

Unveiling Genetic Diversity in Grain Amaranth through K-means Clustering and Principal Component Analysis

ABSTRACT

Grain amaranth (*Amaranthus spp.*) is a highly versatile and nutrient-dense pseudo-cereal that has gained increasing importance in global food systems. Developing diverse varieties and hybrids of grain amaranth (*Amaranthus spp.*) is essential for harnessing its full potential as a climate-resilient and nutritionally rich crop. Germplasm studies provide a comprehensive understanding of the genetic diversity within amaranth, facilitating the breeding of improved cultivars that combine desirable traits such as higher yield, enhanced nutritional content, and increased tolerance to biotic and abiotic stresses. Varietal development and hybridization can optimize characteristics like seed size, growth habit, and adaptability, making amaranth more suitable for a range of agroecological zones. In this direction present study conducted by evaluating 180 grain amaranth germplasm accessions for yield and contributing factors during the summer 2024 at the ICAR-IISS Regional Station in Bengaluru adjacent to NSP. Genetic distances among 180-grain amaranth germplasm accessions were determined using K-means clustering, resulting in 06 distinct groupings. Cluster V comprised of more number of accessions (48) and the least number of accessions in cluster VI (15). As per PCA, eight eigen values explains the total variance; among which PC₁ explained maximum variance 82.06% followed by PC₂, PC₃, PC₄, PC₅, PC₆, PC₇ and PC₈ explained 8.87%, 5.39%, 1.63%, 1.02%, 0.51%, 0.45% and 0.07 % respectively.

Keywords: grain amaranth, k means, clustering analysis, genetic diversity, principal component analysis

INTRODUCTION:

Grain amaranth belongs to the *Amaranthaceae* family, which encompasses over 60 species distributed across tropical, subtropical, and temperate regions globally. While several species within the genus possess edible seeds, the most commonly cultivated for grain production include *Amaranthus hypochondriacus*, *A. cruentus*, and *A. caudatus*. These species exhibit considerable

genetic diversity, resulting in variations in plant morphology, seed color, size, and nutritional composition[1]. Amaranth is renowned for its adaptability to diverse growing conditions, thriving in regions with high temperatures, limited water availability, and poor soil fertility too. Its ability to withstand environmental stressors, such as drought, heat, and pests, makes it an attractive crop for smallholder farmers in marginal lands. Furthermore, grain amaranth demonstrates a short growing season, typically maturing within 90 to 120 days, thus allowing for multiple harvests in a single year.

Beyond its agronomic virtues, grain amaranth stands out for its exceptional nutritional composition and potential health benefits. The seeds are rich in high-quality proteins, containing all essential amino acids, particularly lysine, which is deficient in many other cereals. Additionally, grain amaranth is a good source of dietary fiber, vitamins (such as vitamin E, B-complex vitamins, and folate), minerals (including calcium, iron, magnesium, phosphorus, and potassium), and bioactive compounds like polyphenols and flavonoids. Overall, grain amaranth emerges as a remarkable crop with the potential to address persistent global challenges related to food security, nutrition, and sustainable agriculture. Its resilience, nutritional richness, and versatility make it a valuable addition to diversified cropping systems worldwide, offering opportunities for smallholder farmers to enhance livelihoods and contribute to a more resilient and inclusive food system[2].

Utilizing diverse genetic resources can lead to more sustainable agricultural practices by reducing the reliance on chemical inputs and promoting agro-biodiversity. Breeding programs that utilize diverse genetic resources can produce high-value crops with unique qualities, benefiting farmers and the agricultural economy. By integrating genetic resources into breeding programs, there is a direct contribution to the conservation of plant biodiversity, ensuring the availability of these resources for future generations. To achieve such goals studies related to germplasm collection in crucial; with this aim, the present study has been conducted to analyse the 180-grain amaranth germplasm collection. Isolating the most valuable genotypes from evaluated germplasm pool is a crucial factor when selecting parents in crop breeding. Cluster analysis and principal component analysis assist plant breeders in identifying genetically varied parents to cater the farming community.

MATERIAL AND METHODS

180 grain amaranth germplasm material inclusive of indigenous and exotic used to conduct the experiment.

Experiment layout: A total of 180-grain amaranth germplasm materials, comprising both indigenous and exotic accessions, were used in the experiment. These accessions were evaluated alongside two check varieties, Suvarna and KBGA-15, to assess their performance and variability in key agronomic traits in augmented design [3]. The experiment was structured using a systematic online design developed by the Indian Council of Agricultural Research (ICAR), New Delhi (accessible at <https://drs.icar.gov.in>), to ensure precise and reproducible results. The germplasm entries were organized into 15 blocks, each containing 12 germplasm accessions, which were randomly assigned within the experimental layout.

The crop was cultivated during the summer season of 2024, with a plant spacing configuration of 45 cm between rows and 10 cm between plants within a row. The field experiment was meticulously managed by following the recommended package of agronomic practices, to support healthy crop development.

Five randomly picked plants were chosen to record the data as per Protection of Plant Varieties and Farmers' Rights Authority (PPVFRA). 09 Quantitative traits such as; days to 50% flowering, leaf length (cm), leaf width (cm), petiole length (cm), inflorescence length (cm), plant height (cm), yield per plant (g) and volume weight of seeds (g/10ml) were recorded for each accession and check varieties as well.

Data analysis: The following statistical procedure were followed in the present investigation. The average values for each accession were used to compute further statistical analysis.

Clustering Analysis is a statistical method that groups objects or data points based on their similarities or dissimilarities. Cluster analysis divides a set of objects into clusters; those within one subset are more "similar" to one other, while those inside other subsets are "dissimilar." There must be a means to distinguish between "dissimilar" and "similar" objects. K-means clustering is a key approach for analysing data. K-means is a popular algorithm for clustering data based on known median locations[4].

Principal Component Analysis (PCA): Principal components analysis is a method for identifying hypothetical variables (components) that explain as much of the variance in multidimensional data as possible. The new variables are linear combinations of the prior ones. [5] described the principle component analysis [6] technique, which was used to condense 07 quantitative features reported in 180 grain amaranth germplasm collections into a small number of uncorrelated variables. In the present investigation, 180 grain amaranth germplasm accessions evaluated were subjected for K-means clustering and PCA by using 'R' software package.

RESULTS AND DISCUSSION

The analysis revealed significant variation among the germplasm accessions for key agronomic traits in the present study.

K-means clustering: K-means clustering is widely used in genetic and agronomic studies for classifying genotypes into distinct groups based on their trait similarities. It is particularly effective because it allows researchers to control the number of clusters, providing flexibility in grouping complex datasets [7]. In the present study, 180-grain amaranth genotypes were classified into 6 distinct clusters using the k-means algorithm, which facilitated the identification of genetic diversity patterns among the accessions. The grouping pattern is detailed in Table 1, revealing variations in the number of accessions assigned to each cluster[8].

Among the clusters, Cluster V emerged as the largest group, containing 48 accessions, indicating a higher level of similarity or common traits among these genotypes. This was followed by Cluster I, which consisted of 38 accessions, representing another diverse yet distinct set of genotypes. Clusters II and IV each comprised 30 accessions, demonstrating comparable diversity levels and trait groupings. Cluster III included 25 accessions, while Cluster VI, the smallest cluster, contained only 15 accessions, suggesting that these genotypes may possess unique traits or characteristics that differentiate them from the others.

The clustering pattern illustrated that some clusters were more densely populated, potentially indicating genotypic redundancy or close genetic relationships among accessions within those groups. Conversely, smaller clusters like Cluster VI may represent unique or rare genotypes that could be valuable for breeding programs aimed at enhancing specific traits. The proportion of accessions within each cluster, along with the overall cluster sizes, is visually represented in Figure 1, providing a clear overview of the distribution pattern. The distinct grouping of the genotypes reflects the underlying genetic structure and diversity present in the germplasm, which can be crucial for guiding future breeding strategies. The variability in cluster sizes also suggests that while some clusters might have a broad genetic base, others are likely more specialized, requiring targeted efforts for genetic conservation or improvement.

Overall, k-means clustering not only facilitated a robust classification of the genotypes but also highlighted the diversity and complexity within the grain amaranth collection, providing valuable insights for germplasm management and the selection of promising parental lines for hybridization[9] and [10].

Table 1: Clusters distribution and size formed by k-means of 180-grain amaranth germplasm accessions.

Cluster number	Size
C1	38
C2	30
C3	25
C4	30
C5	42
C6	15
Total	180

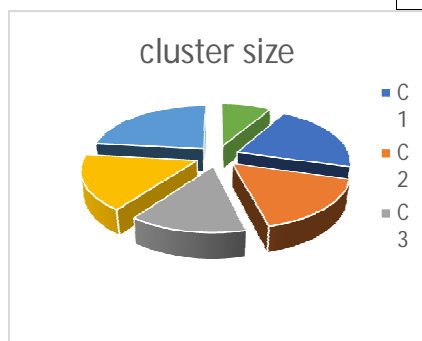


Fig. 1: Pie diagram depicting different clusters and size in 180 grain amaranth germplasm accessions.

Table 2 displays the accessions categorized into six distinct clusters, illustrating the genetic diversity within the grain amaranth germplasm collection. This classification is essential for understanding the relationships and variations among the different genotypes. By grouping the accessions based on their phenotypic traits and genetic similarities, the table reveals underlying patterns of diversity that are critical for effective breeding strategies.

Table 2: List of genotypes belonging to different clusters formed by k-mean clustering from 180 grain amaranth genotypes

Cluster no.	Size	Genotypes
C1	38	IC0095325, IC38301, EC0519542, IC00382665, NIC33418, IC38408, IC0554125, IC0038416, EC322050, EC223651, IC0554058, IC35643, IC26266, IC0038255-5, IC5596, IC35457, EC223659, IC0554033, IC35713, IC35399, NIC22559, IC0042282-7, IC37514, EC277969, EC519509, IC5564, IC0038621, IC19585, IC326899, EC322993, IC120702, IC003851, IC195016, IC0021958, IC195822, IC13899, IC313253 and IC0035577
C2	30	EC34183, IC95255, IC0042311-7, IC0615024, IC17316, IC031100, IC0554102, IC0035701,

		NIC33487, EC10238, IC120617, NIC130270, IC35588, IC120671, IC95446, IC35680, IC0553999, IC0035461, IC35755, EC170310, IC0616907, IC95262, IC0448736, IC0024262, IC32886, IC0038539, IC341496, IC36824, IC415592and IC0553987
C3	25	IC42344, IC95454, IC81701, IC95372, EC038702, IC0038134, EC359410, IC107607, IC95630, IC38356, EC223644, EC359408, IC95299, IC81694, EC289388, IC37150, IC0554010, IC395399, IC540817, IC19588, IC38014, IC038385, IC37145, NIC11318 and EC519511
C4	30	IC0554102, IC21981, IC0038265, IC415268, IC0024262, IC0095564, EC387000, IC1384528, IC35777, IC95284, IC325933, IC0108432, IC35699, NIC22536, IC35659, IC35426, EC130848, IC42337, IC120610, EC170326, IC395391, IC95634, IC33861, IC0281936, IC007933, EC323001, EC389899, IC0554124, IC422836 and IC031100
C5	42	IC0278926, IC396961, EC328874, NIC16168, IC328945, IC93945, IC0035556, IC195810, IC0042315-3, IC0035436, IC0120653, IC0035506, IC42309, IC120690, IC120668, IC120671, IC120748, IC0696, NIC21584, IC0528306, IC0035586, IC0120573, IC0281933, EC38261, IC0038573, IC0038206, IC35590, EC386997, IC0033758, IC0198131, IC35638, IC0035547, IC94662, IC0448663, IC0554124, IC95499, IC0037320, IC396972, IC95634, IC0038065, IC333749 and IC95372
C6	15	IC447680, IC95624, IC120664, IC249602, IC120671, IC0024262, IC0448639, IC0616907, IC0035470, IC95690, IC35755, IC0038554, IC108435, EC277970 and IC38259

The estimates of various quantitative traits mean across distinct clusters are summarized in **Table 3**, providing valuable insights into the phenotypic characteristics of the grain amaranth accessions. Notably, **Cluster 2** exhibited the highest mean values for key traits such as leaf length, plant height, and volume weight of seeds. This indicates that the accessions within this cluster may possess superior growth attributes and seed quality, making them potential candidates for breeding programs aimed at enhancing these desirable characteristics.

In terms of leaf width and petiole length, **Cluster 2** again showcased the best performance, suggesting that accessions in this cluster have well-developed foliage, which can contribute positively to photosynthetic efficiency and overall plant vigor. Conversely, **Cluster 4** was distinguished by its significantly longer inflorescence length, which is an important trait for both aesthetic appeal and reproductive success. This characteristic could be advantageous for breeding initiatives focusing on ornamental or high-yielding varieties.

The analysis of flowering times revealed interesting trends as well. Clusters 4 and 6 had a lower number of days to 50% flowering, indicating that the accessions within these clusters are likely to mature earlier. Early flowering can be beneficial for cultivation in regions with shorter growing seasons or for farmers aiming to achieve multiple cropping cycles within a single year. In contrast, **Cluster 2** exhibited a longer duration before reaching 50% flowering, which may be attributed to its robust growth and developmental traits. Lastly, **Cluster 3** stood out with the highest grain yield per plant, emphasizing its potential for high productivity. This finding highlights the importance of this

cluster in breeding programs focused on increasing grain yield, which is a primary objective for enhancing food security and economic viability in grain amaranth production.

Overall, the data presented in **Table 3** illustrates significant variability among the clusters regarding various quantitative traits, underscoring the genetic diversity within the grain amaranth germplasm collection. Understanding these trait means is crucial for guiding future breeding strategies, allowing researchers to select accessions that exhibit desirable combinations of traits for improved crop performance and resilience.

Table 3: Estimate of quantitative trait means of 180-grain amaranth germplasm accessions belonging to different clusters.

Sl. No.	Traits	Mean of clusters						'F' statistic	Probability
		C1	C2	C3	C4	C5	C6		
1	Leaf length (cm)	15.76	19.42	14.47	9.93	15.2	10.24	25.88	6.2 × 10 ⁻³
2	Leaf width (cm)	9.88	9.36	7.99	6.11	7.64	8.52	15.25	0.9 × 10 ⁻³
3	Petiole length (cm)	12.38	13.33	9.78	7.82	11.94	10.98	13.11	0.2 × 10 ⁻³
4	Inflorescence length (cm)	35.14	35.53	34.25	38.65	33.49	31.28	2.21	1 × 10 ⁻³
5	Plant height (cm)	128.69	135.28	125.77	126.26	124.27	115.28	11.89	1 × 10 ⁻³
6	Days to 50% flowering	35.62	38.28	39.14	31.23	32.43	31.26	11.03	1 × 10 ⁻⁴
7	Grain yield plant-1 (g)	21.77	25.61	26.85	22.07	25.13	20.89	41.99	1 × 10 ⁻³
8	Volume weight of seeds (g/10ml)	6.94	7.06	6.89	6.54	6.48	6.99	7.56	1 × 10 ⁻³

The estimates of variance among the clusters provided further insights into the genetic diversity within the germplasm collection (table 4). For **leaf length**, the highest variance was observed in **Cluster 3**, reflecting a broad genetic range that could facilitate the selection of individuals with desirable leaf characteristics. **Cluster 1** exhibited the greatest variance for both **leaf width** and **plant height**, indicating a diversity of morphological traits that may enhance adaptability to different environmental conditions. Additionally, **Cluster 3** displayed significant variance in **petiole length**, which plays a crucial role in leaf arrangement and support. In terms of **inflorescence length**, **Cluster 2** showed the highest variance, highlighting the diversity in reproductive traits among the accessions within this group. **Cluster 6** exhibited the greatest variance for **days to 50% flowering**, grain yield, and volume weight, suggesting a rich source of traits that can be utilized for breeding programs focused on improving both productivity and seed quality.

In summary, the combination of mean estimates and variance analysis across clusters underscores the substantial genetic diversity present within the grain amaranth germplasm collection. This diversity is essential for informing breeding strategies and selection practices, ultimately contributing to the development of improved varieties with enhanced agronomic performance, adaptability, and consumers' choice. Understanding both average trait performance and the extent of variability among

accessions will significantly influence the success of breeding programs aimed at optimizing grain amaranth cultivars [11], [12], [13] and [14].

Table 4: Estimate of quantitative trait variance of 180-grain amaranth germplasm accessions belonging to different clusters.

Sl. No.		Variance of clusters						'F' statistic	Probability
		C1	C2	C3	C4	C5	C6		
1	Leaf length (cm)	20.82	10.31	17.03	0.34	19.58	10.28	11.29	1 × 10 ⁻³
2	Leaf width (cm)	7.28	6.58	5.72	0.16	4.85	2.36	5.98	1 × 10 ⁻³
3	Petiole length (cm)	5.27	7.66	27.72	1.62	19.95	8.56	10.98	1 × 10 ⁻²
4	Inflorescence length (cm)	78.91	89.46	43.93	9.56	57.97	35.29	1.96	0
5	Plant height (cm)	423.83	399.01	276.36	164.71	487.98	400.28	19.7	1 × 10 ⁻⁴
6	Days to 50% flowering	6.95	2.38	1.02	1.15	35.1	28.96	10.95	1 × 10 ⁻⁴
7	Grain yield plant-1 (g)	3.24	0.92	5.84	2.83	7.23	6.28	20.36	1 × 10 ⁻³
8	Volume weight of seeds(g/10ml)	0.28	0.2	0.31	0.14	0.61	0.58	0.54	2 × 10 ⁻³

Principal Component Analysis: Principal Component Analysis (PCA) is a powerful statistical technique used to identify hypothetical variables, known as components, that account for the maximum variance within multidimensional datasets. These new variables are derived as linear combinations of the original variables, allowing researchers to simplify complex data structures while preserving essential information. The foundational work of Hotelling (1933) built upon the earlier contributions of Pearson (1901) to develop PCA, establishing it as a robust tool for data reduction and analysis. In this study, PCA was applied to condense nine quantitative traits collected from a diverse set of 180 grain amaranth germplasm accessions into a smaller number of uncorrelated variables. This approach enables a more straightforward interpretation of the relationships among accessions based on their phenotypic traits. To ensure that each of the nine numerical characteristics contributed equally to the analysis, all traits were standardized. Standardization is crucial in PCA, as it eliminates biases that could arise from differences in measurement scales among the traits. The correlation matrix was constructed to represent the covariance between these standardized variables. This matrix serves as the foundation for calculating the Eigenvalues and standardized corresponding Eigenvectors, which are essential components of PCA. Eigenvectors provide the coefficients that define the principal components, while the Eigenvalues indicate the amount of variance accounted for by each principal component.

The analysis (table 5) yielded eight Eigenvalues, each corresponding to a principal component. The Eigenvalue of a principal component reveals how much variance it explains relative to the total variance among the nine quantitative traits. By focusing on the first two principal components, which capture the largest portions of variance, researchers can gain significant insights into the underlying structure of the data. To visualize the results of the PCA, a scatter plot was created using the first two principal components for each germplasm accession. This two-dimensional

graph (figure 2), allows for a clear visual representation of the relationships among the accessions. In the scatter plot (figure 3), closely related accessions will appear grouped together, indicating similarity in their trait expressions, while those that are distantly located will suggest greater dissimilarity. This visualization is instrumental in identifying clusters of accessions that share common characteristics, aiding in the selection of diverse parental lines for breeding programs.

Table 5: Number of principal components and their Eigen values and contribution to total variability for 08 quantitative traits in 180 grain amaranth germplasm accessions.

Principal Component	Eigen value	% contribution to total variation
1	406.59	82.06
2	43.96	8.87
3	26.70	5.39
4	8.07	1.63
5	5.05	1.02
6	2.54	0.51
7	2.23	0.45
8	0.35	0.07

Overall, PCA serves as a valuable tool in this study by condensing the complexity of the germplasm data into comprehensible patterns, facilitating further exploration of genetic diversity and trait relationships within the grain amaranth collection. By employing PCA, researchers can make informed decisions regarding breeding strategies and the utilization of germplasm resources, ultimately contributing to the improvement of grain amaranth cultivars [15], [16] and [17].

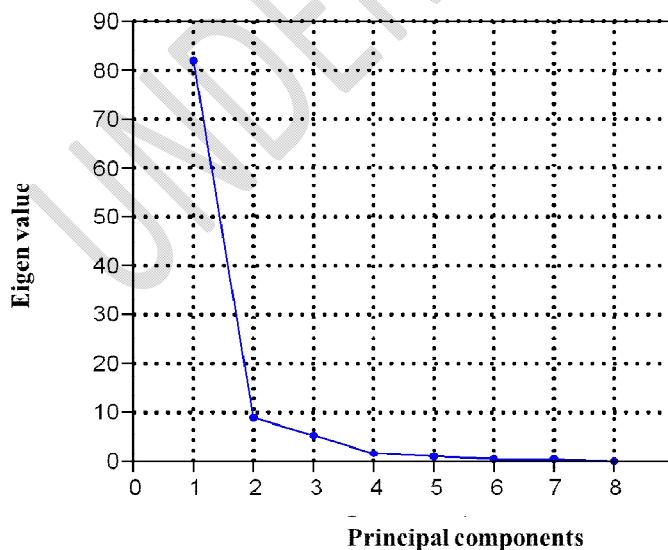


Figure 2. Contribution of each principal component to total explained variance in the phenotypic diversity of *Amaranthus spp.*

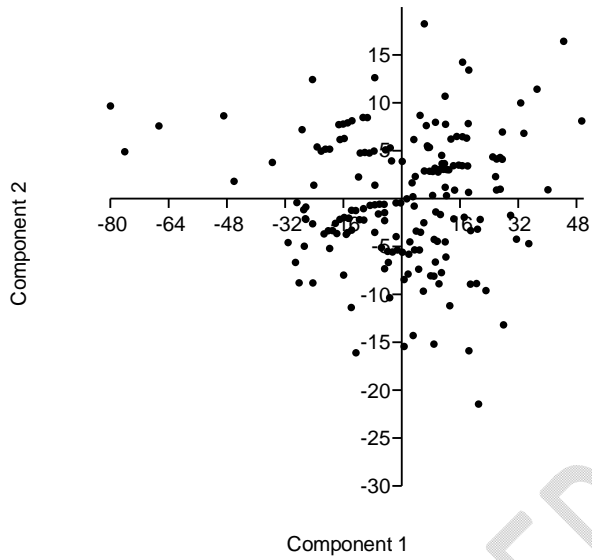


Figure 3. Scatter plot depicting first two principal components explains maximum variance in the phenotypic diversity of *Amaranthus spp.*

CONCLUSION

K-means clustering was selected as the methodology to systematically organize the genetic variability present within a diverse set of 180 genotypes of grain amaranth. This clustering technique is particularly effective for analysing large datasets, as it allows for the classification of genotypes into distinct groups based on their phenotypic or genotypic characteristics. Genetic distances among the 180 grain amaranth germplasm accessions were determined through the application of K-means clustering, which successfully identified six distinct groupings within the dataset. This classification allows researchers to understand the genetic variability present in the collection, facilitating targeted breeding strategies. Among these clusters, **Cluster V** was found to contain the largest number of accessions, totalling **48**, indicating a rich diversity of genetic traits within this group. Conversely, **Cluster VI** included the fewest accessions, with only **15**. This disparity in cluster sizes suggests that certain genetic traits are more prevalent among specific accessions, which can be pivotal for future breeding and selection efforts. In addition to K-means clustering, **Principal Component Analysis (PCA)** was conducted to assess the total variance within the germplasm collection. The PCA results revealed a total of **eight eigenvalues**, each corresponding to a principal component that explains a portion of the overall variance in the data. Notably, **Principal Component 1 (PC1)** accounted for the highest percentage of variance at **82.06%**, indicating that this component captures the most significant trends in the data. This substantial contribution highlights the importance of the traits represented by PC1 in differentiating the accessions. Following PC1, **Principal Component 2 (PC2)** explained **8.87%** of the variance, while **Principal Component 3 (PC3)** accounted for **5.39%**. The remaining principal components contributed progressively less to the overall variance, with **PC4** explaining **1.63%**, **PC5** **1.02%**, **PC6** **0.51%**, **PC7** **0.45%**, and **PC8** just **0.07%**. The substantial variance explained by the first two principal components suggests that a relatively small number of traits drive the overall genetic diversity within the grain amaranth accessions.

The findings from both K-means clustering and PCA provide valuable insights into the genetic structure of the grain amaranth germplasm. By identifying distinct clusters and understanding the principal components contributing to variance, researchers can make informed decisions regarding the selection of accessions for breeding programs. This knowledge not only aids in the enhancement of desirable traits but also contributes to the conservation and utilization of genetic resources in grain amaranth.

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