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Genetic diversity study in M4 mutant lines in pigeon pea (*Cajanus cajan* (L.) Millsp.)

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Abstract
The study titled "Genetic diversity study in M4 mutant lines in Pigeon pea (*Cajanus cajan* L. Millsp.)" was carried out during the *kharif* season of 2022 at the Research and Education farm within the Department of Agriculture Botany, College of Agriculture, Dapoli. In this study, 60 mutant lines and 3 check samples of pigeon pea underwent analysis for 14 distinct characteristics using an Augmented Design approach. These mutants and checks were categorized into 7 clusters, with Cluster I demonstrating the highest count of mutants (34) and Clusters IV, V, VI, and VII containing a solitary mutant each. Notably, Clusters II and VII displayed the greatest average inter-cluster distance of 163.76, succeeded by the distance between Clusters II and V, which measured 152.79. Cluster II exhibited the highest intra-cluster distance (38.56), followed by Cluster III (37.09) and Cluster I (33.74). The most significant genetic divergence was evident within Cluster VII. The study's outcomes suggest that mutants such as T_1 (77) 3-3, T_2 (10)8-1, T_3 (6) 16-11, and T_7 (2) 2-2 found prominent for future varietal enhancement purposes.

Keywords: Diversity, cluster analysis, mutants, pigeon pea

Introduction
Legumes play a crucial role in both human and animal nutrition, serving as a primary source of vegetable protein. Beyond protein, they offer a valuable supply of carbohydrates, minerals, and B-complex vitamins, especially significant within vegetarian diets (Salunkhe *et al*., 1985) [8]. Typically, pulses contain 20-25 percent protein in their dried seeds, a content nearly 2.5-3.0 times higher than what's typically found in cereals. This high protein content in food legumes significantly contributes to ensuring nutritional security among the country's impoverished populations (Chaturvedi and Ali, 2002) [2].

Pigeon pea, known as red gram, tur, or arhar, stands as a vital pulse crop cultivated across tropical and subtropical regions (Sangle, 2016) [9]. Records indicate India as the primary cultivator of pigeon pea. The origins of this crop have sparked a debate between two theories—one advocating for Indian origins and the other favoring Africa. Supporters of the Indian origin theory, including Rheede (1686) [7], Linnaeus (1737) [4] base their argument on the extensive natural genetic variability in pigeon pea and the presence of its wild relatives within the Indian subcontinent.

The pigeon pea stands as a crucial *kharif* crop within the nation, holding the position as the second most vital pulse crop following gram. India proudly claims its spot as the largest global producer, contributing roughly 63% to the overall production. Within India, Maharashtra, Karnataka, Madhya Pradesh, Telangana, Uttar Pradesh, Andhra Pradesh, Gujarat, Odisha, and Chhattisgarh collectively represent over 80% of pigeon pea cultivation. Maharashtra leads in terms of land area devoted to this crop (12.29 lakh ha), succeeded by Karnataka (8.85 lakh ha), and Madhya Pradesh (6.47 lakh ha). When production is considered, Maharashtra secures the top rank with 10.59 Lakh tones, trailed by Madhya Pradesh with 8.39 Lakh tones and Karnataka with 7.27 Lakh tones (Prospects for *kharif* pulses in 2020–2021).

Pigeon pea plants exhibit an erect stature with considerable height, boasting lateral branches alongside a deeply embedded taproot. Their juvenile stems display angularity and a covering of hair, while the leaves cluster spirally. The trifoliate leaves, arranged in a spiral pattern, feature smooth undersides on the leaflets. Despite being self-compatible, pigeon pea relies predominantly on insects as its primary pollinators. The flowers, which possess a cleistogamous nature, actively encourage self-pollination. Insects visiting these flowers determine the cross-pollination potential, with approximately 5 to 40% of the blossoms

achieving cross-pollination based on their visitation frequency.

Materials and Methods
During the *kharif* season of 2022, the assessment took place at the Educational and Research farm within the Department of Agricultural Botany, College of Agriculture, Dapoli. Employing an augmented design, the experiment featured a spacing arrangement of 60 x 60 cm among mutants, covering a plot size of 37.2 × 14.4 m. The experimental plot encompassed 63 lines, each consisting of 25 seeds. The experimental setup comprised 60 mutant lines derived from the M_3 generation of Pigeon pea (*Cajanus cajan* L.), developed through irradiation of the Konkan Tur-1 variety. These mutants were sourced from the Educational and Research farm within the Department of Agricultural Botany, College of Agriculture, Dapoli. The M_4 generation encompassed seeds derived from specifically chosen M_3

plants, exhibiting traits such as early maturity, high yield, resistance to pod borer damage, and various other qualitative and quantitative characteristics. Consequently, the M_4 generation involved the cultivation of 60 mutant lines, alongside the control varieties Konkan Tur-1, Godavari, and BDN-711.

Results and Discussion
The assessment of genetic divergence finds expression through the robust statistical tool, Mahalanobis D^2 statistics, offering a comprehensive insight into the diversity among the mutants. The cohort of 60 mutants and three checks samples underwent categorization into seven distinct groups. Notably, Cluster I boasted the highest count of mutants (34), succeeded by Cluster III (with 19 mutants), Cluster II (comprising 6 mutants), while Clusters IV, V, VI, and VII each contained a solitary mutant.

Table 1: Grouping of Pigeon pea mutant into different clusters by tocher method

Cluster Group	No. of Mutant	List of mutant and check
1 Cluster	34	T_1 (68) 1-2, T_1 (82) 7-4, T_2 (11) 3-3, T_4 (9) 5-1, T_2 (9) 2-7, T_2 (36) 5-10, T_5 (106) 1-2, T_1 (67) 11-5, T_5 (96) 11-2, T_7 (2) 2-3, T_3 (19) 12-3, T_1 (67) 11-7, T_6 (26) 9-4, T_2 (36) l15-2, T_2 (25) 1-6, T_2 (11) 3-4, T_3 (5) 7-5, T_3 (11) 6-5, T_2 (47) 8-5, T_1 (67) 11-6, T_2 (9) 2-1, T_2 (85) 2-3, T_2 (9) 5-2, T_1 (67) 11-2, T_3 (19) 8-3, T_9 (30) 5-7, T_1 (1) 13-4, T_5 (56) 4-5, T_2 (63) 9-5, T_9 (37) 10-4, T_1 (27) 16-4, T_2 (36) 17-1, T_1 (77) 9-5 & T_1 (67) 11-4
2 Cluster	6	T_1 (77) 3-3, T_2 (10) 8-1, T_3 (6) 16-11, T_7 (2) 2-2, T_3 (6) 16-6 & T_5 (56) 4-2
3 Cluster	19	T_2 (10) 8-5, T_3 (19) 8-1, T_3 (55) 3-1, T_2 (23) 5-4, T_3 (48) 4-7, T_3 (55) 3-2, T_1 (51) 9-4, T_5 (48) 2-4, T_6 (14) 98-1, T_1 (47) 3-4, T_5 (96) 11-1, Konkan Tur-1, T_4 (9) 5-3, T_1 (20) 5-5, Godavari, BDN-711, T_1 (67) 2-2, T_1 (21) 7-8 & T_1 (20) 5-4
4 Cluster	1	T_1 (77) 9-3
5 Cluster	1	T_2 (11) 3-2
6 Cluster	1	T_2 (47) 11-1
7 Cluster	1	T_2 (9) 2-3

The average intra and inter cluster D^2 and D values for each of the seven clusters respectively showed in Table No 2.
The highest disparities were evident between various clusters, notably between clusters II and VII (163.76), followed by cluster II and cluster V (152.79), cluster II and cluster IV (145.19), cluster I and cluster II (128.16), cluster II and cluster VI (114.47), cluster III and cluster VII (100.87), cluster III and cluster V (89.40), cluster III and cluster IV (82.17), cluster II and cluster III (78.04), cluster I and cluster III (64.95), cluster VI and cluster VII (58.23), cluster I and cluster VII (57.87), cluster III and cluster VI (57.31), cluster I and cluster V (49.23), cluster I and cluster IV (43.40), cluster

I and cluster VI (43.08), cluster V and cluster VII (42.10), cluster IV and cluster VII (40.25), cluster IV and cluster VI (36.13), cluster V and cluster VII (28.36), and cluster IV and cluster V (15.70). These findings imply potential variations in the genomic architecture among the mutants within these clusters. Notably, Cluster II exhibited the greatest intra-cluster distance (38.56), followed by Cluster III (37.09) and Cluster I (33.74). Conversely, Clusters IV, V, VI, and VII showed the shortest distances (0.00). Cluster VII emerged as the most diversified, displaying significant inter-cluster distances with several other clusters. Nag and Sharma (2012) [6], Saroj *et al*. (2013) [10] ended with similar kind of results.

Table 2: Average intra and inter cluster $\sqrt{D^2}$ values (D Values) in 7 clusters in 60 mutants and 3 checks of pigeon pea.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	33.74	128.16	64.95	43.40	49.23	43.08	57.87
Cluster 2		38.56	78.04	145.49	152.79	114.47	163.76
Cluster 3			37.09	82.17	89.40	57.31	100.87
Cluster 4				0.00	15.70	36.13	40.25
Cluster 5					0.00	42.10	28.36
Cluster 6						0.00	58.23
Cluster 7							0.00

Table No. 3 shows mean performance of clusters with their contribution towards total divergence.
The cluster mean performance for days to flower initiation ranged from 112.80 days (Cluster VII) to 131.84 days (Cluster I), with a mean population of 126.90 days. Cluster VII mutant showed early flowering, but cluster I mutant showed late flowering.

The cluster mean performance for days to 50 per cent flowering ranged from 126.00 days (Cluster VII) to 146.79 days (Cluster III) with population mean of 140.52 days. The mutant from cluster VII reported for early 50 per cent flowering whereas mutant from cluster III were recorded for late 50 per cent flowering.
The cluster mean performance for days to maturity varied

from 155.00 days (Cluster VII) to 176.00 days (Cluster IV) with population mean of 169.68 days. The mutant from cluster VII recorded early maturity whereas mutant from cluster IV were reported for late maturity.

The cluster mean performance for plant height was ranged from 148.32 cm (Cluster I) to 184.17 cm (Cluster II) with the mean population of 169.16 cm. The mutant from cluster I were dwarf plant stature whereas mutant from cluster II were reported for tallness among all.

The cluster mean performance for the number of branches per plant ranged from 5.94 (Cluster I) to 8.98 (Cluster II), with a population mean of 7.42. The mutant from Cluster I had fewer branches per plant, whereas the mutant from Cluster II had more branches per plant.

The cluster mean performance for number of pods per plant ranged from 105.30 (Cluster VII) to 248.45 (Cluster II), with a population mean of 151.97. Cluster VII mutant produced fewer pods per plant, whereas mutant from Cluster II produced more pods per plant.

The cluster mean performance in terms of number of seeds per pod ranged from 3.00 seed per pod cluster (Cluster VII) to 3.70 seed per pod cluster (Cluster II), with a population mean of 3.28. Cluster VII mutant were documented for a lower number of seed per pod, whereas cluster II mutant were reported for a higher number of seed per pod.

The cluster mean pod length performance ranged from 4.60 cm (Cluster V) to 5.92 cm (Cluster II), with a population mean of 5.14 cm. Cluster V mutants were found to have shorter pod lengths, whereas cluster II mutants had longer pod lengths.

The cluster mean performance for 100 seed weight ranged from 7.10 gm (Cluster IV) to 8.10 gm (Cluster VI), with a population mean of 7.53 gm. Cluster IV mutants were documented for low 100 seed weight, while cluster VI mutants were reported for high 100 seed weight.

The number of pods per cluster performance ranged from 3.00 clusters per plant (Cluster IV) to 3.70 clusters per plant (Cluster VI), with a population mean of 3.44. Cluster IV mutants were detected for a lower number of pods per cluster, whereas cluster VI mutants were observed for a higher number of pods per cluster.

The cluster mean performance for percent pod borer damage varied from 9.80 (Cluster VI) to 25.00 (Cluster VII) with population mean of 18.07. The mutant from cluster VI were observed for less percent pod borer damage whereas mutant from cluster VII were recorded for more percent pod borer damage.

The cluster mean performance for number of pod clusters per plant ranged from 32.10 (Cluster V) to 74.32 (Cluster II), with a mean population of 44.65. Cluster V mutants had fewer pod clusters per plant, but cluster II mutants had more pod clusters per plant.

The cluster mean performance for seed yield per plant ranged from 21.60 g (Cluster VII) to 69.52 g (Cluster II), with a mean population of 36.31 g. Cluster VII mutants had low seed yield per plant, but cluster II mutants had good seed yield per plant.

The cluster mean performance for harvest index varied from 18.50% (Cluster VII) to 22.28% (Cluster II) with mean population of 19.85%. The mutants from cluster VII were reported for low harvest index whereas mutants from cluster II were observed for high harvest index.

Character contribution towards divergence
The primary contributor to divergence was seed yield per plant (15.88%, 311), occupying the top position in terms of its impact, followed by 100-seed yield weight (12.55%, 176). Harvest index (8.76%, 171), percent pod borer damage (8.54%, 87), number of pods per plant (7.70%, 133), number of pods per cluster (7.66%, 150), plant height (7.65%, 415), number of clusters per plant (6.98%, 137), number of seeds per pod (5.98%, 78), pod length (5.87%, 115), days to flower initiation (5.00%, 39), days to 50% flowering (3.20%, 63), number of branches per plant (3.00%, 59), and days to maturity (1.23%, 24) followed in their respective contributions to the observed divergence. Kumar *et al*. (2014) founded similar results.

Table 3: Mean performance of clusters with their contribution towards total divergence

Sr. No.	Characters	Clusters							Mean population	Contribution towards divergence (%)	Times ranked first
		I	II	III	IV	V	VI	VII			
1	Days to flower initiation	131.84	129.23	131.26	132.00	125.20	126.00	112.80	126.90	5.00	39
2	Days to 50% flowering	145.74	143.17	146.79	144.00	140.00	138.00	126.00	140.52	3.20	63
3	Days to maturity	174.09	171.50	174.21	176.00	167.00	170.00	155.00	169.68	1.23	24
4	Plant height (cm)	148.32	184.17	164.63	174.00	174.00	181.00	158.00	169.16	7.65	415
5	Number of branches per plant	5.94	8.98	7.02	8.00	7.00	8.00	7.00	7.42	3.00	59
6	Number of pods per plant	142.13	248.45	191.11	117.20	112.40	147.20	105.30	151.97	7.70	133
7	Number of seeds per pod	3.16	3.70	3.30	3.40	3.10	3.30	3.00	3.28	5.98	78
8	Pod length (cm)	4.86	5.92	5.14	5.20	4.60	5.50	4.80	5.14	5.87	115
9	100 seed weight (g)	7.34	7.83	7.55	7.10	7.30	8.10	7.50	7.53	12.55	176
10	Number of pods per cluster	3.53	3.58	3.60	3.00	3.50	3.70	3.20	3.44	7.66	150
11	Pod borer damage (%)	16.15	12.53	19.01	19.60	24.40	9.80	25.00	18.07	8.54	87
12	Number of clusters per plant	40.58	74.32	53.96	39.00	32.10	39.70	32.90	44.65	6.98	137
13	Seed yield per plant (gm)	31.15	69.52	44.00	26.20	23.40	38.30	21.60	36.31	15.88	311
14	Harvest index	19.32	22.28	20.81	18.60	18.80	20.70	18.50	19.85	8.76	171

Conclusion
The study found extensive genetic divergence among mutants, with sixty mutants and three checks categorized into seven groups according to their D^2 levels determined by Mahalanobis D^2 analysis. The first cluster (I) contained 34 mutants, followed by cluster (III) with 16 mutants and 3 checks clusters (II) with 6 mutants. Clusters (IV), (V), (VI), and (VII) each comprised one mutant. Emphasizing these characteristics more during the selection program would be beneficial.

Hence, the mutants identified as T1 (77)3-3, T2 (10)8-1, T3 (6)16-11, and T7 (2)2-2 emerged as top performers in the

studied mutant group. They exhibited the highest seed yield per plant and displayed exceptional yield-related traits. Notably, T7 (2)2-2 boasted the highest pod count per plant, while T2 (85)2-3 showcased the maximum 100-seed weight. Moreover, T2 (10)8-1 demonstrated minimal pod borer damage. All these mutants exhibit significant potential as valuable genetic resources for forthcoming breeding programs. This investigation highlighted substantial and diverse characteristics among the mutants examined.

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