



Morphological and Molecular Evaluation of Potential Indigenous Grain Amaranth (*Amaranthus hypochondriacus* L.) Genotypes of India

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ABSTRACT: The presence of genetic variation and its maintenance are essential components in plant breeding. Considering these, ten phenotypic characters, one biochemical component and seven microsatellite primers were employed to investigate the genetic diversity in forty-six grain amaranth genotypes. The values of GCV were lower than that of PCV for each individual character under investigation. Seed yield plant⁻¹ (g) demonstrated a strong genotypic level association with days to flowering, leaf area plant⁻¹(cm²), length of inflorescence (cm), plant height (cm) and harvest index (%). The path coefficient study revealed that harvest index (%) had the strongest direct positive impact on seed yield plant⁻¹ (g). Seven ISSR primers resulted in a total of 46 DNA amplicons, from which 40 were polymorphic. The data obtained from all markers and related amplicons classified the genotypes into six UPGMA based clusters. Among the grain amaranth genotypes RGA-16 and RGA-17 evident maximum similarity; while the genotypes BGA-10 and BGA-4-9 shown minimum similarity on the basis of Jaccard's co-efficient result. Breeding of new cultivar using this varieties will helpful in the crop improvement programmes. The results of the present investigation concluded that to obtain actual genetic diversity, the molecular and morphological markers should be used in tandem.

Keywords: DNA amplicons, Genetic variation, Grain amaranth, ISSR, Path co-efficient.

INTRODUCTION

Grain amaranth is a nutritionally prospective C₄ photosynthetic plant with wide adaptability to various climatic conditions. Most of the cultivated species are monoecious and pollinated through wind, but the grain species with colourful inflorescence are occasionally visited by bees (Khoshoo and Pal 1972). Diploid genus amaranth with x = 16 and x = 17 chromosomes, almost equally disseminated in section Amaranth (Rastogi and Shukla, 2013). From total 30 different species of Amaranthus, four have 2n = 32, while all others have 2n = 34. Among the 30 species, *Amaranthus caudatus* L. (2n = 32), *Amaranthus cruentus* L. (2n = 34) and *Amaranthus hypochondriacus* L. (2n = 32) are domesticated species, which are the most important for the production of amaranthus grain. Amaranthus is certainly a unique crop in terms of agricultural production, requiring specific consideration and attention to detail in every step of its cultivation, from sowing to harvesting and storing (Shellikeri *et al.*, 2022).

The collection, regular maintenance as well as assessment of germplasm are most important and

primary steps in any crop improvement programme (Sarker *et al.*, 2017; Venkatesh *et al.*, 2014). The genetic advance, heritability, and genotypic and phenotypic coefficients of variation are useful in understanding the nature of inheritance of different traits. To estimate the amount of heritable variation and for efficient genotype selection, heritability can be used in combination with estimates of genetic advance and genetic advance over a mean (Mawblei *et al.*, 2022). Yield being a complex trait, its expression affected by the several allied traits. Correlation coefficient is the tool to study this association; while, path analysis might elucidate direct and indirect relations among the characters (Farshadfar and Farshadfar 2008). Although it corresponds to the variation that is passed down to the next generation, genotypic variation, which is measured as genotypic variance, is essential for every crop improvement effort. Heritability, which measures the ratio of genetic variation to the total amount of phenotypic variation in the traits, is a useful indicator of how traits are passed down from parents to their offspring (Rana *et al.*, 2022). ISSR produces multiple bands during a single amplification, and widely used in intra-specific diversity analysis in Amaranthus (Singh

et al., 2013). With above views, current experiment was undertaken to find the morphological and molecular genetic diversity followed by path analysis for some important agronomics and biochemical traits in amaranthus to accelerate the future breeding programs.

MATERIALS AND METHODS

Plant material and field trial. A set of forty-six genotypes of grain amaranthus (Table 1), obtained from AICRN on potential crops at Centre for Crop Improvement (24°19' N latitude; 27°19' E longitude and at an altitude of 154.52 metres exceeding sea level), S. D. Agricultural University, Sardarkrushinagar, Gujarat were used in the current experiment during rabi 2018-19. With three replications, the RBD design was employed to examine all forty-six genotypes. All seeds were sown in a seed bed with a row length of 3.0 m and a spacing of 45 cm × 15 cm (two rows per genotype). All the cultural practices were performed as per recommendation. From each genotype in each replication, five competitive plants were chosen on plot basis, and eleven phenotypic trait was collected for days to flowering, leaf area plant⁻¹ (cm²), days to maturity, length of inflorescence (cm), width of inflorescence (cm), plant height (cm), stem girth (cm), harvest index (%), test weight (g10ml⁻¹), protein content (%), seed yield plant⁻¹ (g).

DNA extraction and ISSR-PCR. Using the cetyl trimethyl ammonium bromide extraction method (CTAB) described by Doyle and Doyle (1990) with some modifications, the genomic DNA of each of the forty-six genotypes of grain amaranth was extracted from tender, fresh leaves of each genotype (Dharajiya *et al.* 2017). 1X TE (Tris-EDTA) buffer was used to dissolve in and store all the genomic DNA samples at -20 °C for further use. To assess the genomic DNA purity, agarose gel electrophoresis (0.8%) was employed (Primrose and Twyman 2013). Using a nano-drop spectrophotometer, the quantity and quality were analyzed (Eppendorf, Germany). A PCR reaction was comprising of 50 ngμl⁻¹ template DNA, 20 pmoleμl⁻¹ concentration of forward and reverse primers, 10X PCR buffer, 10 mM of dNTPs, 2.5 mM MgCl₂, and 1.0 U of Taq DNA polymerase. Seven ISSR (inter simple sequence repeat) markers were employed to determine the molecular diversity of the genotypes under examination (Table 4). Using the Fluor Chem FC gel documentation system, electrophoresis on a 1.5% agarose gel was carried out to separate the amplification products from the standard DNA marker. Then gel was photographed under UV light (Alpha Innotech Corporation, USA).

Statistical assessment. Analysis of variance, genotypic and phenotypic correlation using phenotypic data of 11 characters was performed as suggested by Panse and Sukhatme (1978). Path analysis was carried out as per the method suggested by Dewey and Lu (1959) for partitioning the genotypic correlation coefficient into direct and indirect effects. DNA fragments amplified through PCR amplification were assessed as binary data. Jaccard's similarity coefficient (Jaccard, 1908)

was used to construct matrix from binary data, and the unweighted pair group method with arithmetic mean was employed for clusters analysis (UPGMA).

RESULTS AND DISCUSSION

Genetic variability. The existence of considerable genetic variation in the experimental material was noticeable from the analysis of variation (Table 2). For all the characters under study, the values of the phenotypic coefficient of variation (PCV) remained marginally greater than the genotypic coefficient of variation (GCV), implying a lesser impact of the environment on the expression of traits (Kumar *et al.* (2018). The same findings have been reported by Venkatesh *et al.* (2014); Sheelamary and Phogat (2016); Kumar and Murthy (2017); Shrivastav *et al.* (2017); Kumar *et al.* (2018), Nevani *et al.* (2022). The magnitude of PCV and GCV was moderate to high for seed yield plant⁻¹ (40.49 % and 40.89 %, respectively), leaf areaplant⁻¹(39.59 % and 40.15 %, respectively), and harvest index (15.64 % and 17.39 %, respectively). Kumar *et al.* (2018) observed similar result for seed yield plant⁻¹(g) test weight (g10 ml⁻¹) and stem girth (cm). Heritability H²_(bs) (%) was measured highest for seed yield plant⁻¹ (98.06%) followed by leaf area plant⁻¹ (97.20%) and days to maturity (87.86%). Similar significant heritability was found by Gelotar (2018) for days to flowering, days to maturity, plant height (cm), leaf areaplant⁻¹(cm²), inflorescence length (cm) and seed yield plant⁻¹ (g). The seed yield plant⁻¹(82.605%) also had largest genetic advance (GA) expressed as a percentage of mean values, followed by leaf area plant⁻¹ (80.405%) whereas, test weight showed lowest value (7.945%) of it (Table 2). The existence of additive gene action is confirmed by the high heritability and high genetic advance as a percentage of the mean for traits *viz.*, leaf area plant⁻¹ (cm²), length of inflorescence(cm), harvest index and seed yield plant⁻¹(g). Shah *et al.* (2018) reported the similar result for days to flowering, plant height (cm), inflorescence length(cm) and test weight (g10 ml⁻¹). Graphical comparison of GCV (%), PCV (%), heritability (%) and GAM (%) for eleven characters in grain amaranth represented in Fig. 1.

Correlations and path analysis. The multifaceted characteristic of seed yield is determined by a number of influencing characteristics. In the current experiment, the genotypic correlations were larger than the corresponding phenotypic correlations, demonstrating a significant genetic association between the various characteristics (Table 3). Seed yield plant⁻¹ demonstrated a strong significant and positive association with days to flowering ($r_g = 0.409$), leaf area plant⁻¹($r_g = 0.767$), length of inflorescence ($r_g = 0.604$), plant height ($r_g = 0.503$) and harvest index ($r_g = 0.782$) at genotypic level. Similar results were also observed by Prajapati *et al.* (2005); Shrivastav *et al.* (2017), Sagar *et al.* (2018); Gelotar (2018). The path coefficient study showed that harvest index (0.607) noted the highest significant positive direct impact on seed yield plant⁻¹ followed by leaf area plant⁻¹ (0.354). Leaf area plant⁻¹(cm²) exhibited highest positive indirect effect (0.276) on seed yield plant⁻¹(g) along

with harvest index (%). On other hand, test weight (-0.012) and protein content (-0.045) exhibited negative direct effect on seed yield plant⁻¹ (Fig. 3). Residual effect estimated was 0.069, indicating the involvement of most of the characteristics were examined explaining the diversity. The strongest positive direct impact on days to flowering for seed yield plant⁻¹(g) was also reported by Gelotar (2018). Tiwari (2018) reported similar result for days to maturity, length of inflorescence(cm) and plant height (cm) on seed yield plant⁻¹(g).

ISSR analysis. The ISSR primers utilized in the investigation were polymorphic, and the results of these primers are shown in Table 4. Seven ISSR markers yielded a total of forty-six DNA amplicons, forty of which have been polymorphic, according to the data collected from them. Average bands per primer were 6.57. On an average, each primer indicated 5.71 polymorphic loci. Collectively, the range of PCR-amplified DNA fragments was 125 to 2429 bp. Primer ISSR 1 demonstrated the maximum number of amplicons (8), while primer ISSR 3 demonstrated the lowest amplicons (3). Primer ISSR 1 had the greatest polymorphism (100%) whereas primer ISSR 4 demonstrated the least polymorphism (75.00%). In the current study, the overall percentage of polymorphism among *Amaranthus* genotypes found by the ISSR primers was 85.79%. The mean PIC value of the primers in the study was 0.572, indicating acceptable

primer selection for the evaluation of genetic diversity (Faseela and Joseph 2007).

The analysis indicated that the ISSR markers employed in the current study had produced a significant percentage of polymorphism, allowing their use to assess genetic variability. Similar result for PIC was also reported by Gelotar (2018) (87.15%). Singh *et al.* (2013) reported 77.15 per cent polymorphism in amaranth genotype which is quite lower than the present study. To compare a pair of parameters, a similarity matrix was created using Jaccard's coefficients. Seven ISSR markers were employed to determine similarity indices, with values in the range from 0.320 (between BGA-4-9 and BGA-10) to 0.930 (between RGA-16 and RGA-17). The Jaccard's coefficient and the dendrogram were derived using software NTSYSpc (Version 2.20 N). The forty-six genotypes were categorised into six clusters, namely clusters A, B, C, D, E, and F (Fig.2) by the dendrogram (Jaccard's distance, paired-group) clustered with the data produced by all markers and their amplicons. The dendrogram showed a correlation co-efficient of 0.720. The cluster A divided into A₁, A₂ and A₃ sub cluster. A₁ contains 9 genotypes, A₂ contains 22 genotypes and A₃ contains 4 genotypes whereas cluster B, C, D, E and F contains 4, 2, 1, 2 and 2 genotypes, respectively. Gelotar (2018) also reported a comparable result for the genotype distribution. Hence, this work can help in the molecular characterization and diversity analysis of *Amaranthus* species at large scale.

Table 1: List of genotypes under diversity studies.

Sr. No.	Genotypes	Sr. No.	Genotypes	Sr. No.	Genotypes
1.	SKGPA-61	17.	SKNA-1407	32.	KGBA-4
2.	SKGPA-73	18.	SKNA-1414	33.	KGBA-5
3.	SKGPA-150	19.	SKNA-1501	34.	KGBA-7
4.	SKGPA-155	20.	SKNA-1502	35.	RMA-62
5.	SKNA-401	21.	SKNA-1503	36.	RMA-63
6.	SKNA-403	22.	SKNA-1508	37.	RGA-13
7.	SKNA-808	23.	SKNA-1510	38.	RGA-14
8.	SKNA-813	24.	GA-2	39.	RGA-16
9.	SKNA-903	25.	GA-3	40.	RGA-17
10.	SKNA-908	26.	BGA-4-9	41.	RGA-18
11.	SKNA-1201	27.	BGA-7	42.	RGAG-12-22
12.	SKNA-1207	28.	BGA-9	43.	RGAG-14-3
13.	SKNA-1305	29.	BGA-10	44.	RHGA-13-3
14.	SKNA-1313	30.	BGA-14	45.	MGA-502
15.	SKNA-1403	31.	BGA-20	46.	Suvama
16.	SKNA-1406				

Source: -Indigenous collection of germplasm of grain amaranth from AICRN on potential crops at Center for Crop Improvement S.D.A.U, S.K. NAGAR.

Table 2: Analysis of variance and genetic parameters of variation in grain amaranth.

Characters	Genetic parameters					Mean sum of square		
	GCV (%)	PCV (%)	H ² (%)	GA	GAM (%)	Replications	Treatments	Error
						DF - 2	DF - 45	DF - 90
Days to flowering	8.24	9.69	72.37	7.928	14.44	9.66	69.20**	7.81
Leaf areaplant ⁻¹ (cm ²)	39.6	40.2	97.2	1261.7	80.4	5867.17	1168839.87**	11103.4
Days to maturity	6.19	6.6	87.86	12.84	11.95	6.61	138.90**	6.11
Length of inflorescence (cm)	11.6	13.6	72.54	12.41	20.38	44.22	169.06**	18.94
width of inflorescence (cm)	6.81	9.71	49.18	1.94	9.79	5.74	7.35**	1.87
Plant height (cm)	8.32	9.86	71.21	21.74	14.47	182.63	532.724**	63.26
Stem girth (cm)	10.8	12.3	76	0.78	19.25	0.15	0.64**	0.06
Harvest index (%)	15.6	17.4	80.87	5.9	28.97	1.62	32.87**	2.39
Test weight (g10 ml ⁻¹)	5.07	6.68	57.69	0.6	7.94	0.022	0.58**	0.11
Protein content (%)	10.3	11.8	76.62	2.4	18.63	1.37	5.85**	0.54
Seed yield plant ⁻¹ (g)	40.5	40.9	98.06	20.04	82.6	3.96	291.48**	1.89

*, ** Significant at 5 and 1 per cent levels of significance, respectively.

sTable 3: Genotypic and phenotypic correlation coefficient for different characters in grain amaranth.

Characters	Days to flowering	Leaf areaplant ¹ (cm ²)	Days to maturity	Length of inflorescence (cm)	Width of inflorescence (cm)	Plant height (cm)	Stem girth(cm)	Harvest index (%)	Test weight (g10ml ⁻¹)	Protein content (%)	Seed yield plant ⁻¹ (g)
rg		0.425**	0.445**	0.482**	0.491**	0.591**	0.375**	0.015	0.176*	0.061	0.409**
rp		0.356**	0.361**	0.297**	0.287**	0.416**	0.255**	0.002	0.158	0.021	0.354**
rg			0.378**	0.516**	0.355**	0.339**	0.140	0.454**	0.389**	0.199*	0.767**
rp			0.348**	0.433**	0.279**	0.290**	0.128	0.405**	0.300**	0.185*	0.755**
rg				0.514**	0.404**	0.591**	0.437**	-0.121	0.081	-0.006	0.309**
rp				0.372**	0.249**	0.493**	0.381**	-0.104	0.020	-0.005	0.282**
rg					0.293**	0.610**	0.256**	0.360**	0.085	0.168*	0.604**
rp					0.273**	0.487**	0.209*	0.281**	0.088	0.133	0.520**
rg						0.404**	0.284**	0.081	0.478**	-0.035	0.443**
rp						0.300**	0.232**	0.076	0.240**	0.002	0.320**
rg							0.620**	0.170*	0.078	0.126	0.503**
rp							0.488**	0.143	0.084	0.089	0.427**
rg								0.050	0.024	0.035	0.344**
rp								0.029	0.072	0.064	0.313**
rg									0.258**	0.334**	0.782**
rp									0.163	0.230**	0.711**
rg										0.167	0.371**
rp										0.096	0.287**
rg											0.239**
rp											0.203*

*, ** Significant at 5 and 1 per cent level of significance, respectively.

Table 4: Polymorphism study used with 7 ISSR primers for forty-six genotypes of grain amaranth.

Primer name	Sequence	Total No. of loci	No. of polymorphic loci	No. of monomorphic loci	% of polymorphic loci	Total fragment amplified	PIC	Range of molecular weight (bp)
ISSR1	CACACACACACACACAGT	8	8	0	100.00	209	0.592	141 to 2429
ISSR2	CACACACACACACACAGC	8	7	1	87.50	174	0.641	125 to 1626
ISSR3	CACACACACACACACAGG	7	6	1	85.71	139	0.589	168 to 761
ISSR4	CACACACACACACACAGA	4	3	1	75.00	100	0.558	170 to 854
ISSR21	GCCTCTCTCTCTCTCT	7	6	1	85.71	235	0.542	308 to 1970
ISSR22	GACTCTCTCTCTCTCT	6	5	1	83.33	166	0.456	232 to 934
ISSR23	GTCTCTCTCTCTCTCT	6	5	1	83.33	150	0.624	308 to 2395
Total		46	40	6	-	-	-	-
Mean		6.57	5.71	0.85	85.79	-	0.572	-
Range		-	-	-	75.00 to 100.00	-	0.456 - 0.641	-

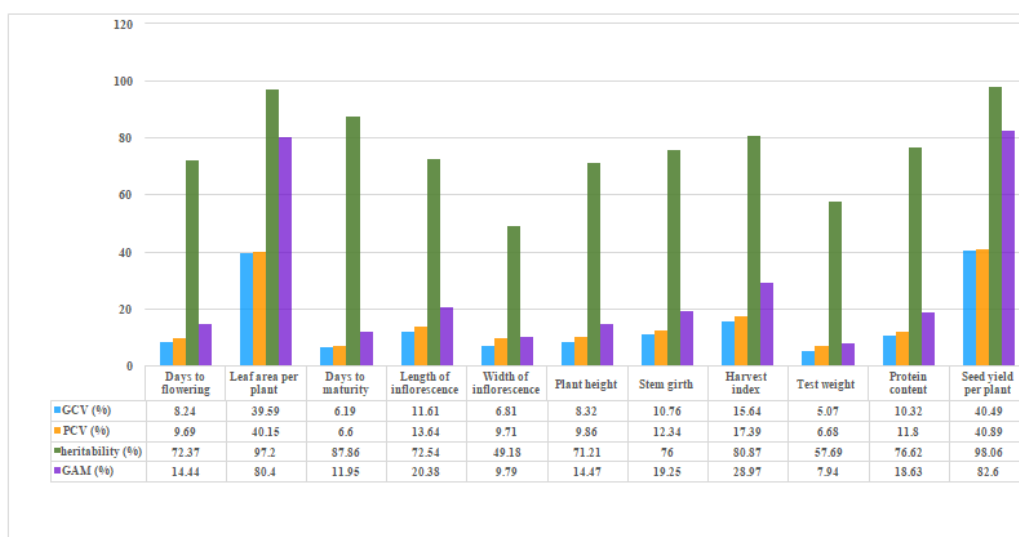


Fig. 1. Graphical comparison of GCV (%), PCV (%), heritability (%) and GAM (%) for eleven characters in grain amaranth.

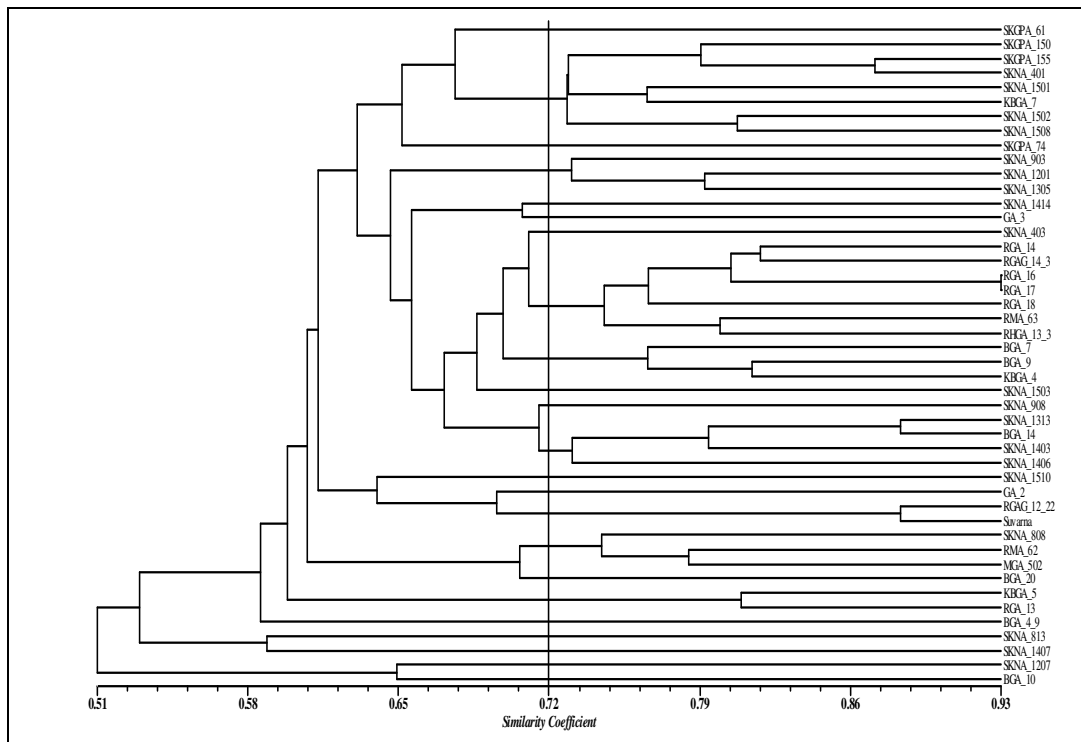


Fig. 2. UPGMA-based dendrogram of grain amaranth genotypes.

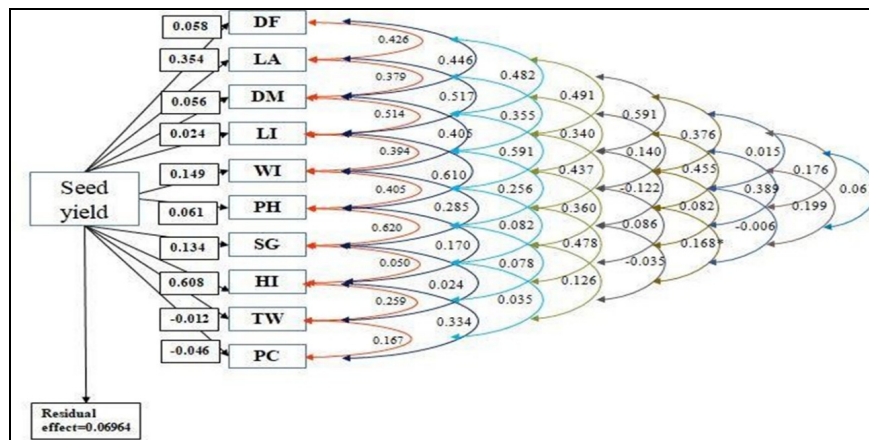


Fig. 3. Path diagram of grain amaranth genotypes.

Where,

DF = Days to flowering, LA = Leaf area per plant (cm²), DM = Days to maturity, LI = Length of inflorescence (cm), WI = Width of inflorescence (cm), PH = Plant height (cm), SG = Stem girth (cm), HI = Harvest index, TW = Test weight (g/10 ml) and PC = Protein content (%).

CONCLUSIONS

While imposing selection for genetic improvement of grain amaranth, due weightage should be given on days to flowering, length of inflorescence (cm), leaf area plant⁻¹(cm²) plant height (cm) and harvest index (%). Also, ISSR markers used in this study appeared to be worthy for the molecular assessment and evaluating the genetic relationship among the genotypes of grain amaranth.

FUTURE SCOPE

In grain amaranth genetic diversity analysis can also be used to for particular characteristics through

intergression of alien genes, heterosis breeding, transgressive breeding. In order to differentiate between lines (genotypes) which are used in a future breeding programme so that molecular markers are employed in diversity analysis.

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Conflict of Interest. None.

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