



Assessment of Crop Genetic Diversity Utilizing Multivariate Analysis in Pigeonpea [*Cajanus cajan* (L.) Millsp.] Genotypes

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ABSTRACT

Background: Understanding genetic variability parameters, correlation analysis and employing multivariate techniques such as principal component analysis and diversity analysis are crucial for designing effective breeding programs and conserving germplasm in pigeonpea. Morphological studies, when combined with multivariate analysis, offer valuable insights into genetic diversity, aiding in the selection of diverse parental lines and identifying promising segregants in hybridization programs.

Methods: The study utilized 155 diverse genotypes of pigeon pea, planted in a randomized complete block design with three replications during the *kharif* seasons of 2022-23 and 2023-24. Character association analysis, multivariate analysis like PCA and diversity assessment were utilized.

Result: Principal component analysis was employed to assess variability, where the first two principal components explained 35.63% of the total variability, with traits such as DFF, DM, NSB and NPP contributing significantly to variance. Morphological genetic diversity was assessed using Mahalanobis D² statistics and hierarchical clustering. Morphological diversity assessment grouped the 155 pigeon pea genotypes into 9 clusters, with cluster I being the largest (88 genotypes). Hierarchical clustering identified 6 distinct clusters, with cluster-I housing the highest number of genotypes (43). Interestingly, the study revealed non-consistent connections between morphological diversity assessments and the geographical origin of genotypes. Based on genetic D² values, genotypes in clusters VI and VII, followed by clusters VI and VIII, were found to be the most genetically distant, suggesting their potential use in hybridization programs to create diverse progenies. Selecting desirable genotypes from different clusters based on their performance could lead to the development of superior recombinants through hybridization.

Key words: Genetic diversity, Hierarchical clustering, Pigeonpea, Principal component analysis.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the important grain legume crop cultivated in tropical and subtropical regions of the world. Taxonomically, it is classified as a diploid plant species with a genome size of 853 Mbp, belonging to the subfamily Papilionoideae within the Fabaceae plant family. Within the *Cajanus* genus, the pigeon pea is the only cultivated species among all the 32 species identified within its sub-tribe *Cajaninae* (Zavinon *et al.*, 2020; Van der Maesen, 1990; Bohra *et al.*, 2017). Both archaeological evidences and molecular studies, conducted by Saxena *et al.* (2014), supported the assertion that pigeon pea originated from India. Furthermore, India holds the global distinction of being the largest consumer and producer of pigeon pea, with a production of 3.31 million tonnes during 2022-23 (Directorate of Economics and Statistics, 2024).

The conventional method for evaluating genetic diversity involves the examination of agro-morphological traits coupled with robust multivariate statistical procedures. This approach, which measures genetic variance through the lens of similarity or dissimilarity, is a fundamental way of assessing the genetic divergence within a population. It allows for the grouping of germplasm without prior knowledge of their geographic origins or genetic backgrounds. By employing biometrical analysis,

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including genotypic and phenotypic coefficient of variances, correlation studies, heritability and genetic advance, researchers can elucidate the underlying variability within a population. This empowers plant breeders to identify reliable yield-contributing characteristics those are suitable for effective selection (Singh *et al.*, 2019). In any crop improvement programme, genetic diversity serves as a prerequisite. Interestingly, geographical diversity does not always correlate with genetic diversity, underscoring the importance of selecting parents for hybridization based more on genetic rather than geographic diversity.

Multivariate analysis, particularly through techniques like principal component analysis (PCA) and hierarchical cluster analysis (HCA), proves valuable in selecting genetically distant parents for hybridization. PCA, a potent dimension reduction and unsupervised linear transformation method, extracts crucial information from complex phenotypic traits while minimizing redundancy and preserving pertinent information. By reducing a large set of initially correlated variables into a smaller set of uncorrelated or orthogonal variables known as principal components, PCA facilitates the identification of unique genotypes for different traits (Kumar *et al.* 2022). Present study aims to assess different genetic parameters, principal components and genetic diversity among pigeon pea genotypes based on quantitative traits to identify distinct genotypes suitable for pigeon pea improvement programme.

MATERIALS AND METHODS

The present study was conducted at Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar during *Kharif*, 2022 and *kharif* 2023 crop seasons. A total of 155 pigeon pea genotypes developed at various research institutes across the country were acquired for the experimental work from the pulse breeding project at GBPUA and T, Pantnagar. These 155 genotypes comprised of 97 genotypes from GBPUA and T, Pantnagar; 17 from ICRISAT, Hyderabad; 11 from ICAR-IARI, New Delhi; 10 from PAU, Ludhiana; 5 from CCSHAU, Hisar; 3 each from TNAU, Tamil Nadu and ARS, Kota; two each from RVSKV, Gwalior; Anand Agricultural University, Gujarat; and IIPR, Kanpur; and one each from ICAR-VPKAS, Almora; ARS-Virinipuram, Vellore and SKNAU, Jobner. Additionally, these genotypes were again classified broadly into 4 categories based on their origin in India *i.e.* North zone, West zone, Central zone and South zone. In the present study, randomized complete block design with three replications was used in the experimental setup. Each genotype was planted in a two row plot of four-meter length spaced 60 cm apart. A plant-to-plant spacing of 20 cm was maintained. Ten quantitative traits *i.e.* days to 50% flowering (DFF), days to maturity (DM), plant height in cm (PH), number of primary branches per plant (NPB), number of secondary branches per plant (NSB), pod length in cm (PL), number of pods per plant (NPP), number of seeds per pod (NSP), hundred seed weight in g (HSW) and seed yield per plant in g (SYP) were recorded. Standard agronomic package of practices was followed throughout the crop season to ensure excellent crop growth. Three plants from each genotype were randomly selected for recording the observations at maturity except for DFF and DM for which data was recorded on a plot basis.

Statistical analysis entailed performing an analysis of variance (ANOVA) for ten quantitative traits. Correlation coefficients were computed following the procedure advocated by Pearson (1897). Principal component

analysis was executed and principal components with eigenvalues exceeding one were selected, in line with Jeffers' recommendation (1967). All statistical analyses were performed using R Studios software version 4.3.3. The ANOVA was conducted utilizing the '*agricolae*' package, while the creation of the correlogram was facilitated by the '*GGPlot2*' and '*GGally*' packages. PCA analysis was executed employing the '*factoextra*' and '*FactoMineR*' packages. The D² analysis was carried out utilizing the '*biotools*' package. Lastly, hierarchical clustering was performed with the aid of the '*factoextra*' and '*cluster*' packages (R Core Team, 2024).

RESULTS AND DISCUSSION

Genetic variability studies provide basic information regarding the genetic properties of the population based on which breeding methods are formulated for improving the targeted characters. The analysis of variance for both years indicated highly significant differences ($p < 0.001$) among the genotypes for all the evaluated traits, indicating the presence of substantial genetic variability in the experimental material that can be further utilized for pigeon pea improvement programme. Variances for both years when compared, revealed non-significance differences and therefore, the data of both years can be combined for further analysis.

Association of different quantitative characters

Correlation analysis was done to find out interrelations among quantitative traits including yield and its component traits. Notably, significant Pearson's correlation coefficients were observed for nearly all the traits, with variations noted when different origins were considered, contributing to the overall correlation. Traits such as DFF, DM, PH and HSW demonstrated significant and positive correlations with SYP (Fig 1). These distinguished traits exhibited considerable promise in the selection process of high-yielding genotypes within the pigeonpea species from the primary gene pool. These findings corroborate with the earlier studies conducted by Verma *et al.* (2018a), Gaur *et al.* (2020) and Ranjani *et al.* (2018), particularly regarding the attributes of DFF and DM. Among the component traits, PH showed significant correlations with six traits, namely NPB, NSP, DFF, DM, HSW and SYP followed by DM and SYP with four significant correlations each. These results highlight the crucial roles played by PH, DM and SYP in influencing yield in the present study, emphasizing the importance of prioritizing these traits in hybridization programs aimed at improving crop productivity.

It can be observed that the genotypes belonging to the north zone were the main determinant for the correlation to be significant between most of the traits. A highly significant positive correlation ($p < 0.001$) was documented between DFF and DM (0.587***), followed by a correlation between DFF and SYP (0.323***). These results validated the earlier findings of Pal *et al.* (2018); Vanniarajan *et al.* (2021) and

Reddy *et al.* (2023). Furthermore, a significant negative correlation was observed between NPB and DM (-0.095^{**}), as well as between PH and HSW (-0.079^{*}). Consequently, these traits hold potential for effective utilization either independently or in tandem, to augment the yield potential of this crop.

Multivariate analysis - Principal component analysis

Principal component analysis (PCA) is a multivariate statistical method for analyzing and simplifying complex and large datasets. Based on the association between studied characteristics and extracted clusters, the variation patterns in pigeon pea genotypes were investigated using PCA to determine the genotypes' genetic diversity and relationship with the studied traits. PCA was conducted using data from ten quantitative traits and the outcomes are summarized in Fig 5. Hierarchical clustering on principal components (HCPC) was employed to elucidate the existing variability within the collection and to explore the similarities and differences between individuals based on

the ten quantitative traits. HCPC, being a multivariate method, integrates PCA and clustering techniques to delineate stable morphological groups (Fig 4). Consequently, a PCA was conducted based on the ten quantitative traits and the first four principal components, each possessing eigenvalues greater than 1.0, collectively elucidated approximately 62.37% of the overall variation, as depicted in Fig 2. The PC-1 attributing to the most variability (18.8%), displayed notable positive Rotational component (RC) loadings with DFF (0.919) and DM (0.905). Consequently, these traits exerted considerable influence on the observed divergence and contributed substantially to the variability, aligning with previous findings (Fig 3).

The PC-2 accounted for 16.8% of the total variation. It was characterized by traits such as NPB, NSB, NPP and PH. PC-3 elucidated 15.1% of the variation and was associated with traits like HSW and SYP. PC-4 explained 11.7% of the total variation and featured traits like PL and NSP. PCA was utilized by Manyasa *et al.* (2008); Zovinson *et al.* (2019) and Upadhyaya *et al.* (2007) to find out the



Fig 1: Correlogram showing Pearson's correlation and scatter plot of ten quantitative traits of 155 pigeon pea genotypes based on their geographical origin in India.

Table 1: Average intra- and inter-cluster D^2 distance between genotypes grouped in 13 distinct clusters.

Cluster	C1	C2	C3	C4	C5	C6	C7	C8	C9
C1	11.65	17.33	19.97	20.33	17.06	41.48	34.81	42.03	28.00
C2		11.28	32.46	37.57	18.69	25.34	53.49	45.61	16.86
C3			12.98	29.59	35.84	74.27	25.19	26.62	52.51
C4				12.08	22.20	65.79	37.87	70.51	47.55
C5					13.66	31.38	54.28	67.06	23.17
C6						15.99	103.98	91.13	24.68
C7							25.86	46.83	77.10
C8								29.53	71.61
C9									0.00

relative importance of different traits in the collections they analysed. Similar findings were reported by them in their study.

The clustering analysis performed on the principal components *i.e.* hierarchical clustering on principal components (HCPC) led to the classification of the 155 pigeon pea accessions into three primary clusters (Fig 4). The individuals projected onto the axis system, defined by the first two principal components, displayed a mixed distribution among the genotypes. Notably, genotypes originating from various groups were associated with all three clusters, as illustrated on the individual factor map (Fig 4). This clustering pattern underscores a significant degree of variability within the different pigeon pea genotypes. Furthermore, the comparative analysis of phenotypic mean values revealed noteworthy variations among clusters for all quantitative variables, except for PL and NSP.

The principal component biplot, illustrating the quantitative traits among the pigeon pea genotypes, is presented in Fig 5. Two variables showed lower magnitude with shorter vector lengths, *i.e.*, HSW and PL, whereas

DFF and DM, with longer vector lengths, showed a higher magnitude (more variance) than the rest of the traits. In a biplot diagram, vector angles are key: the cosine of the angle approximates the correlation coefficient between two characters (Yan and Kang, 2019). A $<90^\circ$ angle indicates a positive correlation, $>90^\circ$ indicates a negative correlation and 90° signifies independence (Yan and Rajcan, 2002). Four trait vectors *i.e.* NPB, NSB, PH and PL had small angles between them, indicating positive correlations. Similarly, DFF and DM also had a small angle between their vectors, signifying a positive correlation. However, DFF and DM formed angles greater than 90° with NPB, NSB, PH and PL, indicating negative correlations. Thus, smaller angles between vectors indicate stronger positive correlations among traits and larger angles indicate negative correlations. Notably, genotypes 132 (PA 676), 75 (PA 471), 151 (RVSA 2014-2), 74 (PA 470), 101 (PA 500), 84 (PA 483), 83 (PA 482), 22 (ICPL 8501s0) and 126 (PA 652) occupied distinct positions, situated far from the remaining genotypes in the biplot indicated their potential for further use in breeding programs (Fig 5). According to the principal component analysis, traits such as DFF and

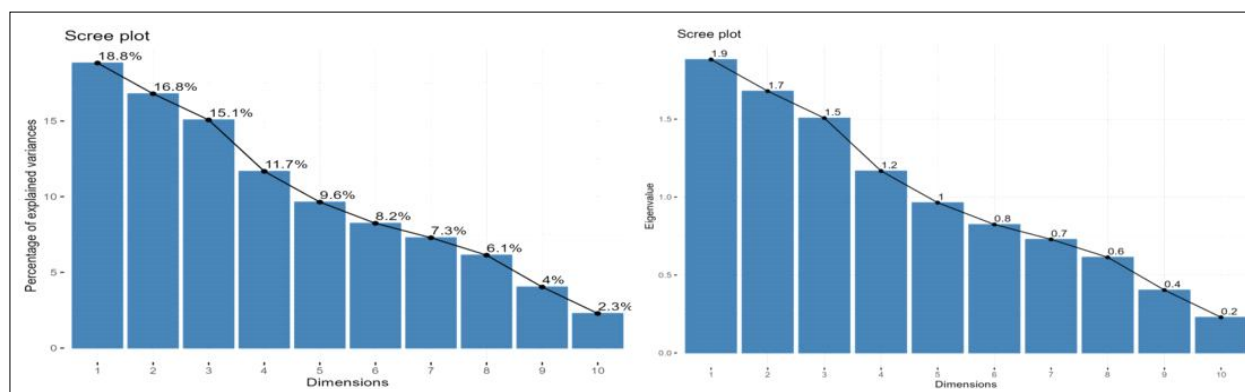


Fig 2: Scree plot based on per cent variation explained and the eigenvalue by each principal component.

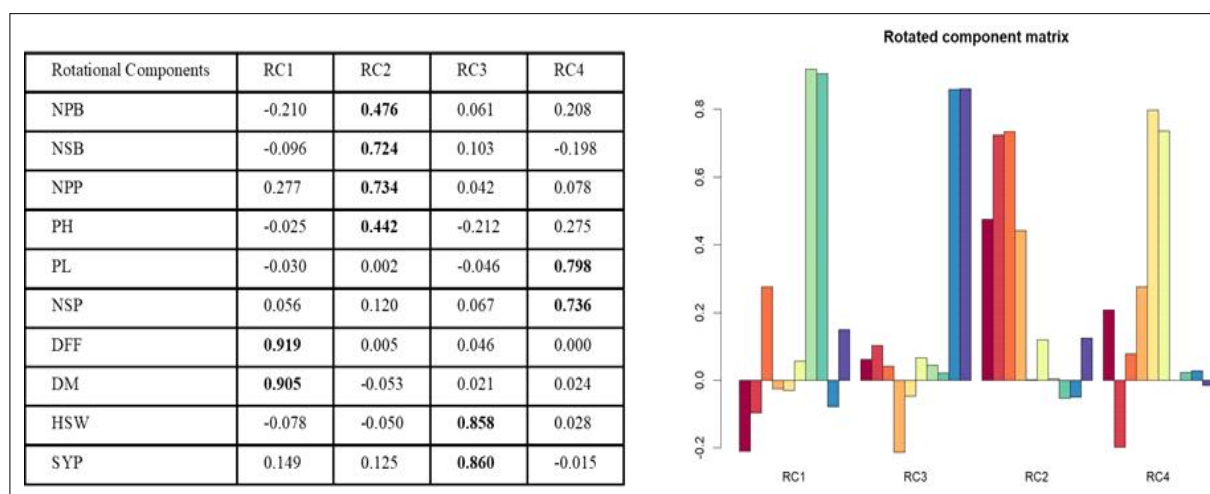


Fig 3: Rotational components loading for the first four principal components selected for the study.

DM in PC-1 and traits such as the NPB, NSB, NPP and PH in PC-2, accounted for a significant portion of the variation.

Genotypes in the top right quadrant were closely related to PH, PL, NSB, NSP, NPP, HSW and SYP traits. The top left quadrant contained varieties related to the NPB trait. The bottom right quadrant included varieties associated with DFF and DM traits. Overall, the biplot analysis effectively visualized the relationships among genotypes and yield contributing traits.

Diversity assessment through D² analysis and complete hierarchical clustering

The Mahalanobis D² values have classified the 155 pigeon pea genotypes into nine discrete clusters. Analysis of the average intra- and inter-cluster distances among these clusters (Table 1) indicated minimal genetic variation

among genotypes within the same cluster across ten quantitative traits. The maximum intra-cluster distance of 29.53 was observed for cluster VIII. Conversely, significant genetic diversity exists between genotypes from different clusters. The maximum inter-cluster distance was observed between clusters VI and VII (103.98), followed by clusters VI and VIII (91.13). Optimal genetic divergence is preferred between parental lines for hybridization to increase the likelihood of producing favourable segregants. Therefore, hybridization between genotypes from clusters with maximum inter-cluster distances is recommended to enhance the probability of isolating desirable segregants in subsequent generations. The substantial inter-cluster distance in comparison to the intra-cluster distance indicated the presence of a considerable amount of genetic diversity among the studied genotypes.

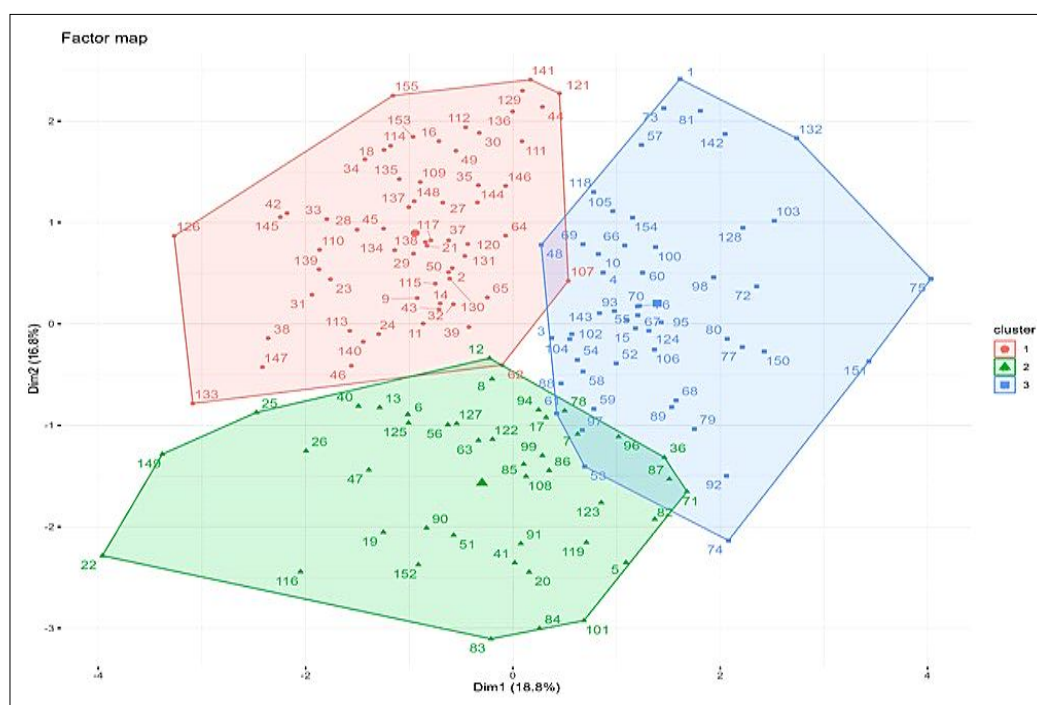


Fig 4: Factor map of HCPC for first two principal components showing the grouping of accessions into three clusters.

Table 2: Cluster mean of genotypes belonging to different clusters for ten quantitative traits.

Cluster means	NPB	NSB	NPP	PH	PL	NSP	DFF	DM	HSW	SYP	Number of genotypes
Cluster 1	5.45	13.40	134.29	234.70	5.52	3.94	84.81	149.36	9.10	38.15	87
Cluster 2	5.43	12.56	135.87	230.29	5.45	3.86	92.40	155.80	9.72	40.75	21
Cluster 3	5.38	12.96	127.73	244.69	5.37	3.94	78.64	144.71	10.13	39.76	20
Cluster 4	5.31	12.31	125.02	236.70	5.55	3.74	79.04	148.11	7.73	30.93	9
Cluster 5	4.73	12.10	131.53	246.59	5.13	3.73	89.30	154.03	8.38	32.21	5
Cluster 6	6.00	13.40	169.30	243.97	5.58	4.47	100.20	158.57	8.87	38.02	5
Cluster 7	5.27	12.17	103.93	194.19	5.41	4.20	75.53	137.37	9.66	36.81	5
Cluster 8	5.42	14.75	159.25	235.96	5.58	4.08	81.17	149.17	12.18	47.00	2
Cluster 9	5.83	11.50	149.50	221.46	4.98	2.50	96.00	153.17	8.68	47.88	1
Contribution (%)	5.86	6.11	6.82	8.27	5.14	6.99	27.92	6.81	15.25	10.77	

The present observation is in alignment with the earlier findings of Verma *et al.* (2018b), Naing *et al.* (2022) and Pushpavalli *et al.* (2017), who also noted a high magnitude of inter-cluster distance relative to intra-cluster distance. Clusters VI and VII, as well as clusters VI and VIII, demonstrated considerable divergence, suggesting that hybridization programs involving genotypes from these clusters are more likely to yield superior segregants or desirable combinations for the development of valuable genetic resources or varieties.

Analysis of genotype mean values across clusters (Table 2) highlights notable differences. Cluster VII shows the shortest maturity duration (137.37 days) and days to 50% flowering (75.53 days), making it ideal for

developing short-duration varieties. In contrast, Cluster VI has the longest maturity duration (158.57 days) and days to 50% flowering (100.20 days). Cluster VI also boasts the highest number of primary branches (6.00) and pods per plant (169.30). Cluster VIII excels in secondary branches (14.75) and hundred-seed weight (12.18 g), while Cluster V has the tallest plants (246.59 cm). Pod length and the number of seeds per pod are highest in Cluster VI (5.58 cm and 4.47 seeds). Cluster IX exhibits the highest seed yield per plant (47.88 g). These findings suggest that genotypes of Clusters VI, VII and VIII are valuable for hybridization programs aiming to develop heterotic combinations, higher yield and short-duration varieties.

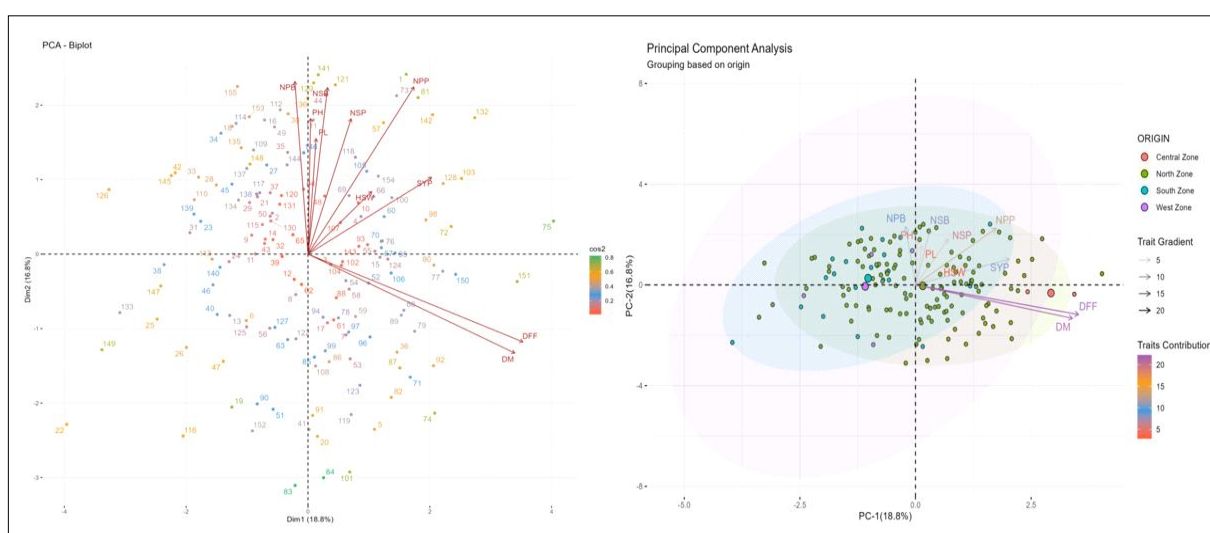


Fig 5: PCA biplot of ten quantitative traits of pigeon pea and grouping of 155 genotypes based on their geographical origin.

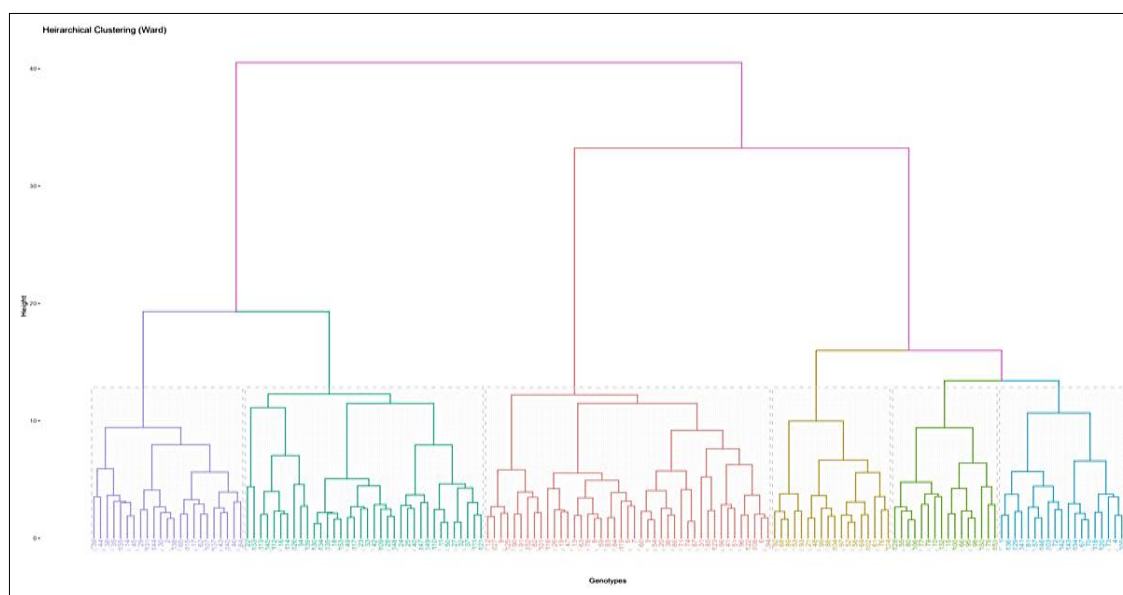


Fig 6: Hierarchical clustering of 155 genotypes using the ward method of clustering.

Analysis of the D^2 statistic revealed the traits contributing to genetic divergence (Table 2). The greatest contribution to genetic divergence comes from days to 50% flowering (27.92%), followed by hundred-seed weight (15.25%), seed yield per plant (10.77%) and plant height (8.27%). These traits collectively account for over 62% of the total divergence observed in the pigeon pea genotypes. Among the genotypes used in the present study, it was observed that cluster I contained the highest number of genotypes (87) followed by cluster II (21). The lowest number of genotypes were grouped in cluster IX containing only one genotype (Table 2).

A dendrogram was also constructed using Euclidean distance and the “ward” method of the hierarchical clustering of 155 genotypes into 6 distinct clusters. Ward method of clustering was the preferred method of choice since it had the highest correlation between cophenetic distance and the original distance (0.34) when different methods of clustering were compared. The highest number of genotypes was in cluster III (43), followed by 36 genotypes in cluster II and the lowest number of genotypes was grouped in cluster V (16 genotypes) (Fig 6). The genotypes were categorized into six distinct clusters, providing an opportunity to identify divergent parental lines for hybridization and the establishment of novel pigeon pea breeding populations. There exists a necessity to introduce new genetic variations or incorporate genes from genetically divergent parents to capitalize on the genetic diversity observed within the evaluated pigeon pea genotypes.

The clustering of 155 genotypes was conducted using two distinct methods: clustering based on Mahalanobis D^2 values utilizing the modified Tocher's method and hierarchical clustering employing the wardD method. In this study, both methodologies were compared, revealing an inability to consistently group genotypes of similar origins into the same clusters. While certain genotypes from the same origin were occasionally grouped together in both methods but the frequency of such occurrences was not substantial enough to draw a definite conclusion.

This suggested that further investigation is needed and consequently, it is inferred that the grouping of genotypes into distinct clusters primarily hinges on their genetic diversity rather than their geographical origins.

CONCLUSION

In conclusion, the identified traits, including HSW, DFF, DM, NPP and SYP through significant ANOVA and PCA, present promising avenues for enhancing crop improvement initiatives. The PCA revealed that the first four principal components collectively explained a substantial portion (62.37%) of the total variation, indicating the significance of these traits. Furthermore, Mahalanobis D^2 analysis proved effective in delineating genotype diversity. Thus, genetic variations emerged as the primary driver of genotype diversity, outweighing geographical factors in this

study. It emphasises the need for targeted breeding efforts to harness genetic potential for crop improvement.

Conflict of interest

All authors declare that they have no conflict of interest.

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