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Assessment of Morphological and Molecular Genetic Diversity in Pigeonpea [*Cajanus cajan* (L.) Millspaugh]

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ABSTRACT

Background: The estimation of genetic diversity in pigeonpea is crucial for desiging breeding programmes and germplasm conservation. Morphological studies alone do not provide sufficient information to understand genetic diversity. Molecular analysis using SSRs can provide additional information on genetic diversity that can be used for selection of diverse parents.

Methods: The experimental material for present study consisted of 50 diverse genotypes of pigeonpea. The fifty genotypes were planted in Randomized Complete Block Design consisted of three replications during the *kharif* 2020-21. The estimation of morphological genetic diversity was done by Mahalanobis D^2 statistics. The molecular genetic diversity analysis was done by using 30 molecular markers in same genotypes.

Result: The assessment of morphological diversity revealed that the fifty pigeonpea genotypes were grouped into four different clusters with cluster I as the largest cluster (33 genotypes). The molecular markers differentiated the 50 genotypes in five major clusters with cluster II as the largest cluster (24 genotypes). The results of present study suggested that morphological and molecular diversity in pigeonpea is different. On the basis of genetic distance, the genotypes RVSA 2014-1 and PA 406 were found most genetically distant and may be used in hybridization programme to create diverse progenies.

Key words: Genetic diversity, Molecular, Morphological, Pigeonpea.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a popular *kharif* pulse of India, as this crop is highly suitable for subsistence agriculture. Globally, India is the largest producer and consumer of pigeonpea with an area of 4.4 m ha, annual production of 3.68 mt and productivity of 832 kg/ha (Anonymous, 2019). The development of improved cultivars with the desirable traits largely depends upon the availability of adequate genetic variation. The study of literature suggested that cultivated pigeonpea varieties exhibit relatively low levels of genetic diversity (Yohane *et al.,* 2020). The loss of genetic diversity may be due to continuous artificial selection and breeding for a few targeted economic traits to meet the market requirements. Hence, in order to broaden the genetic base of its cultivated varieties there is an urgent need to assess the genetic diversity of pigeonpea.

The assessment of genetic diversity in pigeonpea is crucial for effective breeding and germplasm conservation. The initial studies of genetic diversity in pigeonpea were mainly based on morphological traits (Verma *et al.,* 2018). Morphological studies alone do not provide sufficient information to understand genetic diversity within the species as well as its relatedness to other species. Molecular analysis using SSRs can provide additional information on genetic diversity that would be useful for breeding programs through selection of diverse parents. Recently DNA markers have been extensively used for study of genetic diversity since they are readily available and are not influenced by the environment (Saxena *et al.,* 2010). Cultivated pigeonpea are known to have low polymorphism and hence SSR markers are ideal for studying the genetic diversity. Therefore, the aim of present study was to assess the genetic diversity among 50 pigeonpea varieties/advance breeding lines on the basis of morphological traits and SSR markers.

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MATERIALS AND METHODS

Estimation of morphological diversity

The experimental material for present study consisted of 50 diverse genotypes of pigeonpea (Table 1). The fifty genotypes were planted in Randomized Complete Block

Design consisted of three replications. The experimental material was grown during the *kharif* season of year 2020-21 at N.E.B.C.R.C., G.B.P.U.A.T, Pantnagar, Uttarakhand. Observations were recorded on five randomly selected competitive plants from each genotype in each replication for the seven different morphological characters *viz.,* plant height (cm), number of primary branches/plant, number of secondary branches/plant, number of pods/plant, number of seeds/pod, 100 seed weight (g) and seed yield/plant (g) while for days to 50 per cent flowering and days to maturity observations were recorded on whole plot basis. The data recorded for various yield and attributing traits was subjected to the estimation of genetic diversity using the Mahalanobis D^2 statistics (Mahalanobis, 1928). The clusters were prepared by following the Tocher's method as suggested by Rao (1952).

Estimation of molecular diversity

The molecular diversity was estimated by using 30 molecular markers. Molecular diversity experiment was conducted in Pulse Breeding Laboratory, Department of Genetics and Plant Breeding, GBPUAT, Pantnagar during 2020-21. The genomic DNA from leaves was extracted by using the Cetyl tri-methyl ammonium bromide (CTAB) method of Doyle and Doyle (1987) with slight modifications. A single PCR reaction contains a total volume of 10.0 µl and consisted of 1.0 µl DNA template (100 ng/ µl), 1.5 µl Taq buffer (10X) with 15mM $MgCl_2$, 0.3 µl dNTPs 10 mM, 0.2 µl Taq polymerase 3U/µl and 1.0 µl primers (50 ng) and 6 µl part ddH_2O was used for each PCR reaction. The binary data were used to compute pair wise similarity coefficients (Jaccard, 1908). The similarity matrix thus obtained was subjected to hierarchical cluster analysis using the Unweighted Pair Group Method with Arithmetic average (UPGMA) algorithm using NTSYS-pc software (Rohlf, 1988).

RESULTS AND DISCUSSION

Genetic diversity at morphological level by using D^2 statistics

The major goal of any plant breeding programme is the generation and exploitation of genetic variability for crop improvement. The study of ANOVA indicated that the mean sum of squares due to genotypes were highly significant for all the characters except number of seeds per pods (Table 2). These results indicated the presence of sufficient genetic variability for most of the characters in the experimental material. The existence of significant genetic variability of these different traits in pigeonpea was also reported earlier by Meena *et al.* (2017), Pal *et al.* (2018) and Gaur *et al.* (2020). The present findings proved the suitability of the experimental materials chosen for the present investigation. The assessment of genetic diversity by using the D^2 statistics revealed that the fifty pigeonpea genotypes were grouped

Table 1: Pigeonpea genotypes used in present study along with their pedigree information.

Genotype	Pedigree	Genotype	Pedigree
AL 1932	AL 201× *C. acutifolius*	PA 429	UPAS 120 × AL 201
AL 1758	AL 201 × early local collection	PA 439	UPAS 120 × PUSA 971
AL 2046	AL 1455 × P 2003-2	PA 440	PUSA 992 × UPAS 120
CORG 99-4	-	PA 441	UPAS 120 × H 82-1
CORG 2012-20	Vamban 3 × H 2001-4	PA 443	UPAS 120 × ICPL 88039
ICPL 98-15	-	PA 444	UPAS 120 × H 82-1
ICPL 86005	-	PA 449	UPAS 120 × H 82-1
ICPL 87115	-	PA 452	UPAS 120 × PUSA 971
ICPL 88039	-	PA 454	UPAS 120 × ICPL 88039
ICPL 91031	-	PA 455	UPAS 120 × PUSA 991
ICPL 98009	-	PA 469	H 82-1 × UPAS 120
ICPL 98024	-	PA 473	H 82-1 × UPAS 120
IPA 94-4	-	PA 475	H 82-1 × UPAS 120
PAU 881	H 89-5 × ICPL 85024	PA 476	H 82-1 × UPAS 120
PA 3	Selection from UPAS 120	PA 477	H 82-1 × UPAS 120
PA 111	UPAS 120 × KPBR 80-2-1	PA 483	H 82-1 × UPAS 120
PA 288	UPAS 120 × ICPL 88039	PA 492	PUSA 992 × UPAS 120
PA 291	UPAS 120 × KPBR 80-2-1	PA 493	PUSA 992 × UPAS 120
PA 435	UPAS 120 × PUSA 855	PUSA 2003-1	-
PA 337	UPAS 120 × ICPL 88039	PUSA 2015-1	Pusa dwarf × H 2001-4
PA 374	UPAS 120 × PA 291	RGT-1	Allapur local × BSMR 853
PA 406	AL 201 × UPAS 120	RVSA 2014-1	ICP 88039 × 88022
PA 415	Pusa 992 × AL 201	RVSA 2014-2	ICP 88039 × ICPL 149
PA 421	Pusa 992 × ICPL 88039	RKPV 310-03	BDN 2 × ICP 7035
PA 426	UPAS 120 ×AL 201	SJP 102	ASJ 105 × ICPL 88039

into four different clusters (Table 3). The discrimination of genotypes into discrete clusters suggested presence of high degree of genetic diversity in the material evaluated. Earlier workers have also reported substantial genetic divergence in the pigeonpea materials (Pushpavalli *et al.,* 2017 and Verma *et al.,* 2018). Presence of substantial genetic diversity among the experimental material in the present study indicated that this material may serve as a good source for selecting the diverse parents for hybridization programme aimed at isolating desirable segregants for seed yield and other important characters. The cluster I (33 genotypes) was largest cluster followed by cluster II (15 genotypes) while cluster III (RVSA 2014-1) and cluster IV (PA 406) each contained one genotype, respectively. The inter-cluster distance ranged from 109.86 between cluster II and cluster IV to 1017.47 between cluster III and cluster IV (Table 4). In the present study, highest inter cluster distance was recorded between cluster III and IV (1017.47) and minimum between cluster I and III (100.96). The high inter cluster distance as compared to intra cluster distance suggested the presence of sufficient amount of genetic diversity among genotypes under study. The high magnitude of inter cluster distance as compared to intra cluster distance was also reported earlier by Pushpavalli *et al.,* 2017. These results indicated that if hybridization is attempted between the genotypes RVSA 2014-1 included in the cluster III and PA 406 in cluster IV, lot of genetic diversity will be produced in the segregating generations and the selection for desirable genotypes can be practiced.

Cluster means of different characters and their per cent contribution

The cluster mean for days to 50% flowering ranged from 74.67 to 98.33 days while days to maturity ranged from 126.67 to 154.67 days (Table 5). The cluster IV was found to be the earliest flowering cluster (flowering= 74.67 days; maturity=126.67 days). These results indicated that the genotype present in cluster IV can be used as donors for earliness in pigeonpea breeding programme. The cluster mean for plant height ranged from 259.33 to 300.00 cm. The cluster III was found to have the highest height (300.00 cm). The more plant height is a desirable character in pigeonpea as it results in more biomass and ultimately in more yield and hence the cluster III can be used as donors for more plant height in pigeonpea. The cluster mean for number of primary branches per plant ranged from10.00 to 15.42 while for secondary branches per plant ranged from 10.67 to 23.33. The cluster II was found to have the maximum primary branches (15.42) while cluster IV was found to possess the maximum number of secondary branches (23.33). The genotypes included in these clusters can be used as donors for more number of primary and secondary branches in pigeonpea. In case of pods per plants, the cluster IV (390.00) was found to have the highest number of pods and as more number of pods is directly related to high yield in pigeonpea and, hence, the genotype PA 406 present in cluster IV can be used as donor for more pods in pigeonpea. The cluster mean for number of seeds per pod ranged from 4.00 to 4.09. The cluster II was found to have the highest seeds/pod (4.09). The cluster mean for 100-seed weight ranged from 8.20 to 8.57g. The cluster IV was found to have the highest 100-seed weight (8.57g) and hence the genotype included in cluster IV can be used as donors for higher 100-seed weight in pigeonpea. The cluster mean for seed yield ranged from 38.33 to 76.60 g. The cluster IV (76.60 g) was found to have the highest yield followed by cluster I (75.31g), Cluster II (75.17g) and Cluster III (38.33g). Thus the high yielding genotype PA 406 in cluster IV can be used as donors for higher seed yield in pigeonpea. The present results indicated that the cluster IV was the most desirable cluster as it was the earliest maturing cluster along with highest number of secondary branches per plant, number of pods per pant, 100- seed weight and seed yield per plant and hence the genotype included in cluster IV *i.e.* PA 406 can be used as parent in pigeonpea improvement programme. The contribution of different characters towards the divergence is presented in Table 6**.** The character days to maturity (52.24%) showed maximum contribution followed by number of pods/ plant (17.96%), days to 50% flowering (14.37%), seed yield /plant (10.20%), 100-seed weight (2.45%), plant height (1.80%), number of secondary branches (0.57%), number of seed /pod (0.24%) and number of primary

Table 2: Analysis of variance for nine different characters for fifty pigeonpea genotypes.

Characters	Mean sum of squares		
	Replication (2)	Treatment (49)	Error (98)
Days to 50% flowering	0.45	105.77**	0.50
Days to maturity	5.36**	130.29**	0.71
Plant height (cm)	1752.32	1554.91**	422.73
Number of primary branches / plant	11.33	52.65**	29.79
Number of secondary branches/ plant	978.98**	229.52**	94.80
Number of pods/ plant	939.92*	9967.47**	280.40
Number of seeds/ pod	0.06	0.471	0.72
100 - seed weight (g)	0.04	0.74**	0.17
Seed yield/ plant (g)	45.19	1038.15**	50.88

Values in parenthesis indicates degree of freedom; *, ** refers to significance at 5 and 1% level of probability respectively.

Table 3: Comparison of clustering pattern on basis of morphological and molecular diversity.

Morphological diversity			Molecular diversity		
Clusters	Number of genotypes	Genotypes	Clusters	Number of genotypes	Genotypes
I	33	ICPL 91031, CORG 99-4, IPA 94-4, ICPL 98-15, ICPL 98009, PUSA 2003-1, RGT 1, PA 111, ICPL 87115, ICPL 86005, ICPL 98024, PA 337, PA 415, PA 444, PA 449, PA 441, PA 452, RKPV 310-03, AL 1758, RVSA 2014-2, PA 455, PUSA 2015-1, PA 3, CRG 2012-20, PA 493, SJP 102, PA 483, PA 469, AL 2046, AL 1932, PAU 881, PA 476 and ICPL 88039	I	12	ICPL 91031, PA 291, PA 429, PA 439, CORG 2012-20, AL 1932, PA 469, SJP 102, PA 406, PUSA 2003-1, PA 443 and PA 444
II	15	PA 288, PA 492, PA 374, PA 421, PA 291, PA 426, PA 429, PA 439, PA 443, PA 440, PA 435, PA 454, PA 475, PA 477 and PA 473	II	24	CORG 99-4, ICPL 86005, PA 454, PA 493, PA 477, PA 455, RVSA 2014-2, PA 475, PA 441, AL 2046, RKPV 310-03, PA 483, RVSA 2014-1, AL 1758, PA 473, PA 476, ICPL 88039, PAU 881, PA 337, PA 440, PA 452, PA 426, PA 435 and PA 449
III	1	RVSA 2014-1	III	5	IPA 94-4, ICPL 98009, PA 374, ICPL 98-15 and RGT 1
IV	1	PA 406	IV	8	PA 111, ICPL 87115, ICPL 98024, PA 288, PA 3, PA 492, PA 415 and PUSA 2015-1
			V	1	PA 421

Table 4: Inter and Intra cluster distances among different clusters.

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	28.01	213.07	100.96	568.57
Cluster II		22.34	496.64	109.86
Cluster III			0.00	1017.47
Cluster IV				0.00

branches (0.16%). Thus, the characters days to maturity, number of pods/ plant, days to 50 % flowering and seed yield /plant were identified as major contributing characters towards the genetic divergence.

Genetic diversity at molecular level

The major drawback of morphological markers is that they are influenced by environment and fail to give true and accurate results. This problem can be easily addressed by using molecular markers as these markers bypass the problems related to environment effects. Molecular markers can be effectively used for genetic diversity analysis in pigeonpea (Sharma *et al.,* 2018). Among these markers, SSRs are mostly preferred due to their tremendous desirable properties like multi-allelic, abundance and co-dominant nature. These properties make SSRs as the genetic marker of choice for the genetic diversity analysis among crop plants. In the present study 50 pigeonpea genotypes were evaluated for estimating genetic diversity by using 30 SSR markers.Out of the 30 markers used in present study, seven markers *viz*., ASSR 3, ASSR 148, ASSR 281, ASSR 352, ASSR 390, CCac003 and CCB 1 were found to be polymorphic in the experimental material used.These seven polymorphic SSR markers yielded a total of 14 polymorphic bands. The Polymorphism Information Content (PIC) value of markers ranged from 0.499 (CCac003) to 0.927 (ASSR 390). Marker ASSR 390 (80-100bp) was found as most informative primers on the basis of highest PIC value of 0.927 followed by marker CCB 1 (0.582), ASSR 3 (0.574), ASSR 148 (0.524), ASSR 281 (0.519), ASSR 352 (0.519) and CCac003 (0.499). The ASSR markers were also used earlier by Singh *et al.* (2013) and Singh *et al.* (2016) and their study revealed that these markers were highly polymorphic.

The dendrogram obtained in the present study revealed that the used markers differentiated the 50 genotypes up to a good extent. The Jaccard's similarity coefficient ranged from 0.62 to 1.00 (Fig 1). The dendrogram analysis classified the 50 genotypes in five major clusters (Table 3). The cluster II contains the largest number of genotypes *i.e.* 24 followed by cluster I *i.e*. 12 genotypes, cluster IV *i.e*. 8 genotypes, cluster III *i.e*. 5 genotypes while cluster V has only one genotype *i.e*. PA 421. Similar kind of results for genetic diversity by using SSR markers were also reported by other workers in their experimental material [Sharma *et al.,* 2020 and Zavinon *et al.* (2020)]. A comparative insight on clustering pattern on the basis of morphological and molecular diversity exhibited that there is no direct relationship between morphological and molecular diversity

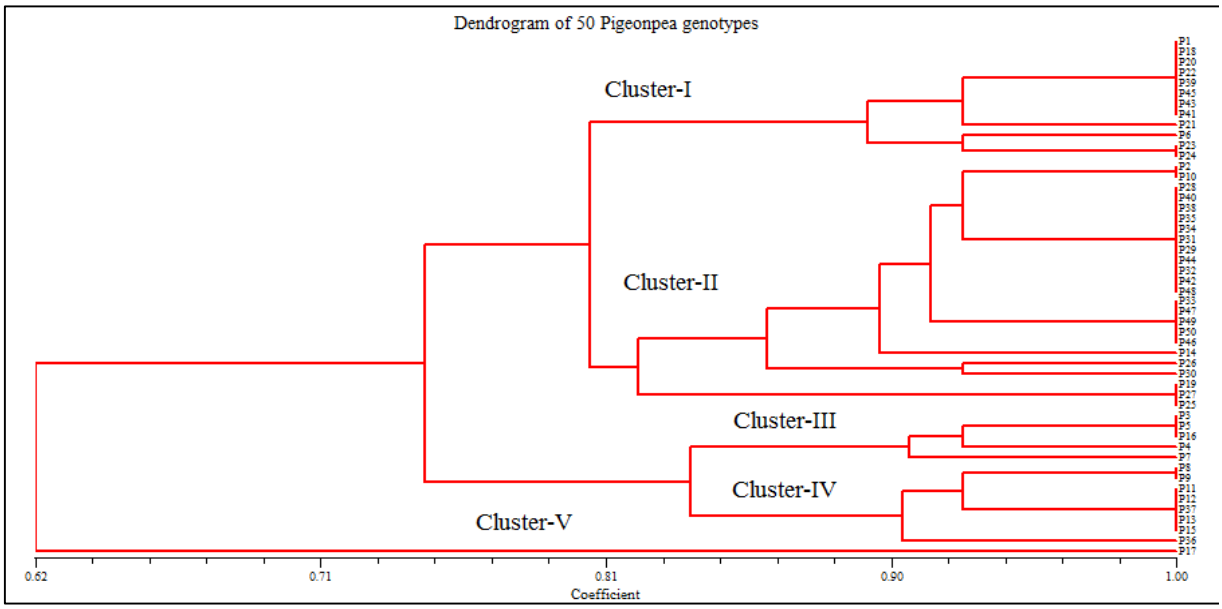

Fig 1: Dendrogram of 50 pigeonpea genotypes obtained by using SSR molecular markers

P1 to P50 refers to genotypes ICPL 91031, CORG 99-4, IPA 94-4, ICPL 98-15, ICPL 98009, PUSA 2003-1, RGT 1, PA 111, ICPL 87115, ICPL 86005, ICPL 98024, PA 288, PA 492, PA 337, PA 415, PA 374, PA 421, PA 291, PA 426, PA 429, PA 406, PA 439, PA 443, PA 444, PA 449, PA 440, PA 435, PA 454, PA 441, PA 452, PA 475, RKPV 310-03, AL 1758, RVSA 2014-2, PA 455, PUSA 2015-1, PA 3, PA 477, CRG 2012-20, PA 493, SJP 102, PA 483, PA 469, AL 2046, AL 1932, PAU 881, PA 473, RVSA 2014-1, PA 476 and ICPL 88039

Table 5: Cluster means of different characters of pigeonpea genotypes.

CharactersClusters	DF	DM	PH	NPB	NSB	NPP	NSP	HSW	SYP
Cluster I	92.71	147.79	267.23	13.64	12.72	359.04	4.02	8.29	75.31
Cluster II	81.67	135.64	259.33	15.42	21.56	343.22	4.09	8.31	75.17
Cluster III	98.33	154.67	300.00	10.00	10.67	238.33	4.00	8.20	38.33
Cluster IV	74.67	126.67	272.50	14.00	23.33	390.00	4.00	8.57	76.60

Where, DF, DM, PH, NPB, NSB, NPP, NSP, HSW, SYP refers to Days to 50% Flowering ; Days to Maturity; Plant Height (cm); Number of Primary Branches/plant; Number of Secondary Branches/plant; Number of Pods/Plant; Number of Seeds/Pod; 100-Seed Weight (g) and Seed yield/Plant (g).

Table 6: Contribution of different characters towards the total divergence.

Characters	Contribution %
Days to 50% Flowering	14.37
Days to Maturity	52.24
Plant Height	1.80
No. of Primary Branches	0.16
No. of Secondary Branches	0.57
No. of Pods/ Plant	17.96
No. of Seeds/ Pod	0.24
100 Seed Weight	2.45
Seed yield/ Plant	10.20

as the genotypes those are grouped in same cluster on the basis of morphological diversity, grouped into different clusters on molecular diversity basis. This may be ascribed to different parents used in pedigree of these genotypes and effect of environment on expression of different morphological traits. The results of the present study revealed that the experimental material has enough and sufficient genetic diversity. The coefficient of genetic similarity obtained in the present study ranged from 0.62 to 0.90, indicating the presence of sufficient genetic diversity among the experimental genotypes. The highest estimated genetic distance could be ascribed to differences between genotypes due to diversification in the pedigree. The results of present study suggested that crossing or hybridization between the genotypes selected from diverse clusters may result in heterotic progenies. The results of morphological as well as molecular studies suggested that the molecular markers differentiating the genotypes in more number of clusters as compared to morphological markers.

CONCLUSION

The assessment of genetic diversity by using the D^2 statistics

revealed that the fifty pigeonpea genotypes were grouped into four different clusters. As the maximum intercluster distance existed between the cluster III and IV, the hybridization between the genotype included in these cluster *i.e.* RVSA 2014-1 and PA 406 will produced wide range of genetic variation in F_2 and succeeding generations and desirable segregants may be obtained. The characters days to maturity, number of pods/ plant, days to 50% flowering and seed yield /plant were identified as major contributing characters towards the genetic divergence. The used markers differentiated the 50 genotypes in five major clusters with cluster II as the largest cluster (24 genotypes). The results of morphological as well as molecular studies suggested the morphological and molecular diversity in pigeonpea is different.

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