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CORRELATION AND GENETIC DIVERGENCE FOR YIELD-ATTRIBUTING AND NUTRITIONAL TRAITS IN GRAIN AMARANTH (AMARANTHUS SPP.) ACCESSIONS

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ABSTRACT
Grain amaranth (Amaranthus spp.) is a nutrient-dense, climate-resilient pseudocereal whose genetic improvement depends on the identification of reliable indirect selection criteria and genetically divergent parents. A total of 50 accessions and two check varieties (Suvarna and KBGA-15), were evaluated in an Augmented Block Design at the College of Agriculture, Shivamogga, during Kharif 2025–26, to work out the phenotypic correlation of grain yield with nine yield-attributing and eleven nutritional traits and to assess the pattern of genetic divergence among the accessions using Mahalanobis' D^2 statistic. Grain yield per plant recorded a highly significant and strong positive correlation with seed volume (r = 0.889) and inflorescence length (r = 0.526), followed by significant positive associations with days to 50 per cent flowering, leaf area, leaf length, petiole length and zinc content, whereas calcium content (r = −0.261) exhibited a highly significant negative association with yield. The D^2 analysis grouped the 52 accessions into five clusters, Cluster III being the largest (29 accessions) and Cluster I represented by a solitary accession, Suvarna. The maximum inter-cluster distance was recorded between Cluster I and Cluster IV (D^2 = 77.911) and the minimum between Cluster II and Cluster III (38.889). Cluster IV recorded the highest mean grain yield (38.26 g), protein (16.86%) and zinc content (4.83 mg/100g), while Cluster V combined the longest inflorescence with the highest iron and calcium content. Seed volume and inflorescence length emerged as reliable indirect selection criteria for yield improvement, and hybridization between genotypes of the widely divergent Clusters I, IV and V is suggested to generate desirable transgressive segregants combining high grain yield with superior nutritional quality in grain amaranth.

KEYWORDS: Amaranthus, Correlation coefficient, Mahalanobis D^2, Genetic divergence, Grain yield

INTRODUCTION

Agriculture worldwide is under increasing pressure to deliver not only greater food quantity but also better nutritional quality, particularly in the face of climate variability, shrinking arable land and the rising burden of micronutrient malnutrition. Grain amaranth (*Amaranthus* spp.), an ancient C-4 pseudocereal of the family Amaranthaceae, is increasingly recognised as a climate-resilient, nutrient-dense crop that can complement cereal-based diets. The grain contains 13 to 18 *per cent* protein with a relatively balanced amino acid profile, is naturally gluten-free and is rich in dietary fibre, calcium, iron and zinc, although it also carries anti-nutritional factors such as tannins and oxalates.

Grain yield in amaranth is a complex, polygenically controlled trait that is the resultant of several interacting component and nutritional characters, and direct selection based on yield alone is often inefficient owing to its comparatively low heritability and pronounced sensitivity to environment. Correlation analysis, which quantifies the nature and magnitude of association between yield and its component and nutritional traits, therefore assumes considerable importance in identifying reliable indirect selection criteria and in revealing traits whose simultaneous improvement may involve trade-offs, both of which have direct relevance for designing an efficient selection strategy.

Grain amaranth nevertheless remains a relatively underexploited and under-researched crop, and systematic information on the association among yield, yield-component and nutritional traits, together with an understanding of the extent of genetic divergence within the available germplasm, is still limited, particularly under Indian conditions. The genetic improvement of any crop through hybridization depends critically on the judicious choice of genetically divergent parents, since crosses between diverse parents are more likely to produce desirable transgressive segregants and greater heterosis in the resulting progeny. Mahalanobis' D^2 statistic provides an objective, multivariate measure of genetic divergence among genotypes based on several traits simultaneously and, being largely independent of the confounding effects of environment, allows grouping of genotypes into relatively homogeneous clusters, thereby helping the breeder identify genetically distant parents suitable for hybridization.

Considering the increasing relevance of grain amaranth as a nutrient-dense crop and the comparatively limited information available on character association and genetic divergence in indigenous grain amaranth germplasm, the present investigation was undertaken with the objectives of (i) working out the phenotypic correlation of grain yield with its component and nutritional traits and among the component and nutritional traits themselves, and (ii) assessing the pattern of genetic divergence among the accessions using Mahalanobis' D^2 statistic, so as to identify effective indirect selection

criteria and suitable divergent parents for future hybridization programmes in grain amaranth.

MATERIAL AND METHODS

Experimental site and material

The field experiment was conducted at the Research Farm, College of Agriculture, Shivamogga (13°58′35.52″N, 75°34′37.12″E; altitude 632 m), falling under Agro-climatic Zone VII (Southern Transitional Zone) of Karnataka, during *Kharif* 2025–26. The experimental material comprised 50 grain amaranth accessions procured from AICRN on Potential Crops, UAS, GKVK, Bengaluru, together with two check varieties, Suvarna and KBGA-15, making a total of 52 genotypes.

Field experimentation

The 52 accessions including 2 check varieties were evaluated using an Augmented Block Design comprising 13 blocks, each accommodating four test accessions and two replicated check varieties. Each block measured 3.6 m × 2.7 m, with a spacing of 45 cm between rows and 15 cm between plants.

Traits recorded

Nine quantitative traits — days to 50 per cent flowering, plant height (cm), inflorescence length (cm), leaf length (cm), leaf width (cm), leaf area (cm^2), petiole length (cm), seed volume (g/10ml) and grain yield per plant (g) — and eleven nutritional traits — moisture (%), protein (%), carbohydrates (%), fat (%), fibre (%), ash (%), iron (mg/100g), zinc (mg/100g), calcium (mg/100g), tannins (%) and oxalates (mg/100g) — were recorded on each accession following standard procedures.

Correlation coefficient

Simple correlation coefficients among grain yield and its component and nutritional traits were worked out at the phenotypic level using genotype mean values, following the standard procedure of Singh and Chaudhary (1977):

$r^{xy} = \text{Cov}(x, y) / \sqrt{\{Var(x)\ Var(y)\}}$

where Cov(x,y) is the covariance between traits x and y, and Var(x) and Var(y) are their respective variances. The significance of each correlation coefficient was tested against tabulated r-values at (n − 2) degrees of freedom (n = 52), and the coefficients were designated as non-significant (ns), significant at the 5 per cent probability level (single asterisk), or significant at the 1 per cent probability level (double asterisk).

Skewness and kurtosis

The nature of the frequency distribution of the nine quantitative and eleven nutritional traits was examined by working out the coefficient of skewness (g_1) and the coefficient of kurtosis (g_2) based on genotype mean values, using R software, following the standard procedure of Snedecor and Cochran (1967):

$g_1 = m_3 / m_2^{(3/2)} \qquad g_2 = (m_4 / (m_2)^2) - 3$

where m2, m3 and m4 are the second, third and fourth central moments of the trait distribution about the mean, respectively. A skewness value close to zero indicates a fairly symmetric, near-normal distribution, whereas positive and negative values indicate a distribution skewed towards higher and lower trait values, respectively. Likewise, a positive kurtosis value indicates a leptokurtic (sharply peaked) distribution and a negative value indicates a platykurtic (flat) distribution relative to the normal curve. Taken together, skewness and kurtosis provide indirect evidence of the type of gene action governing a trait: distributions close to normal, with low skewness and negative or near-zero kurtosis, generally indicate predominantly additive gene action controlled by several genes of small effect, whereas markedly skewed, leptokurtic distributions indicate the involvement of a few genes of major effect or non-additive (dominance/epistatic) gene action.

Mahalanobis' D² analysis

Mahalanobis' D^2 statistic was used to assess genetic divergence among the 52 genotypes based on all the quantitative and nutritional traits studied, using R software. The generalized distance between any two populations is given by:

$D^2 = \Sigma\Sigma\ \lambda_{ij}\ \sigma_{ai}\ \sigma_{aj}$

where D^2 is the square of the generalized distance, λ_{ij} is the reciprocal of the common dispersion matrix, and σ_{ai} and σ_{aj} are the differences between genotype means for the traits concerned. Since computation requires inversion of a higher-order determinant, the original correlated, unstandardized character means were transformed to standardized, uncorrelated variables to simplify the computation, and the D^2 values were obtained as the corresponding uncorrelated values for any two genotypes (Rao, 1952).

All the possible n(n − 1)/2, D^2 values were grouped into clusters by Tocher's method as described by Rao (1952), such that the average intra-cluster distance was minimized relative to the inter-cluster distance. The intra- and inter-cluster distances were computed following Singh and Chaudhary (1977):

Square of intra-cluster distance $= \Sigma D_i^2 / n$

Square of inter-cluster distance $= \Sigma D_i^2 / (n_i \times n_j)$

where ΣD_i^2 is the sum of the distances between all possible combinations of genotypes included in (or between) the cluster(s) concerned, and n_i and n_j are the number of genotypes in clusters i and j, respectively.

RESULTS AND DISCUSSION

Correlation analysis

Grain yield is a complex, polygenically controlled trait that is the resultant of several interacting component characters. Correlation analysis quantifies the nature and magnitude of association among traits and helps identify reliable indirect selection criteria, particularly for traits that are difficult to measure directly or that exhibit low heritability. The phenotypic correlation coefficients among grain yield per plant and its component and nutritional traits are presented in Table 1.

Correlation of grain yield per plant with quantitative and nutritional traits

Grain yield per plant recorded the strongest and highly significant positive correlation with seed volume (r = 0.889), followed by inflorescence length (r = 0.526), indicating that heavier, bolder seeds and longer inflorescences are the traits most directly responsible for higher grain yield in this germplasm. This finding is in close agreement with Showemimo *et*

al. (2021), who reported a strong positive correlation of grain yield with principal yield-related traits in grain and leafy amaranth, and is further supported by Gins *et al.* (2024), who observed a similarly strong association between morphological traits and seed yield in *Amaranthus*. Significant positive correlations were also recorded between grain yield and days to 50 per cent flowering (r = 0.335), leaf area (r = 0.311), leaf length (r = 0.303), petiole length (r = 0.301) and zinc content (r = 0.287), suggesting that improvement in these component traits would contribute favourably to yield enhancement, a pattern broadly consistent with the identification of leaf area as an important yield-associated trait by Savaliya *et al.* (2024).

Notably, grain yield per plant also showed significant positive associations with several nutritional quality traits, namely leaf width (r = 0.242), oxalate content (r = 0.239), tannin content (r = 0.232) and fat content (r = 0.227), indicating that, in the present germplasm, selection for higher yield need not necessarily compromise these nutritional attributes. This is an encouraging observation from a breeding perspective, as it suggests scope for simultaneous improvement of grain yield and certain quality traits, a possibility also highlighted by Sarker *et al.* (2022) with respect to mineral-rich, high-yielding amaranth genotypes. Protein (r = 0.011), carbohydrate (r = 0.024), ash (r = 0.083) and iron content (r = 0.176) exhibited positive but statistically non-significant associations with grain yield, while plant height (r = 0.035) also showed a weak, non-significant positive relationship. Fibre content (r = −0.056) recorded a non-significant negative association, whereas calcium content (r = −0.261) exhibited a highly significant negative correlation with grain yield, indicating that genotypes with higher calcium accumulation tended to record comparatively lower yield in this germplasm. This negative association warrants careful consideration in future breeding programmes seeking to combine high yield with enhanced calcium content, and selection indices that weight both traits appropriately, rather than relying on either trait alone, would be advisable.

Table 1: Correlation between grain yield and its component and nutritional traits among grain amaranth germplasm accessions

Traits	DFPF	PH	IL	LL	LW	LA	PL	SV	MT	PR	CHO	FT	FB
Days to 50% flowering	1												
Plant height	-0.048	1											
Inflorescence length	0.379**	-0.159	1										
Leaf length	0.124	0.137	0.161	1									
Leaf width	0.090	0.022	-0.079	0.537**	1								
Leaf area	0.115	0.098	0.060	0.875**	0.862**	1							
Petiole length	0.432**	-0.024	0.345**	0.276**	0.070	0.198*	1						
Seed volume	0.345**	0.033	0.470**	0.311**	0.291**	0.342**	0.229*	1					
Moisture	0.091	-0.026	0.120	0.102	0.020	0.077	0.222*	0.195*	1				
Protein	-0.253**	-0.077	-0.135	0.056	0.012	0.045	-0.143	-0.053	0.155	1			
Carbohydrates	-0.111	0.473**	-0.207*	0.010	-0.093	-0.017	-0.371**	0.021	-0.057	-0.091	1		
Fat	0.156	0.247*	-0.004	0.339**	0.415**	0.422**	-0.010	0.375**	-0.100	0.121	-0.092	1	
Fibre	-0.233*	0.093	-0.172	-0.186	0.146	-0.026	-0.203*	-0.092	0.007	-0.216*	0.120	-0.208*	1
Ash	-0.044	0.202*	-0.218*	0.022	0.154	0.077	0.084	0.158	-0.024	0.010	0.074	0.118	0.403**
Iron	-0.025	0.100	-0.045	0.020	0.161	0.104	0.177	0.218*	0.010	-0.018	-0.068ns	0.224*	0.010
Zinc	-0.032	0.194*	0.080	0.156	-0.018	0.087	-0.281**	0.255**	-0.031	0.290**	0.292**	0.188	-0.183
Calcium	-0.035	-0.257**	-0.189	0.018	0.137	0.089	0.066	-0.217*	0.160	0.038	-0.143ns	0.006	0.251*
Tannins	0.059	-0.195*	-0.008	0.064	0.298**	0.184	0.043	0.258**	0.053	0.194*	-0.290**	0.143	0.132
Oxalates	0.061	0.249*	0.055	0.102	0.103	0.117	-0.069	0.231*	0.046	-0.032	0.237*	0.183	0.081
Grain yield per plant	0.335**	0.035	0.526**	0.303**	0.242*	0.311**	0.301**	0.889**	0.298**	0.011	0.024	0.227*	-0.056

*DFPF: days to 50% flowering, PH: plant height, IL: inflorescence length, LL: leaf length, LW: leaf width, LA: leaf area, PL: petiole length, SV: seed volume, GYPP: grain yield per plant, MT: moisture content, PR: protein, CHO: carbohydrates, FT: fat, FB: fibre, AH: ash, FE: iron, ZN: zinc, CA: calcium, TA: tannins, OX: oxalates. ns: non-significant; *: significant*

*at 5% level; **: significant at 1% level.*

Skewness and kurtosis of quantitative and nutritional traits

The frequency distribution of grain yield and its nine component and eleven nutritional traits was further examined through the coefficients of skewness and kurtosis, which provide indirect evidence of the type of gene action governing each trait and complement the correlation and divergence analyses discussed above. The skewness and kurtosis values estimated for all the traits studied are presented in Table 2.

Table 2: Skewness and kurtosis for quantitative and nutritional traits in grain amaranth germplasm

Trait	Skewness	Kurtosis
Days to 50% flowering	0.192854	1.018994
Plant height (cm)	-0.03378	-0.525
Inflorescence length (cm)	-0.27216	-0.33917
Leaf length (cm)	0.125552	-0.74311
Leaf width (cm)	0.10883	-0.25235
Leaf area (cm^2)	0.499679	-0.22862
Petiole length (cm)	-0.20399	-0.34761
Seed volume (g/10 ml)	0.001774	-1.2786
Moisture content (%)	0.230567	0.685724
Protein (%)	0.489018	-0.36776
Carbohydrates (%)	1.177117	4.405509
Fat (%)	0.035612	-0.70149
Fibre (%)	0.866234	0.807002
Ash (%)	0.641714	0.736476
Iron (mg/100 g)	1.177377	1.565886
Zinc (mg/ 100 g)	2.528243	9.878177
Calcium (mg/ 100 g)	0.368105	-0.47628
Tannins (%)	0.499759	-0.34725
Oxalates (mg/ 100g)	0.179873	-0.41652
Grain yield per plant	-0.2852	-0.63483

Skewness values were close to zero and low in magnitude for most of the traits studied, including plant height (−0.034), inflorescence length (−0.272), leaf length (0.126), leaf width (0.109), petiole length (−0.204), seed volume (0.002), fat content (0.036) and oxalate content (0.180), indicating a fairly symmetric, near-normal distribution for these traits across the germplasm and suggesting that they are governed predominantly by several genes of small, additive effect, a situation favourable for their improvement through simple selection procedures. Grain yield per plant likewise recorded a low, negative skewness (−0.285) together with a negative (platykurtic) kurtosis (−0.635), consistent with the polygenic, additive inheritance of yield widely reported in the crop and supporting the reliability of seed volume and inflorescence length, themselves near-normally distributed, as indirect selection criteria for yield improvement.

In contrast, several nutritional traits exhibited comparatively higher positive skewness, most notably zinc content (2.528), carbohydrate content (1.177), iron content (1.177), fibre content (0.866) and ash content (0.642), indicating that the distribution of these traits was skewed towards the lower end, with only a few genotypes recording exceptionally high content; such genotypes, being disproportionately rich in these nutrients, are of considerable value as potential donor parents for biofortification breeding. The corresponding kurtosis values reinforced this pattern, being markedly leptokurtic for zinc content (9.878) and, to a lesser extent, for carbohydrate (4.406), iron (1.566), fibre (0.807) and ash content (0.736), as well as for days to 50 per cent flowering (1.019), suggesting that these traits are influenced by relatively few genes of major effect or by non-additive gene action, whereas the remaining traits, which recorded negative (platykurtic) kurtosis, including seed volume (−1.279), leaf length (−0.743), fat content (−0.701) and plant height (−0.525), point towards predominantly additive, polygenic control. These observations are in general agreement with Dhanalakshmi et al. (2025), who also reported positive skewness and high kurtosis for days to 50 per cent flowering in grain amaranth and attributed such departures from normality to the involvement of complementary gene action; a similar inference can be drawn for zinc, carbohydrate and iron content in the present study, whose pronounced positive skewness and leptokurtosis reinforce the highly non-additive correlation pattern already noted for zinc with other mineral traits, and together suggest that recurrent or marker-assisted selection, rather than simple mass selection, would be more effective for improving mineral density, particularly zinc content, in this germplasm. Plant height and inflorescence length, on the other hand, recorded mildly negative skewness and kurtosis in the present germplasm, in contrast to the positively skewed, leptokurtic distribution reported for these two traits by Dhanalakshmi et al. (2025), a difference that may simply reflect differences in germplasm base and sample size between the two studies.

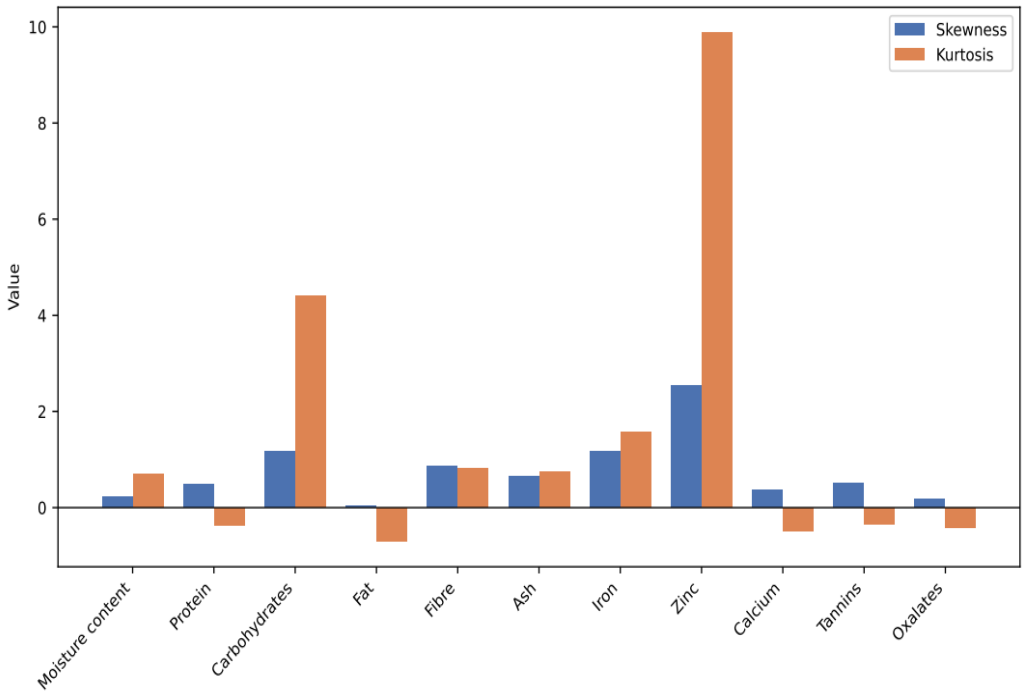


Fig.2- Skewness and kurtosis of nutritional traits in grain amaranth accessions

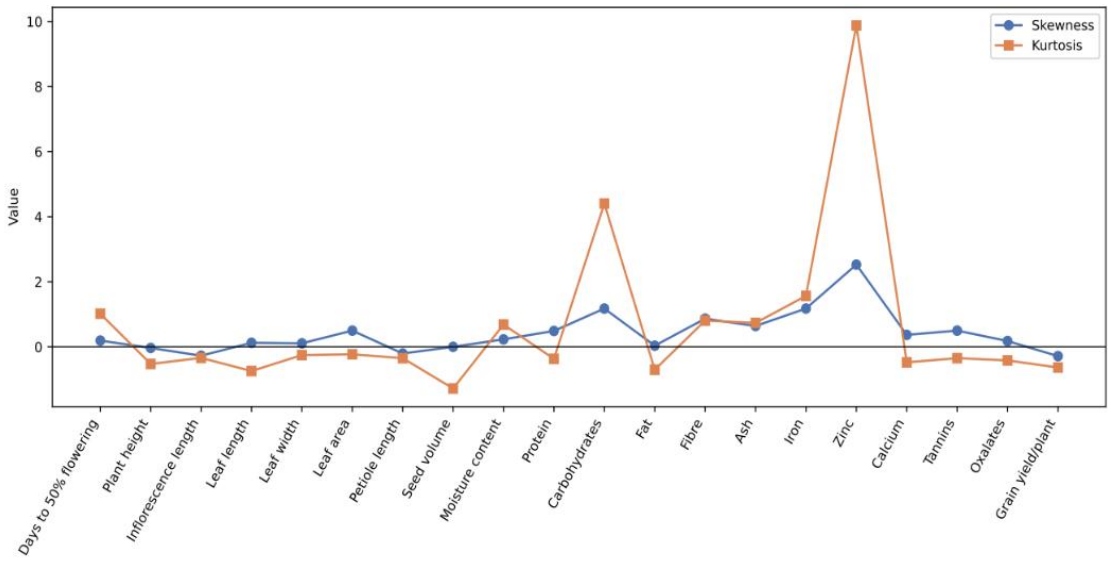


Fig.3- Skewness and kurtosis estimate for quantitative and nutritional traits in grain amaranth accessions

Inter-relationship among yield-attributing and nutritional characters

Days to 50 per cent flowering showed highly significant positive correlation with petiole length (r = 0.432), inflorescence length (r = 0.379) and seed volume (r = 0.345), indicating that relatively late-flowering genotypes tended to develop longer petioles, longer inflorescences and bolder seeds, a pattern consistent with the identification of petiole length and inflorescence length as major contributors to genetic diversity in amaranth reported by Yadav *et al.* (2022). Conversely, days to 50 per cent flowering showed highly significant negative correlation with protein content (r = −0.253) and significant negative correlation with fibre content (r = −0.233), suggesting that delayed flowering may reduce the accumulation of these quality components.

Plant height exhibited a highly significant positive correlation with carbohydrate content (r = 0.473) and significant positive correlations with fat (r = 0.247), ash (r = 0.202) and zinc content (r = 0.194), but significant negative correlations with calcium (r = −0.257) and tannin content (r = −0.195). Among the leaf-related traits, leaf length was highly significantly and positively correlated with leaf area (r = 0.875) and leaf width (r = 0.537), while leaf width and leaf area were themselves closely associated (r = 0.862); all three traits also showed consistent positive correlations with fat content, seed volume and petiole length. This close inter-relationship among leaf dimensions is consistent with the findings of Gerrano *et al.* (2015), who similarly reported leaf length and leaf area as tightly co-varying traits that jointly influence yield potential in

Amaranthus. Seed volume, besides its strong association with grain yield, also recorded significant positive correlations with fat (r = 0.375), zinc (r = 0.255) and iron content (r = 0.218), indicating that bolder seeds in this germplasm are generally associated with better nutritional quality — an association of considerable practical value, since seed volume can be visually or physically assessed with relative ease, making it a convenient morphological proxy for simultaneous selection of yield and mineral density.

Among the nutritional traits, protein content was significantly and positively correlated with zinc content (r = 0.290) but significantly negatively correlated with fibre content (r = −0.216); carbohydrate content showed a positive association with zinc (r = 0.292) but a highly significant negative correlation with petiole length (r = −0.371) and tannin content (r = −0.290); and zinc content exhibited a highly significant negative correlation with calcium content (r = −0.356), pointing to an inverse relationship between the accumulation of these two mineral nutrients. Such antagonistic relationships among mineral traits have a plausible genetic basis, as suggested by Prabhakaran *et al.* (2026), who identified distinct and, in some cases, overlapping marker-trait associations governing calcium, iron and zinc accumulation in grain amaranth through genome-wide association mapping, indicating that simultaneous improvement of all mineral traits may require careful marker-assisted selection strategies rather than simple phenotypic selection alone.

Taken together, the correlation studies clearly establish seed volume and inflorescence length as the most reliable and consistent indirect selection criteria for improving grain yield per plant in this germplasm, given their strong, positive and highly significant correlations with yield. The positive associations of grain yield with several nutritional traits, particularly zinc, fat and tannin content, indicate encouraging scope for combining high productivity with improved nutritional quality, while the significant negative association of grain yield with calcium content, and the inverse relationship between zinc and calcium accumulation, highlight the need for balanced, multi-trait selection strategies rather than single-trait selection when breeding for nutrient-dense, high-yielding grain amaranth cultivars.

Genetic divergence (Mahalanobis D² analysis)

Selection of genetically divergent parents is fundamental to the success of any hybridization programme, as crosses between diverse parents are more likely to yield desirable transgressive segregants and greater heterosis. The Mahalanobis D² statistic, being largely independent of environmental effects, provides a reliable measure of genetic divergence among genotypes based on multiple traits simultaneously.

Clustering pattern

The 52 grain amaranth accessions were grouped into five distinct clusters by Tocher's method, as shown in Table 3. Among the five clusters, Cluster III was the largest, comprising 29 genotypes, followed by Cluster II and Cluster V, each with 10 genotypes, and Cluster IV with 2 genotypes, while Cluster I was represented by a single genotype, Suvarna. This clustering pattern, involving grouping of the germplasm into five clusters, is consistent with several earlier reports in amaranth, including Kujur and Kurrey (2017) and Kumar *et al.* (2019), who also grouped 25 *Amaranthus* genotypes each into five clusters using D² analysis, and Sharma *et al.* (2026), who similarly obtained five clusters among Indian *Amaranthus* genotypes using morpho-molecular data. The predominance of a large proportion of the germplasm within a single cluster (Cluster III) indicates a comparatively narrow genetic base among these accessions, whereas the solitary placement of Suvarna in Cluster I reflects its marked distinctiveness from the rest of the germplasm. Importantly, the distribution of genotypes among clusters did not correspond with their geographic origin, suggesting that factors such as genetic drift, natural and artificial selection pressures, and differential adaptation have played a greater role than geographic separation in shaping the present pattern of diversity.

Table 3: Grouping of 52 grain amaranth accessions into different clusters by Mahalanobis' D² statistic method

Cluster	No. of genotypes	Genotypes
I	1	Suvarna
II	10	KBGA15, IC328893, IC95476, EC519520, IC0038267, IC37156, EC277969, KBGA1, IC35368, GA2
III	29	IC120662, IC120615, IC0106354, IC0554102, IC0120652, IC031100, IC0278926, IC0281939, EC519510, IC107249, IC328965, IC0107322, IC0415331, IC108811, IC0625391, IC108420, BGA49, KBGA4, VL115, KBGA16, BGA2, KBGA18, EC345797, AMT RED, AMT GREEN, MIRKERE, AMT DARKRED, IC0024262, IC415269
IV	2	IC0448736, IC95480
V	10	IC061907, IC415232, EC239385, EC519515, SKNA1803, GA5, SKNA1804, KBGA17, IC107255, NIC35334

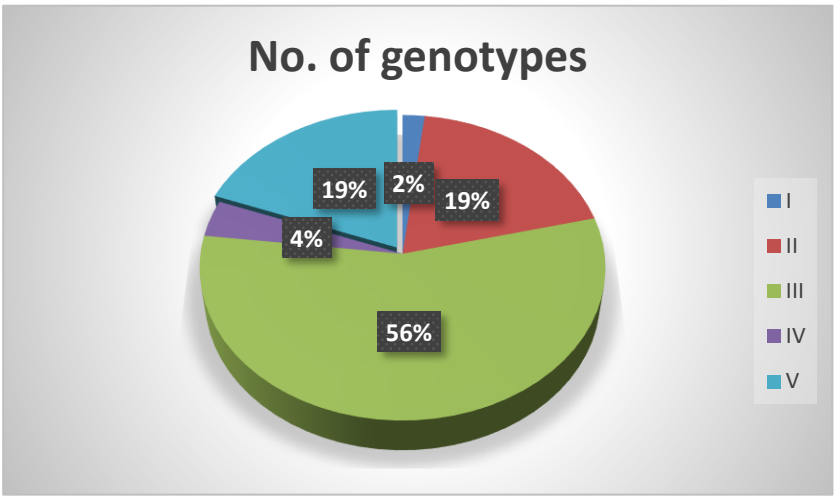


Fig-4 Distribution of genotypes across different clusters

Intra- and inter-cluster distances

The maximum inter-cluster distance was recorded between Cluster I and Cluster IV (77.911), followed by Cluster I and Cluster V (65.446) and Cluster I and Cluster II (64.107), indicating that genotypes placed in these clusters are genetically the most divergent and could be profitably utilized as parents in hybridization programmes to obtain greater heterosis and wider variability in segregating generations. Conversely, the minimum inter-cluster distance between Cluster II and Cluster III (38.889) indicated a comparatively close genetic relationship among genotypes of these clusters, and crosses between such closely related genotypes are less likely to yield substantial heterotic gains. The intra-cluster distance was highest for Cluster IV (40.202), followed by Cluster III (37.043) and Cluster V (35.287), reflecting the presence of considerable genetic diversity even within these clusters, which could be further exploited through intra-cluster selection. Cluster II recorded the lowest intra-cluster distance (34.518) among the multi-genotype clusters, implying a comparatively close genetic relationship among the genotypes within this cluster (Table 4).

Table 4: Average intra (diagonal, bold) and inter-cluster distance in grain amaranth accessions based on Mahalanobis' D² statistic method

Cluster	I	II	III	IV	V
I	**0.00**	64.107	63.196	77.911	65.446
II		**34.518**	38.889	50.942	39.503
III			**37.043**	48.852	39.437
IV				**40.202**	50.893
V					**35.287**

Cluster means for quantitative and nutritional traits

The cluster means for the nine quantitative and eleven nutritional traits are presented in Table 5 and revealed wide variation among the five clusters for all the traits studied, indicating the presence of considerable genetic diversity in the germplasm evaluated. Cluster IV recorded the highest mean grain yield per plant (38.26 g), the tallest plant height (124.50 cm), the highest seed volume (12.70 ml), and superior mean values for protein (16.86%), fat (4.72%) and zinc content (4.83 mg/100g), identifying it as the most promising cluster for combining high yield with improved nutritional quality. Cluster V recorded the highest mean values for iron (9.27 mg/100g), calcium (208.70 mg/100g) and tannin content (17.47 mg/100g), together with the longest inflorescence (42.10 cm), making it a valuable source for mineral biofortification breeding, though its comparatively higher tannin and calcium content, both of which showed negative correlation with grain yield in the present study, would need to be considered while selecting parents. These findings are broadly in agreement with Sarker *et al.* (2022), who identified specific cluster groupings combining high yield with superior iron, zinc and calcium content in amaranth, and with the emphasis placed by Prabhakaran *et al.* (2026) on exploiting genetically divergent accessions for mineral biofortification in grain amaranth.

Table 5: Cluster means for nine quantitative and eleven nutritional traits using Mahalanobis' D² statistic method

Trait	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Days to 50% flowering	43.00	49.20	49.07	46.50	50.40
Plant height (cm)	112.00	107.80	113.78	124.50	105.98
Inflorescence length (cm)	39.00	37.40	41.83	40.00	42.10
Leaf length (cm)	10.25	11.53	12.51	11.60	11.57

Trait	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Leaf width (cm)	8.00	8.17	8.29	7.85	8.50
Leaf area (cm²)	101.50	94.38	104.70	92.55	98.90
Petiole length (cm)	13.86	13.78	16.39	12.93	15.28
Seed volume (ml)	9.50	10.82	10.73	12.70	11.06
Grain yield per plant (g)	25.90	29.99	31.06	38.26	30.55
Moisture content (%)	8.94	8.64	8.93	8.89	8.94
Protein (%)	11.84	7.61	10.51	16.86	12.43
Carbohydrates (%)	51.49	50.52	35.58	49.71	32.78
Fat (%)	2.70	3.61	4.22	4.72	4.49
Fibre (%)	13.45	8.94	8.71	8.31	11.75
Ash (%)	1.85	3.05	3.60	3.38	3.74
Iron (mg/100g)	7.22	6.34	5.52	6.05	9.27
Zinc (mg/100g)	1.61	2.10	2.13	4.83	1.88
Calcium (mg/100g)	188.00	169.00	178.79	148.50	208.70
Tannins (mg/100g)	13.65	15.86	16.41	16.53	17.47
Oxalates (mg/100g)	218.00	229.00	218.17	221.00	225.60

Based on these results, genotypes from Cluster IV and Cluster V, being genetically divergent from each other (inter-cluster distance of 50.893) as well as from the remaining clusters, may be prioritized as parents in hybridization programmes aimed at combining high grain yield with enhanced nutritional quality.

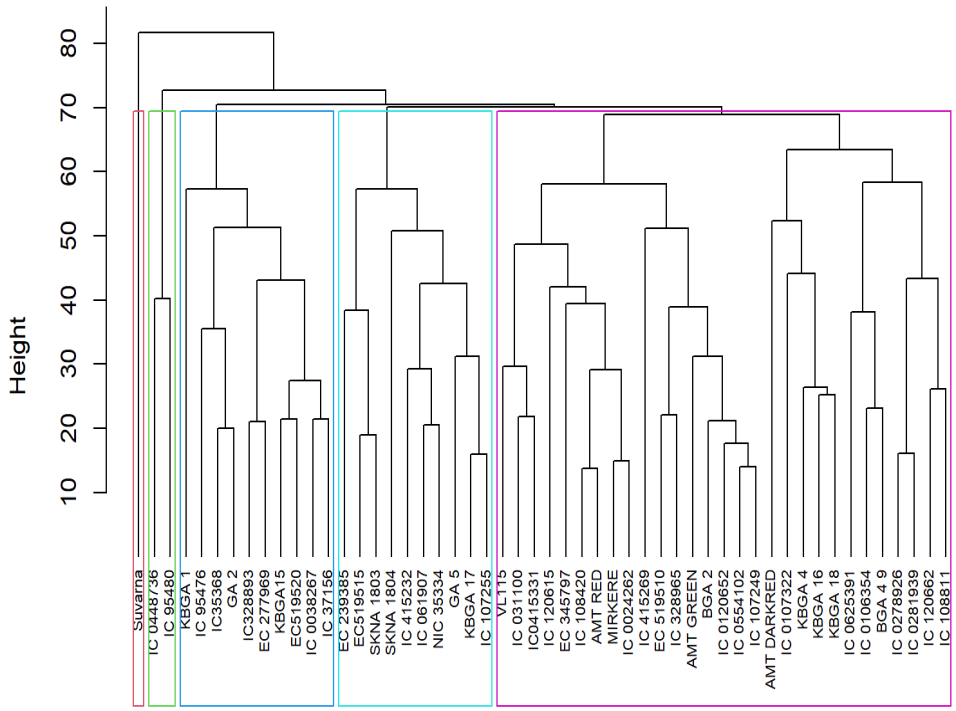


Fig. – 5 : Hierarchical clustering of 52 grain amaranth accessions based on Mahalanobis' D² distances

Breeding implications of correlation and divergence studies

The correlation and D² analyses carried out in the present study are strongly complementary from a breeding standpoint. While correlation analysis identifies seed volume and inflorescence length as the most reliable morphological traits for indirect selection of grain yield, the D² analysis identifies Clusters I, IV and V as the most genetically divergent groups within the germplasm, with Cluster IV combining high yield, protein and zinc content, and Cluster V combining superior iron, calcium and tannin content. Hybridization between genotypes drawn from these widely divergent clusters — for instance, between the high-yielding, protein- and zinc-rich genotypes of Cluster IV and the iron- and calcium-rich genotypes of Cluster V, or between either of these clusters and the distinctive check Suvarna (Cluster I) — is expected to generate wide variability and desirable transgressive segregants in the resulting progeny, given the strong positive correlation of grain yield with seed volume and inflorescence length and the substantial inter-cluster genetic distances observed. Such an integrated selection strategy, combining trait-based indirect selection criteria with divergence-based parental choice, offers a more efficient pathway for developing high-yielding, nutritionally superior grain amaranth cultivars than reliance on

either approach alone.

CONCLUSION

The present study demonstrated that grain yield per plant in grain amaranth is most strongly and reliably associated with seed volume and inflorescence length, both of which can serve as effective indirect selection criteria, while calcium content showed a significant negative association with yield that warrants attention in future selection. Mahalanobis' D^2 analysis grouped the 52 accessions into five genetically distinct clusters that did not correspond with geographic origin, with Cluster III harbouring the majority of the narrow-based germplasm and Clusters I, IV and V standing out as the most divergent groups, Cluster IV combining superior yield, protein and zinc content and Cluster V combining superior iron, calcium and inflorescence length. Hybridization between genotypes drawn from these divergent clusters, guided by the trait associations identified through correlation analysis, is recommended as an effective strategy for developing high-yielding, nutritionally enriched grain amaranth cultivars.

Competing Interests

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Declaration

This study involved field evaluation of plant germplasm only and did not involve human participants, human data, or animal subjects.

Authors' Contributions

Akash Basavaraddi conceived and conducted the field experiment, recorded observations, analysed the data and drafted the manuscript. Dhanalakshmi T.N. and Satish Yadav supervised the study and revised the manuscript critically for important intellectual content and contributed to the experimental design, data interpretation. Dinesh H. B, Rudragouda F. Channagouda, and Sudharani N, contributed for manuscript revision. All authors read and approved the final manuscript.

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REFERENCES

Dhanalakshmi TN, Madhusudan K, Ramesh S, Mohan Rao A, Srinivas Reddy KM, Anand SR. Third- and fourth-degree statistics-based genetics of grain yield and its contributing traits in grain amaranth (*Amaranthus* spp.). Mysore J Agric Sci. 2025;59(1):47-54.

Gerrano AS, Jansen van Rensburg WS, Adebola PO. Genetic diversity of Amaranthus species in South Africa. S Afr J Plant Soil. 2015;32(1):39-46.

Gins EM, Baikov AA, Khasanova SD, Goryunova SV, Gins VK, Gins MS, et al. Amaranthus species assessment for morphological and biochemical parameters. SABRAO J Breed Genet. 2024;56:1387-1399.

Kujur A, Kurrey VK. Genetic divergence analysis in Khedha Bhaji (*Amaranthus dubius*) genotypes for yield and its component characters. Int J Curr Microbiol Appl Sci. 2017;6(12):2248-2253.

Kumar Y, Prasad R, Chameli. Genetic divergence analysis in amaranthus (*Amaranthus* spp.) genotypes for yield and its component characters. Pharma Innov J. 2019;8(12):109-112.

Mahalanobis PC. On the generalised distance in statistics. Proc Natl Inst Sci India. 1936;2:49-55.

Prabhakaran S, Chauhan R, Joshi DC, Kumar S, Nayak JK, Chandora R, et al. Deciphering the genetic determinants of calcium, iron, magnesium and zinc accumulation in grain amaranth (*Amaranthus hypochondriacus*) through a multi-environment GWAS approach. BMC Genomics. 2026;27:287.

Rao CR. Advanced statistical methods in biometrical research. New York: John Wiley and Sons; 1952.

Sarker U, Azam MG, Talukder MZA. Genetic variation in mineral profiles, yield contributing agronomic traits, and foliage yield of stem amaranth. Genetika. 2022;54(1):91-108.

Savaliya PM, Prajapati NN, Solanki RS. Estimation of genetic variability and association of yield and related traits in indigenous grain amaranth (*Amaranthus hypochondriacus* L.) genotypes. Electron J Plant Breed. 2024;15(3):714-719.

Sharma AK, Sagar V, Dwivedi SV, Devi J, Neetu, Tiwari SK, et al. Characterization of genetic diversity in *Amaranthus* species using morpho-molecular markers. Indian J Genet Plant Breed. 2026:1-17.

Showemimo FA, Soyombo MA, Amira JO, Porbeni JBO. Traits selection criteria for genetic improvement of grain and leafy amaranth (*Amaranthus* spp.) using principal component analysis. Egypt J Agric Res. 2021;99(2):170-179.

Singh RK, Chaudhary BD. Biometrical methods in quantitative genetic analysis. New Delhi: Kalyani Publishers; 1977.

Snedecor GW, Cochran WG. Statistical methods. 6th ed. New Delhi: Oxford and IBH Publishing Co.; 1967.

Yadav S, Kumar V, Pooniya M, Kumar A, Chaturvedi SK, Singh A. Genetic variability, correlation and principal component analyses in grain amaranth under water limited conditions of Bundelkhand region. J Cereal Res. 2022;14(3):268-274.