



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 2910-2915
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www.thepharmajournal.com

Received: 09-11-2022

Accepted: 21-12-2022

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Genetic divergence studies and Clustering by Torcher's Method for yield and quality traits in Pigeonpea [*Cajanus cajan* (L.) Mill sp.]

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Abstract

Background: Protein requirement is essential for human growth and development and pulses play a dynamic role in providing protein fulfillment in the human diet. In pulses, Pigeonpea is the second most important crop. It has been recommended for a balanced diet with cereals, especially to fill the nutritional gap for proteins. This crop also possesses medicinal properties and is being used in traditional ways as a medicine. Pigeon pea has diversified uses such as food, feed, fodder, and fuel wood. Also, act as a check for soil erosion and maintain soil fertility. It is a hard, widely adapted, and drought-tolerant crop.

Method: The experiment was conducted on 30 pigeonpea genotypes in the central plain agro-climatic region of Uttar Pradesh in RBD with three replications. Twelve different traits were included in the study. Observed data were analyzed using SPAR 3.0 (Indian Agricultural Statistics research institute ICAR- IASRI).

Result: Based on D^2 statistics, six clusters were formed from 30 genotypes by 'Tocher's method' and the clustering pattern indicated that genotypes were clustered irrespective of their eco-geographical regions. The generalized Inter-cluster distances in the present investigation ranged from 1640.023 - 11271.322 and the Intra-cluster D^2 values showed noticeable differences ranging from 0 to 922.520. Cluster Ist was the largest comprising 18 genotypes, followed by cluster IInd which accommodate 6 genotypes. Clusters IIIrd and IVth comprised 2 genotypes each. Clusters i.e., Vth and VIth out of six clusters were mono genotypic as they accommodate single genotypes. Superior genotypes selected based on Inter-cluster distance and cluster means could be utilized in the exploitation of heterosis and for the hybridization program.

Keywords: Genetic divergence, D^2 Analysis, Torcher's Method

Introduction

Pulses are a rich source of inexpensive plant-based proteins (20-25%), vitamins, minerals, and dietary fibers, besides having low-fat content, zero cholesterol, and gluten and consequently have got a significant place in the human meal where a vegetarian diet is predominant (Anonymous, 2018). Pigeonpea [*Cajanus cajan* (L.) Millsp.], ($2n = 2x = 22$) occupies second place after chickpea in India and has been rated the best regarding its biological value.

Pigeonpea [*Cajanus cajan* (L.) Millsp.] Is the sixth in global legume production and worldwide it is cultivated in about 7 Mha with production and productivity of 6.8 MT and 969 kg ha⁻¹ respectively (FAOSTAT, 2019). India ranks first and accounts for 72% of the world's area grown to pigeon pea or 5.58 million hectares area with an annual production of 3.6 million tonnes followed by Malawi (0.4 mt) and Myanmar (0.3 mt). (Source FAOSTAT 28. Dec. 2021).

In India, Maharashtra has the highest area 1.22 million ha with a production of 1.05 million tons followed by M.P, Karnataka, Gujrat, U.P. with the highest ever Productivity level of 937 kg/ha was achieved during 2017-2018. Uttar Pradesh has a total area of 0.28 million ha, with an annual production of 0.303 million tons of pigeon pea i.e., a contribution of 7.25% towards total national production (Source: Ministry of Agri. & FW, Govt. of India; 2018 3rd Advance Estimate).

The selection of diverse parents of a particular crop provides information about the nature and magnitude of genetic diversity existing in the available germplasm. Obtaining a wide spectrum of gene recombination for quantitatively inherited traits and desirable segregants in segregating generations crossing between divergent parents provides a greater possibility. Therefore, genetically diverse parents are preferred for use in hybridization programs.

It has been repeatedly emphasized to recover transgressive segregants in crops including pigeonpea for recombination breeding and hence genetic diversity for selecting parents is required.

Hence, based on sound statistical procedures, such as D² cluster analysis the characterization of genetic divergence for

the selection of diverse and suitable genotypes should be done. Keeping this visible, an experiment was done for selecting diverse parents for the hybridization program focused on studying genetic diversity and isolating desirable important characteristics in pigeonpea primarily seed yield.

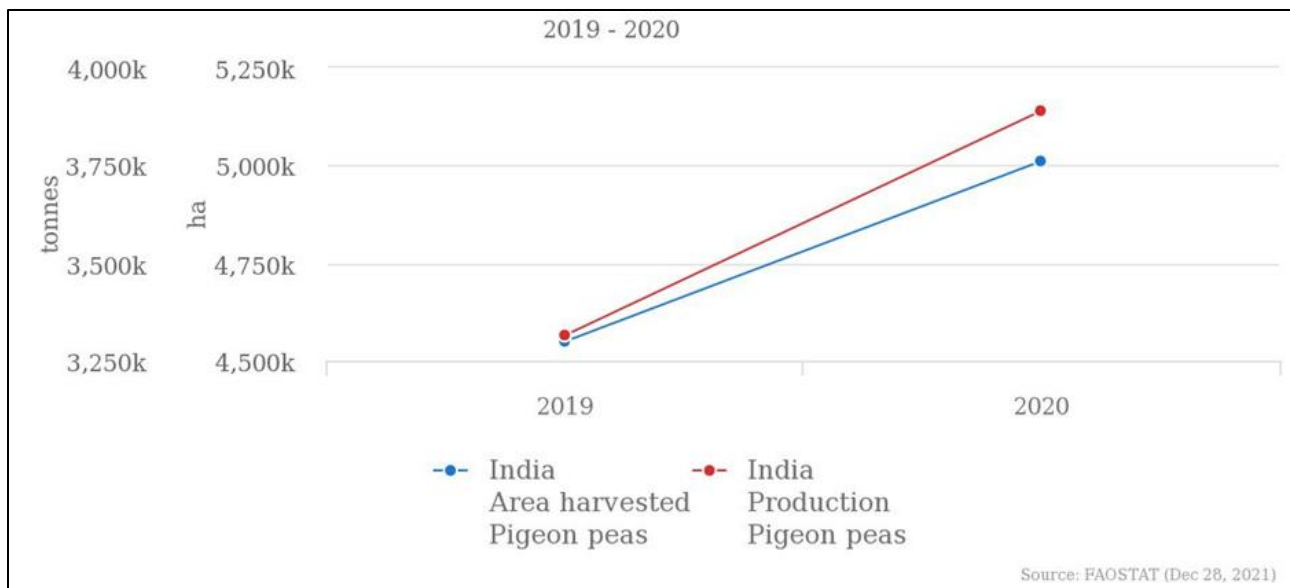


Fig 1: Production/yield quantities of pigeon peas in India

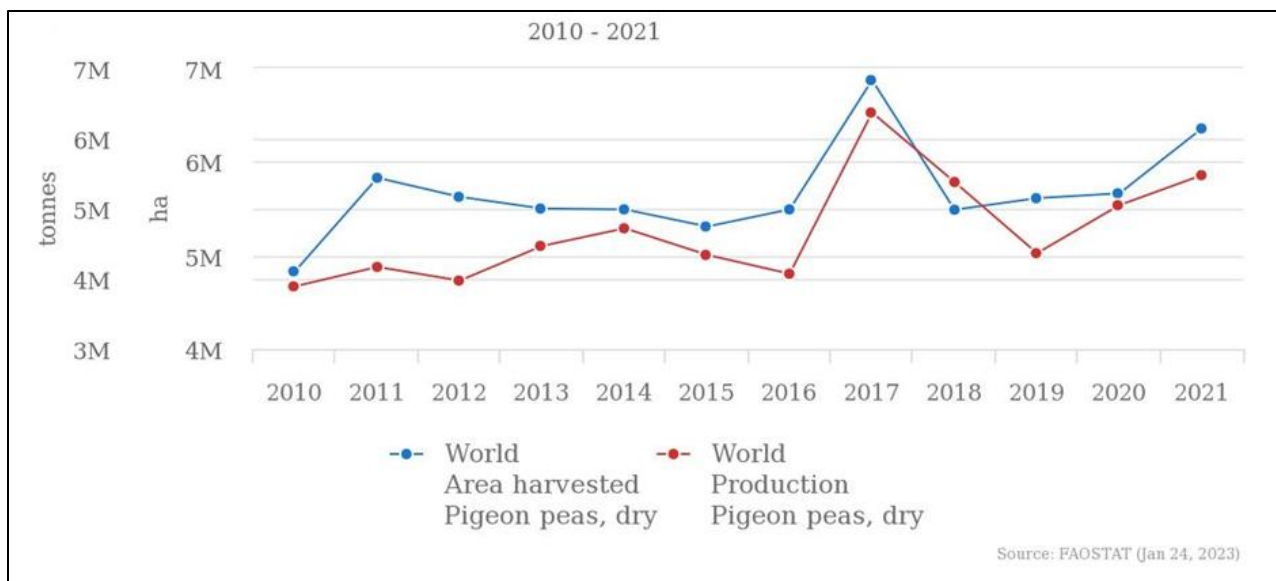


Fig 2: Production/yield quantities of Pigeon peas, dry in World + (Total)

Material and Methods

Experimental Details

The experimental material consisting of 30 genotypes was obtained from the germplasm maintained by Pigeonpea Breeder at the Legume Section of C.S.A., Department of Genetics and Plant Breeding, C.S.A University, Kanpur. The investigation was conducted at Crop Experimental Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, situated at NH 91, Vinayakpur, kalyanpur, Kanpur (26.4950° N lat., 80.2678° E long.) during Kharif season 2019-20 in a randomized block design (R.B.D.) with three replications. Each genotype was raised in a single row of 4m in length and maintained a row-to-row distance of 75

cm and plant-to-plant distance of 25 cm respectively. Standard recommended agronomic practices were followed to raise a good crop.

Collection of data

A number of 5 plants were selected at random from each strain per replication in respect of the characters and Observations were recorded on days to 50% flowering (DF), days to maturity (DM), plant height in cm (PH), number of primary branches (NPBR), number of secondary branches (NSBR), number of pods plant⁻¹ (NPP), pod length in cm (PL), number of seeds pod⁻¹ (NSPP), 100-seed weight in g (HSW), seed yield plant⁻¹ in g (YPP), biological yielding

(BY) and harvest index (HI). Days to 50% flowering, days to maturity, 100-seed weight, and harvest index were recorded on a plot basis. The harvest index was determined as seed yield over biological yield.

Statistical Analysis

The mean values were subjected to statistical analysis. The multivariate analysis of genetic divergence using Mahalanobis's D^2 statistics was first suggested by C.R. Rao (1952).

All the data were analyzed using SPAR 3.0 (*Indian Agricultural Statistics research institute ICAR- IASRI*).

Group Constellation of genotypes based on D^2 values

The genotypes were grouped using Tocher's method (Rao, 1952).

The average intra-cluster was calculated by using the formula:

$$\text{Intra-cluster Distance} = \frac{\sum Di^2}{n}$$

$\sum Di^2$ = Sum of the distance between all possible combinations of the genotypes included in the cluster. N = Number of genotypes in a cluster.

The inter-cluster distance was calculated by measuring the distance between clusters I and II between clusters I and III, between clusters II and III, and so on. Likewise, one by one cluster were taken and their average Intra and inter-cluster D^2 values were used to obtain mutual relationships among clusters.

Average intra and inter-cluster

The square root of average D^2 values was worked out to calculate the average intra and inter-cluster D values. Average intra and inter-cluster are given as under $D = \sqrt{D^2}$ value.

Cluster Mean

The cluster means for a particular trait were calculated by dividing the summation mean values of the strains included in

a cluster by the number of strains in the cluster.

Cluster Diagram

With the help of D^2 values between and within the cluster, a cluster diagram has been drawn that showed the relationship between the different populations.

Result and Discussion

Genetic Divergence: Genetic divergence in a population is always given considerable importance for using the genotypes for a breeding program. It is believed that genetically diverse parents are likely to produce high heterotic effects. Distantly related parents within the same species when utilized in a crossing program are likely to produce a wider spectrum of variability.

The generalized distance between the two populations observed in the present investigation varied from (1640.023 - 11271.322) which indicated that a large amount of diversity was present in the material studied.

Mahalanobis's D^2 values: The mean value of 30 genotypes was transformed into standardized and correlated mean values using the pivot condensation method ($Y_1 - Y_2$). The D^2 values were computed for all the possible pairs of 30 genotypes of genotypes.

$$\frac{30(30 - 1)}{2} = 435 \text{ pairs}$$

Cluster composition

All 30 genotypes were grouped into six clusters by using 'Tocher's method' and the distribution of 30 genotypes in six clusters was presented in (Table 1). Cluster 1st was the largest cluster comprising 18 genotypes followed by cluster 2nd which accommodate 6 genotypes. Clusters 3rd and 4th consist of 2 genotypes each. Two (Cluster 5th and 6th) out of six clusters were mono-genotypic as they accommodate a single genotype. The mean values of clusters are given in Table 3.

Table 1: Grouping of 30 Pigeonpea genotypes into six clusters (By Tocher's method)

Clusters	Number of Genotypes included	Name of Genotypes
I	18	KA-17-3, KA-17-4, KA-12-2, KA-12-1, KA-17-2, KA-16-5, KA-16-1, KA-17-1, KAWR-1, KA-12-3, NDA-1, NDA-2, MA-6, KAWR-2, AZAD, IPA 6-F, IPA-234, DA-11.
II	6	IPA 8-F, MARUTHI (ICPL-8863), MAL-13, ASHA (ICPL-87119), ICPL-13174, BAHAR.
III	2	IPA 16-F, IPA-203.
IV	2	TYPE-17, TYPE-21.
V	1	ICPL 1155-2.
VI	1	AMAR.

Intra and inter-cluster distance: The intra and inter-cluster D^2 and D values among 6 clusters are furnished and illustrated in Table 2 (a) (b). Intra cluster average D^2 values ranged from 0 to 922.520. Among the clusters, cluster IInd had maximum intra-cluster distance (922.520) and the minimum was recorded for cluster IIIrd (347.276), while intra-cluster D^2 value was zero for two clusters viz; cluster Vth and VIth as they comprised single genotype each. The minimum inter-cluster D^2 value was observed between cluster IInd and Vth (1640.023) while the maximum D^2 value was found between cluster IIIrd and Vth (11271.322) followed by cluster Vth and

VIth (10573.021); cluster IInd and IIIrd (9655.162); cluster IInd and VIth (7391.438).

Cluster I: Cluster Ist had the largest distance from cluster IIIrd ($D^2 = 4461.405$), followed by cluster Vth ($D^2 = 4079.236$), cluster IVth ($D^2 = 2981.065$), cluster VIth ($D^2 = 2909.516$) and cluster IInd ($D^2 = 2315.610$).

Cluster II: The distance between cluster IInd and cluster IIIrd was highest ($D^2 = 9655.162$), followed by cluster VIth ($D^2 = 7391.438$), and cluster IVth ($D^2 = 3651.470$). Cluster IInd was

closer to cluster Vth ($D^2=1640.023$).

Cluster III: The highest distance was observed between cluster IIIrd and cluster Vth ($D^2 = 11271.322$), followed by cluster IVth ($D^2 = 6487.837$).

Cluster-IV: The distance between cluster IVth cluster VIth ($D^2 = 5357.761$) followed by cluster Vth ($D^2 = 3508.996$).

Cluster-V: The highest distance was observed between cluster Vth and cluster VIth ($D^2 =10573.021$).

Cluster Mean: Cluster means for 12 characters are presented in Table 3. It revealed a wide range of variability for most of the characters.

Clustering pattern and cluster mean

On the basis of D^2 statistics, six clusters were formed from 30 genotypes by ‘Tocher’s method’ and the clustering pattern indicated that genotypes were clustered irrespective of their eco-geographical regions i.e., genotypes of the different geographical regions were also included in the same cluster and those of same region were included in different clusters. This reflects that there was no relationship between eco-geographical differences and genetic diversity. Similar conclusions were also derived by Sawant *et al.* (2009) [13].

In the present study, Cluster Ist was the largest comprising 18 genotypes, followed by cluster IInd which accommodates 6 genotypes. Clusters IIIrd and IVth comprised 2 genotypes each. Clusters i.e., Vth and VIth out of six clusters were mono genotypic as they accommodate single genotypes.

The intra-cluster D^2 values showed noticeable differences ranging from 0 to 922.520. The D^2 value for characters studied showed significant differences indicating the presence of substantial genetic diversity even within a cluster. The minimum intra-cluster value was recorded for cluster IIIrd (347.2764). Suggesting that genotypes within this cluster exhibited a close relationship. These results were similar to the earlier findings of Singh *et al.* (2010) [14] and Kumar *et al.* (2019) [2].

Inter-cluster distances ranged from (1640.023 - 11271.322) with a maximum inter-cluster distance between clusters IIIrd and Vth, followed by clusters Vth and VIth. In the present study, selecting genotypes from the different clusters as from

clusters IIIrd and Vth may create a wide spectrum of variability and also provide high heterotic for yield advantage. The results are in agreement with earlier findings of Patel and Acharya (2011) [11]; Pandey *et al.* (2013) [10]; Pushpavalli *et al.* (2018) [12] and H.B. Shruthi *et al.* (2020) [6].

Inter-cluster distance is the main criterion for the selection of genotypes using D^2 analysis. Genotypes belonging to the cluster with maximum distance are genetically more divergent and hybridization between genotypes of the divergent cluster is likely to produce a wider range of variability with desirable segregants (Gartan *et al.* 1989) [4].

The highest cluster mean values for 50% flowering, were recorded by cluster Ist. The highest mean value for days to maturity, seed yield per plant, and biological yield was recorded by cluster VIth. The highest mean values for the number of secondary branches, pod length, and 100-seed weight were recorded by cluster IIIrd. The highest mean values for the number of seeds per pod were recorded by cluster IInd. The highest mean values for the number of primary branches and harvest index were recorded by cluster Vth. The highest mean value for plant height, the number of pods per plant was recorded by cluster IVth. Intercrossing of genotypes among these clusters may produce variability for the respective characters (Gohil, 2006). Refer to Table 2.

The clustering pattern could be utilized to identify the best cross-combination for generating variability regarding various traits.

In the present study, the genotypes clubbed in cluster IIIrd may be intercrossed with the genotypes placed in cluster Vth for generating wide variability and consequently transgressive segregants may be achieved in advanced generations.

Further, on the basis of cluster mean performance it is discussed here that genotypes clustered in IInd, IVth, and VIth may be inter-crossed for creating maximum variability for yield contributing traits.

Cluster mean performances of cluster Ist are near about highest mean value for the number of secondary branches and the number of seeds per pod. So, genotypes of this cluster can also be utilized for inter-crossing with other clusters to make the hybridization program more effective and generate maximum variability. Thus, the hybridization program could utilize superior genotypes selected on the basis of inter-cluster distance and cluster means.

Table 2 (a): Average intra-cluster (diagonal) and inter-cluster distance (D^2) of 30 Pigeonpea genotypes among six clusters (By Tocher’s method).

Clusters	I	II	III	IV	V	VI
I	718.440	2315.610	4461.405	2981.065	4079.236	2909.516
II		922.520	9655.162	3651.470	1640.023	7391.438
III			347.276	6487.837	11271.322	3152.648
IV				416.502	3508.996	5357.761
V					0.000	10573.021
VI						0.000

Table 2 (b): Average intra-cluster (diagonal) and inter-cluster distance (D) of 30 Pigeonpea genotypes among six clusters (by Tocher’s method) $D=\sqrt{D^2}$

Clusters	I	II	III	IV	V	VI
I	26.80	48.12	66.79	54.59	63.86	53.94
II		30.37	98.26	60.42	40.49	85.97
III			18.63	80.54	106.17	56.14
IV				20.40	59.24	73.19
V					0.000	102.82
VI						0.000

Table 3: Cluster means performance for 12 quantitative characters of 30 pigeonpea genotypes

Characters Clusters	Days to 50% flowering	Days to Maturity	Plant Height (cm)	No. of Primary Branches	No. of Secondary Branches	No. of Pods/Plant	Pod Length (cm)	No. of Seeds/Pod	100-Seed Weight (g)	Seed Yield/Plant (g)	Biological Yield (g)	Harvest Index (%)
I.	128.148	244.666	170.988	11.975	23.574	157.481	5.033	3.259	10.380	22.844	126.685	18.004
II.	118.444	228.555	143.300	12.433	22.300	97.166	5.088	3.344	11.222	17.300	88.394	19.402
III.	122.333	235.833	185.566	14.866	27.000	175.500	5.700	2.700	13.400	28.800	186.900	15.371
IV.	91.500	183.500	190.433	7.866	21.766	200.000	5.150	3.166	8.633	18.016	107.333	16.765
V.	106.000	216.000	139.933	16.266	24.600	154.000	4.133	2.733	8.066	16.300	75.866	21.476
VI.	123.000	245.000	172.466	15.800	26.600	199.000	5.133	2.866	11.600	42.700	203.900	20.936

Table 4: Percentage contribution of each character towards total genetic divergence in 30 pigeonpea genotypes

S. No.	Source	Time Ranked 1 st	Contribution (%)
1.	Days to 50% flowering	14	3.21
2.	Days to Maturity	65	14.94
3.	Plant Height (cm)	1	0.22
4.	No. of Primary Branches	0	0.01
5.	No. of Secondary Branches	6	1.37
6.	No. of Pods/Plant	40	9.19
7.	Pod Length (cm)	0	0.01
8.	No. of Seeds/Pod	0	0.01
9.	100-Seed Weight (gm)	5	1.14
10.	Seed Yield / Plant (gm)	18	4.13
11.	Biological Yield (g)	260	59.77
12.	Harvest Index (%)	26	5.97

The relative contribution of characters towards diversity

The relative contribution of the characters toward genetic diversity has been depicted in *Table 4*. The greater number of times that every 12 characters appeared in 1st rank the more it contributed toward the diversity. Among all the characters, biological yield contributed the maximum (59.77%) to the diversity followed by days to maturity (14.94%) and number of pods per plant (9.19%) together contributing 24.13% of total divergence. The character Harvest index, seed yield/plant, days to 50% flowering, number of secondary branches, 100-seed weight, plant height, number of primary branches, pod length, and number of seeds per pod contributed 5.97%, 4.13%, 3.21%, 1.37%, 1.14%, 0.22%, 0.01%, 0.01%, 0.01% respectively towards total diversity in decreasing order.

Conclusion

On the basis of D² statistics, all 30 genotypes were grouped into six clusters by using 'Tocher's method'. Cluster Ist was the largest comprising 18 genotypes, followed by cluster IInd which accommodate 6 genotypes. Clusters IIIrd and IVth comprised 2 genotypes each. Clusters i.e., Vth and VIth out of six clusters were mono genotypic as they accommodate single genotypes. Maximum inter-cluster distance between cluster IIIrd and Vth, followed by cluster Vth and VIth.

Selecting genotypes from clusters IIIrd and Vth may be intercrossed for creating a wide spectrum of variability, which may provide an opportunity for selecting high-yielding genotypes in an advanced generation. These clusters also reflect that there was no relationship between eco-geographical differences and genetic diversity. The variability among the genotypes will also help to select the parents for hybridization.

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