cluster X (87.00) and cluster XI (79.00) had lowest mean values and were comprised of early flowering genotypes. Cluster II (105.00) had lowest mean value indicating early maturing genotypes followed by cluster VI (111.00) and cluster VII (111.50). The lowest mean value for plant height was recorded in cluster XI (99.50) whereas highest value in cluster IV (243.67). The

highest cluster mean for pods per plant was found in cluster VI (197.00) followed by cluster II (184.50). Cluster IV had highest mean value (294.25) for yield per plot followed by cluster VI (284.00). The highest cluster mean for 100-seed weight was recorded in cluster X (8.85g) followed by cluster VIII (8.80g). For number of branches cluster V, cluster VIII and cluster X were observed to be superior with high cluster mean values. Grain iron content had highest cluster mean value in cluster III (27.95 ppm), followed by cluster X (27.61 ppm) and cluster VI (27.09 ppm). The highest value for grain zinc content was recorded in cluster III (29.59 ppm) followed by cluster VIII (25.98 ppm) and cluster IV (25.97 ppm). It was clearly indicated that cluster III had genotypes containing higher iron and zinc content therefore these genotypes can be used for enhancement of iron and zinc

simultaneously in pigeonpea breeding programme. The results in the present investigation revealed that all desirable traits cannot be selected from single cluster. Hence, ideal cross combination should be conducted to get improvement for target traits effectively. Our results were in agreement with the results of Muniswamy *et al.* (2014), Chaudhary *et al.* (2016) and Shruthi *et al.* (2020). Several researchers viz., Mohan *et al.* (2021), Remzeena *et al.* (2021) and Basavaraja *et al.* (2021) in other legume crops also gave emphasis on need of high genetic diversity to create the high genetic variation and their results were in conformity with our findings.

## CONCLUSION

Mahalanobis's  $D^2$  is statistical tool which helped to reveal genetic divergence in 178 pigeonpea genotypes and these were grouped into eleven clusters by Tocher's method. On the basis of divergence values, genotypes from different clusters can be selected for further breeding programme. Germplasm lines which fell into solitary clusters viz., PantA37, IC245186, Sarita, H057, AL1781, PUSA2001, AL2091 and AL2204 often had some distinct characters which make them more divergent from rest of the lines. From the results it can be concluded that genotypes from cluster II and cluster XI can be used for crossing and expected to generate huge amount of genetic variability with combination of traits for early flowering and maturity, short stature and high grain yield may be achieved. For combining high iron and high zinc content, genotypes for high iron content and high zinc content from cluster III should be used for hybridization. Genotypes in clusters with greater genetic distance will be more diverse and will give better transgressive segregants than genotypes in clusters with moderate genetic distances. However, genotypes with any desirable trait in clusters separated by moderate genetic distance can also be used in crossing programme. The desirable genotypes from corresponding clusters could be further evaluated for selecting high yielding and early maturing genotypes for practicing mass selection and pedigree selection.

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**Conflict of interest:** None.

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