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Morphological Characterization and Genetic Diversity among Indigenous Collections of Pigeonpea [*Cajanus cajan* (L.) Millsp]

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ABSTRACT

Background: Morphological characters are stable across environments owing to oligogeneic nature. Hence, they serve as morphological markers which can be used in varietal or genotypic identification, varietal purification and even in seed production. Genetic diversity analysis is being used to identify the divergent genotypes and to utilize these genotypes to exploit heterosis and also help to select desirable segregants.

Methods: A total of 266 pigeonpea germplasm lines along with three checks were evaluated in augmented block design (ABD) at ZARS, Kalaburagi, during *kharif*, 2020. Observations are recorded on nine quantitative and fourteen morphological triats.

Result: Grouping of 266 germplasm lines into twelve clusters indicated a wider genetic diversity for the traits studied, in which cluster I and cluster II are the largest comprising 105 genotypes each, while clusters V, VI, VIII, IX, X, XI, XII were solitary with one entry each. Germplasm lines IC339062, IC343954, IC339074, IC418976, IC343931, IC437078, IC355599 and IC384490 fall into solitary cluster and are more often likely to have some unique characters which make them divergent. The genotypes IC 355599 and IC 343931 are trait specific for both seed weight and pods per plant.

Key words: Clusters, Diversity, Morphological characterization, Pigeonpea, Variability.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the second most important pulse crop of India, which contributes significantly to the nourishment of Indian population. It plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as food, fodder and fuel (Rao *et al.,* 2002). India is the largest producer and consumer of pigeonpea covering 85% of world pigeonpea share. Globally pigeonpea covers an area of 6.99 mha with an annual production of 5.96 mt coupled with a productivity of 852 kg per ha. In India pigeopea covers an area of 4.23 mha with an annual production of 3.89 mt and productivity of 919 kg per ha. Pigeonpea is mainly grown in the states of Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh and Gujarat. In Karnataka currently it is grown in an area of about 13 lakh ha with production of 9.1 lakh tons. (AICRP, Pigeonpea Annual report, 2020-21).

Variability studies are important to know the genetically divergent genotypes useful in crop improvement. Considering the choice of diverse parental material in the study, lot of diversity is expected to be generated for various characters. Genetic diversity analysis was therefore used to identify the divergent genotypes to utilize these genotypes to exploit heterosis. The genetically diverse parents are likely to produce not only heterotic effect but also desirable segregates to be selected.

Morphological characters are stable across environments owing to oligogeneic nature. Hence, they serve as morphological marker in breeding which can be used in varietal or genotypic identification, varietal purification and even in seed production. Keeping above points in view, the genotypes were categorized based on morphological characters. From characterization, a number of conclusions can be drawn. The variability that is identified by characterization needs to be conserved and should be made available to both germplasm collectors and breeders.

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MATERIALS AND METHODS

Present experiment was carried out during *Kharif* 2020 at Zonal Agriculture Research Station (ZARS), Kalaburagi, which is situated in agro-climatic zone-2 (North Eastern dry zone) of Karnataka state with 17° 20′ Latitude (N), 76° 49′ Longitude (E) and at an altitude of 443.88 meters above mean sea level.

A total of 266 indigenous collections of pigeonpea obtained from ICAR-National Bureau of Plant Genetic Resource (NBPGR), New Delhi and three checks BSMR-736, Asha and PT-012 were sown in augmented block design (ABD) consisting of 9 blocks with 33 genotypes in each block, checks were repeated randomly. Each genotype was sown in two rows of three meter length with spacing of 90 cm between rows and 20 cm between plants. Standard agronomic practices were followed and plant protection measures were taken as and when required by following the recommended package of practices (Anonymous., 2017). Observations were recorded on nine quantitative traits in five randomly selected plants from each genotype, *viz.,* days to 50% flowering, days to maturity, plant height, number of primary branches, number of secondary branches, pod bearing length, number of pods per plant, seed yield per plant and 100 seed weight. Data recorded on five plants were averaged and average values were subjected to statistical analysis. The analysis of variance (ANOVA) was carried out for all characters individually. The data was analyzed using WINDOSTAT ver. 8.5 software developed by Indostat services, Hyderabad as per the principles of Mahalanobis (1936) and clustering by Tocher's method (Rao, 1952) respectively.

Morphological characterization of 14 qualitative traits was recorded as per the key guidelines provided by PPV and FR (protection of plant varieties and farmer rights) authority, New Delhi and seed characteristics were recorded as per ICRISAT/ IBPGR (1993) (international bureau of plant genetic resources) guidelines.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) exhibited highly significant difference among genotypes for all the traits (Table 1). Based on D^2 values the genotypes were grouped into 12 clusters using Tocher's method given by Rao (1952). Of the 12 clusters, cluster I and II are the largest comprising 105 genotypes each followed by cluster IV (28 genotypes), cluster III (20 genotypes), eight clusters (V, VI, VII, VIII, IX, X, XI, XII) were solitary with single genotypes each. These results are similar to observations of Muniswamy *et al.*

Table 1: Analysis of variance for yield and yield attributing traits of pigeonpea germplasm lines.

	DF	DFF	DM	PH	PB	SB	PBL	NPP	100SW	SYP
Block (ignoring treatments)	8	2514.8***	3169.8***	7009.4***	38.0***	71.0***	364.6***	14388.2***	8.6***	1049.1***
Treatment (eliminating blocks)	265	444.6***	470.6***	663.9***	6.8*	21.4	101.8***	5084.9	3.2***	624.8***
Checks+var vs. var.	263	447.7***	473.8***	667.5***	6.8*	21.0	100.3***	5121.5	3.2***	622.3***
Block (eliminating check+var.)	8	7.1	15.7	581.3***	8.3*	14.4	38.4	1795.6	0.3***	300.7
Entries (ignoring blocks)	265	614.9***	684.9	1100.5***	8.9**	25.3*	124.0***	5940.3	3.8***	675.6***
Checks	2	37.3***	56.0***	179.5	8.2	81.5**	299.8***	279.8	0.003	948.1*
Varieties	262	612.1***	690.7***	1055.8***	7.8*	19.1	122.8***	4728.7	3.4***	275.9
Checks vs. varieties	1	2512.0***	413.0***	14658.4***	281.3***	1534.6***	69.1	334694.6***	115.5***	04843.7***
Error	36	3.8	5.6	154.4	4.1	14.8	34.9	3798.3	0.11	244.330

*-Significant at 5 per cent **-Significant at 1 per cent. DFF: Days to 50% flowering; PB: Number of primary branches; NPPP : Number of pods per plant; DM: Days to maturity; SB: Number of secondary branches; 100 seed wt: 100 seed weight (g); PH: Plant height (cm); PBL: Pod bearing length (cm); SY/PP: Seed yield per plant (g).

Table 2: Clustering pattern of pigeonpea germplasm lines based on D^2 analysis.

Cluster number	No. of lines germplasm	Name of germplasm lines									
I	105	1	IC323296	23	IC339109	45	IC355598	67	IC397314	89	IC468007
		2	IC328726	24	IC339110	46	IC369222	68	IC397532	90	IC468016
		3	IC329121	25	IC339112	47	IC369226	69	IC397552	91	IC468034
		4	IC337447	26	IC339114	48	IC369574	70	IC397574	92	IC468045
		5	IC338562	27	IC343094	49	IC369575	71	IC397603	93	IC468047
		6	IC339054	28	IC343818	50	IC369600	72	IC398660	94	IC468048
		7	IC339056	29	IC343862	51	IC369679	73	IC398906	95	IC468049
		8	IC339058	30	IC343863	52	IC369681	74	IC398907	96	IC468048
		9	IC339061	31	IC343890	53	IC382856	75	IC399016	97	IC468099
		10	IC339066	32	IC343898	54	IC384030	76	IC400159	98	IC468120
		11	IC339067	33	IC343902	55	IC384488	77	IC410027	99	IC468122

Table 2: Continue...

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		12	IC339069	34	IC343906	56	IC384504	78	IC413021	100	IC468124
		13	IC339071	35	IC343908	57	IC385713	79	IC415007	101	IC468125
		14	IC339072	36	IC343911	58	IC385885	80	IC424442	102	IC468126
		15	IC339073	37	IC343913	59	IC385888	81	IC426954	103	IC468128
		16	IC339075	38	IC343916	60	IC395740	82	IC427777	104	IC468151
		17	IC339076	39	IC343938	61	IC395830	83	IC438342	105	IC468152
		18	IC339081	40	IC343944	62	IC396014	84	IC441979		
		19	IC339085	41	IC343966	63	IC396020	85	IC442099		
		20	IC339095	42	IC345579	64	IC396818	86	IC442215		
		21	IC339101	43	IC345621	65	IC396829	87	IC442216		
		22	IC339107	44	IC355595	66	IC397303	88	IC468005		
II	105	1	PT-0012	23	IC369576	45	IC447926	67	IC468036	89	IC468112
		2	BSMR-736	24	IC369673	46	IC448188	68	IC468037	90	IC468113
		3	IC323293	25	IC369677	47	IC448275	69	IC468038	91	IC468118
		4	IC323295	26	IC385790	48	IC467991	70	IC468039	92	IC468127
		5	IC323300	27	IC385791	49	IC467992	71	IC468047	93	IC468129
		6	IC323305	28	IC395310	50	IC467993	72	IC468048	94	IC468130
		7	IC323310	29	IC395805	51	IC467994	73	IC468049	95	IC468131
		8	IC323311	30	IC395815	52	IC468002	74	IC468048	96	IC468132
		9	IC328727	31	IC395843	53	IC468006	75	IC468046	97	IC468133
		10	IC329118	32	IC396021	54	IC468008	76	IC468047	98	IC468134
		11	IC329119	33	IC396022	55	IC468013	77	IC468048	99	IC468135
		12	IC329708	34	IC398905	56	IC468014	78	IC468051	100	IC468136
		13	IC337159	35	IC399644	57	IC468017	79	IC468047	101	IC468138
		14	IC339097	36	IC405216	58	IC468019	80	IC468051	102	IC468143
		15	IC343819	37	IC405217	59	IC468020	81	IC468047	103	IC468144
		16	IC343848	38	IC405219	60	IC468021	82	IC468097	104	IC468146
		17	IC343969	39	IC437077	61	IC468022	83	IC468104	105	IC468153
		18	IC355601	40	IC441969	62	IC468023	84	IC468105		
		19	IC361402	41	IC442102	63	IC468024	85	IC468036		
		20	IC361407	42	IC442160	64	IC468030	86	IC468037		
		21	IC361408	43	IC442217	65	IC468033	87	IC468038		
		22	IC369527	44	IC446483	66	IC468035	88	IC468039		
III	21	1	IC323289	5	IC323297	9	IC346274	13	IC382808	17	IC384509
		2	IC323290	6	IC323298	10	IC355600	14	IC382963	18	IC384510
		3	IC323291	7	IC323299	11	IC361404	15	IC384491	19	IC398908
		4	IC323292	8	IC343859	12	IC369538	16	IC384508	20	IC411500
IV	28	1	ASHA	7	IC323309	13	IC369601	19	IC442083		
		2	IC323288	8	IC329120	14	IC369674	20	IC467988	25	IC468145
		3	IC323301	9	IC339096	15	IC384493	21	IC468009	26	IC468147
		4	IC323302	10	IC339103	16	IC396017	22	IC468046	27	IC468148
		5	IC323303	11	IC342947	17	IC405218	23	IC468114	28	IC468150
		6	C323304	12	IC369539	18	IC441994	24	IC468137		
V	1	1	IC339062								
VI	1	1	IC343954								
VII	1	1	IC339074								
VIII	1	1	IC418976								
IX	1	1	IC343931								
X	1	1	IC437078								
XI	1	1	IC355599								
XII	1	1	IC384490								

Table 3: Intra (diagonal) and inter cluster distances (D^2 value) of 266 germplasm lines along with checks of pigeonpea.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	4932.5	14624.4	19646.3	40810.3	11096.5	10074.9	7066.1	11709.3	9421.5	10041.6	103323.9	21524.9
II		4694.3	12789.8	14668.5	35720.4	35521.9	10350.2	39938.0	30593.2	16965.1	52158.0	37386.9
III			5306.7	28186.6	36941.0	34696.5	22811.3	45279.6	24801.6	9951.4	74208.2	20144.7
IV				6881.6	69628.7	72529.5	25727.0	82021.6	65261.8	47304.1	20877.8	67614.2
V					0.00	738.6	14069.1	5821.3	2870.3	21680.1	149790.6	15073.8
VI						0.00	16193.1	3762.2	1761.0	17053.8	154971.7	13859.1
VII							0.00	19896.1	16629.4	20805.1	76110.0	29822.7
VIII								0.00	8354.2	20887.1	167349.4	28865.4
IX									0.00	11507.3	144435.1	6367.2
X										0.00	111833.5	15217.2
XI											0.00	142998.8
XII												0.00

*Diagonal values indicate intra cluster distances.

*Above diagonal values indicate inter cluster distances.

(2014), Patel *et al.* (2018) and Satish *et al.* (2020). Katiyar *et al.* (2004), observed 14 clusters while grouping of 221 genotypes, Nethravathi and Patil (2014) obtained nine clusters using 196 genotypes.

Generation of more clusters in general and sole clusters in specific is a representative of existence of huge amounts of diversity between the set of genotypes. The genotypes *viz.,* IC339062, IC343954, IC339074, IC418976, IC343931, IC437078, IC355599 and IC384490 belongs to solitary cluster, which more usually have some distinctive characters which make them divergent. Furthermore, the genotypes which have congregated into a cluster which exhibit narrow range of genetic diversity among them while, broad range of variability was recorded between clusters. The generation of such clusters may be due to total isolation arresting the gene flow or rigorous natural or human selection for diverse adaptive complexes. These results are similar to the findings of Muniswamy *et al.* (2014), Patel *et al.* (2018) and Satish *et al.* (2020).The grouping of 266 germplasm lines into twelve clusters is presented in Table 2.

The highest intra cluster distance was noticed in cluster IV (6881.6) followed by III (5306.7), I (4932.53) and II (4694.3). The inter cluster D^2 values were maximum (167349.3) between clusters VIII and XI indicating these two clusters distantly placed, followed by cluster V and XI (149790.6), IX and XI (144435.1). It is sensible to select genotypes from clusters showing high inter cluster distance for further crossing programme (Table 3). These results are in agreement with the earlier findings of Sreelakshmi *et al.,* (2010) Muniswamy *et al.* (2014), Patel *et al.* (2018) and Satish *et al.* (2020).

Cluster mean analysis (Table 4) indicated that cluster VIII (80.00) and VII (95) are comprised of early flowering genotypes. Cluster VIII (133.00) comprised early maturing genotypes followed by cluster VII (148.00). The highest cluster mean for number of pods per plant was recorded in cluster XI (418.00) followed by cluster IV (291.43). The highest cluster mean for seed yield per plant was recorded in cluster IV (43.32) followed by cluster XI (42.40). The maximum cluster mean for 100 seed weight was recorded in cluster X (13.00) followed by cluster IX (12.50). Thus, the genotypes IC 343931 and IC437078 of the cluster IX and X have more seed weight. The cluster XI was solitary cluster which contains IC 355599 genotype and it is unique among all genotypes as indicated by more inter cluster distances and highest cluster means for number of pods per plant. Similar results were also obtained by Muniswamy *et al.* (2014) Patel *et al.* (2018) and Satish *et al.* (2020).

Contribution of individual characters towards divergence have been calculated (Table 5) which revealed that relative contribution of number of pods per plant was maximum (63.75%) followed by plant height (17.89%), days to 50 per cent flowering (6.84%), number of days to maturity (6.80%), seed yield per plant (3.61%) and rest of the characters

Table 4: Cluster means of 12 clusters for yield and its related traits in pigeonpea germplasm lines.

Cluster no.	No. of germplasm lines	DFF	DM	PH	PB	SB	PBL	NPPP	SY/PP	100 seed wt	Overall score	Rank
I	105	110.71 (4)	165.35 (4)	153.63 (6)	8.06 (6)	4.37 (6)	41.18 (4)	112.62 (6)	19.43 (5)	9.50 (6)	47	7
II	105	126.40 (6)	187.56 (5)	193.64 (3)	11.20 (4)	8.72 (5)	46.63 (1)	197.90 (3)	33.00 (3)	9.42 (7)	37	10
III	20	184.85 (11)	239.60 (9)	186.52 (4)	12.90 (1)	12.50 (3)	36.26 (6)	162.10 (5)	13.97 (8)	9.30 (8)	55	5
IV	28	133.07 (7)	194.46 (7)	186.35 (5)	12.63 (2)	12.61 (2)	43.16 (2)	291.43 (2)	43.22 (1)	9.73 (5)	33	11
V	1	106.00 (3)	158.00 (3)	78.70 (12)	6.00 (9)	1.30 (9)	13.70 (11)	66.00 (9)	14.90 (7)	10.50 (3)	66	4
VI	1	116.00 (5)	158.00 (3)	97.70 (10)	5.00 (10)	0.00 (10)	18.70 (9)	51.00 (11)	9.90 (10)	10.50 (3)	71	2
VII	1	95.00 (2)	148.00 (2)	126.30 (8)	8.00 (7)	8.30 (6)	39.00 (5)	170.00 (4)	23.40 (4)	9.50 (6)	44	8
VIII	1	80.00 (1)	133.00 (1)	130.00 (7)	6.00 (9)	0.00 (10)	39.00 (5)	32.00 (12)	5.70 (12)	8.00 (9)	66	4
IX	1	135.00 (8)	193.00 (6)	103.30 (9)	7.00 (8)	2.00 (8)	25.70 (8)	60.00 (10)	11.20 (9)	12.50 (2)	68	3
X	1	166.00 (10)	205.00 (8)	200.70 (2)	12.00 (3)	9.00 (4)	34.70 (7)	87.00 (7)	16.80 (6)	13.00 (1)	48	6
XI	1	136.00 (9)	301.00 (11)	224.70 (1)	10.00 (5)	15.70 (1)	42.70 (3)	418.00 (1)	42.40 (2)	9.50 (6)	39	9
XII	1	191.00 (12)	245.00 (10)	89.70 (11)	6.00 (9)	2.70 (7)	16.30 (10)	75.00 (8)	6.60 (11)	10.00 (4)	82	1

DFF: Days to 50% flowering; DM: Days to maturity; PH: Plant height (cm); PB: Number of primary branches; SB: Number of secondary branches; PBL: Pod bearing length (cm); NPPP: Number of pods per plant; SY/PP: Seed yield per plant (g); 100 seed wt: 100 seed weight (g).

Table 5: Per cent contribution of each character towards genetic divergence among pigeonpea germplasm lines.

Characters	Times ranked 1st	Contributions (%)	Cumulative
Number of pods per plant	22467	63.75	63.75
Plant height	6307	17.89	81.64
Days to 50% flowering	2410	6.84	88.48
Days to maturity	2398	6.8	95.28
Seed yield per plant	1271	3.61	98.89
Pod bearing length	387	1.1	99.99
Number of secondary branches	4	0.01	100
Primary branches	0	0.00	100
100 seed weight	0	0.00	100

have shown very negligible or no contribution towards diversity. Therefore, selection of genotypes for character like number of pods per plant should receive maximum importance as they contributed more towards genetic divergence. These findings conformity with pervious results of Muniswamy *et al.* (2014), Singh *et al.* (2010), Bhadru (2011), Hariprasad *et al.* (2018) and Satish *et al.* (2020).

Morphological characterization

Out of 266 genoytpes, morphological variation was not observed for plant growth habit, leaf pubescence and pod pubescence. Unique genotypes were observed for seed colour pattern like mottled (IC 343859) and mottled and specked seeds (IC 343818). The traits like early plant vigour, branching pattern, base flower colour, stem colour, streak pattern of base petal, pod colour, seed characteristics exhibited lot of variation and the results of characterization of genotypes for morphological traits are presented in Table 6. Similar findings for plant vigor and plant growth habit were observed by Muniswamy *et al.* (2014) and Kumar *et al.* (2016). Hariprasad (2018) found similar results for branching pattern and stem colour. Kallihal *et al.* (2016) observed similar morphology in case of, streaks pattern on base petal, pod shape and pod colour. Similar findings for seed colour pattern and base seed colour was recorded by Muniswamy *et al.* (2014) and Satish *et al.* (2020).

Germplasm lines IC339062, IC343954, IC339074, IC418976, IC343931, IC437078, IC355599 and IC384490 fall into solitary cluster and are more often likely to have some unique characters which make them divergent like IC339062 had least plant height, IC418976 was early to flower and mature, IC437078 was having more 100 seed weight, IC355599 had more number of pods *etc.* Based on mean performances for yield and yield related traits, the genotypes IC329120, IC405218, IC468129, IC468130, IC468145 and IC468146 were found promising. The genotypes IC355599 and IC343931 are trait specific for seed weight and pods per plant. Hence, afore said genotypes can be used for the further studies for improving the yield as a parent in the future breeding programme.

Table 6: Morphological characterization of 266 pigeonpea germ plasm lines.

Qualitative characters	Type	No. of genotypes	Frequency (%)/genotype
Early plant vigour	Poor	13	4.89
	Good	130	48.87
	Very good	123	46.24
Branching pattern	Erect and compact	56	21.05
	Semi spreading	141	53.01
	Spreading	69	25.94
Plant growth habit	Determinate	0	0
	Semi determinate	0	0
	Indeterminate	266	100.00
Base flower colour	Light yellow	0	0.00
	Yellow	228	85.7
	Orange yellow	28	10.52
	Red	10	3.76
Stem colour	Green	217	81.58
	Sun red	9	3.38
	Purple	40	15.04
	Dark purple	0	0.00
Leaf pubescence	Glabrous	0	100
	Pubescent	266	100.00
Streaks pattern	Sparse streaks	137	51.50
	Medium streaks	76	28.57
	Dense streaks	35	13.16
	Un. Strk	18	6.77
Pod pubescence	Glabrous	0	0
	Pubescent	266	100.00
Pod shape	Flat	220	82.71
	Cylindrical	46	17.29
Pod colour	Green	11	4.14
	Purple	19	7.14
	Dark purple	0	0.00
	Green and purple	236	88.72
Seed colour pattern	Plain	253	95.11
	Mottled	1(IC343859)	0.38
	Speckled	11	4.14
	Mottled and speckled	1(IC343818)	0.38
Seed eye width	Narrow	98	36.84
	Medium	73	27.44
	Wide	95	35.71
Base seed colour	White	20	7.51
	Cream	34	12.7
	Orange	35	13.15
	Light brown	69	25.93
	Reddish brown	94	35.33
	Dark grey	0	0.00
	Dark purple	0	0.00
	Others (Mix)	14	5.26
Seed shape	Oval	163	61.28
	Globular	60	22.56
	Square	40	15.04
	Elongate	3	1.12

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Author contributions

Conceptualization of research: S. Muniswamy, Neeta Singh, Sushil Pandey, and I.P. Singh. Designing of the experiment: S. Muniswamy, Neeta Singh and Sushil Pandey. Execution of field/lab experiments and data collection: S. Muniswamy and Geeta. Writing-original draft preparation: S. Muniswamy and Geeta. Writing-review and editing: Neeta Singh, Sushil Pandey, and I.P. Singh.

All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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