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Assessment of genetic diversity through D^2 analysis in underutilized indigenous potential grain amaranth (*Amaranthus hypochondriacus* L.) genotypes of India

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Abstract
Genetic diversity analysis was coordinated at Center for Crop Improvement (24°19' N latitude; 27°19' E longitude and at altitude of 154.52 meters above the sea level) Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, District: Banaskantha (Gujarat). In this study, 46 genotypes of amaranth were evaluated for eleven yields and yield accredited traits and grouped into twelve clusters through Mahalanobis D^2 analysis. Cluster II contained maximum number of genotype (10), Cluster I (9), Cluster III (8), Cluster IV (6) and Cluster V has 3 genotypes. Clusters VII, VIII and X have (2 in each) and Clusters VI, IX, XI and XII (1in each case). Among them, clusters VI, IX, XI and XII were monogenotypic, rest cluster I, II, III, IV, V, VII, VIII and X were polygenotypic based on genetic divergence. Intra-cluster distance was highest in cluster VII (63.27) followed by cluster V (58.82) and cluster II (56.20), suggesting there is a lot of extent for swapping of genes among genotypes within these clusters. The inter cluster values ranged from 71.00 to 874.76. Concerning to inter cluster distance the cluster X and cluster I were most diverse with each other as distance between them was 874.76. It is advantageous for genotypes from clusters viewing high inter-cluster distance (cluster X and XI) for advance crop hybridization programme to generate wide range of transgressive segregants in population to develop high yielding grain amaranth varieties. Contribution to total divergence was higher from Leaf area per plant (cm^2), seed yield per plant (g), Days to maturity.

Keywords: Amaranth, Genetic diversity, D^2 analysis, cluster, transgressive segregants

Introduction

Grain amaranth is fast growing, dicotyledonous, belonging to genus *Amaranthus* and family *Amaranthaceae*. The word "Amaranth" is basically derived from Greek word "Anthos" which means "everlasting" according to Sankaran (1943) [12]. Grain amaranth is a nutritionally potential photo-synthetically efficient C_4 plant which is highly adaptable to various climatic conditions. Leaf colour is green or red or with different shades observed. The height of mature plants varies between 0.3 m and 2.5 m, depending on the species, growth habit and environment. Some species have distinct markings on their leaves.

The genus amaranth is dibasic with (x = 16, 17) chromosomes, almost equally distributed in section amaranth. Grant (1959) [3] has reported the information regarding the chromosome number of 30 species of *Amaranthus.* Among these, 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 and among these three species, *Amaranthus caudatus* L. is drooping type and rest two are erect type species. Terminal and auxiliary inflorescences also occur. Most of the cultivated species are monoecious, wind pollinated, but the grain species with colourful inflorescence are occasionally visited by bees (Khoshoo and Pal, 1970) [4].

Sauer (1993) [13] believes the progenitor of *Amaranthus cruentus* L. and *Amaranthus hybridus* L. which is currently found over a wide range of plains and mountains in North, Central and South America. *Amaranthus hypochondriacus* L. has characteristics of both *Amaranthus cruentus* L. and the wild species *Amaranthus powelli* L. and it may be a hybrid of the two. Chemical composition and nutritional value of grain amaranth (Dodok *et al.,* 1994) [2] confirms its high potential for use as human and animal nutrition as well as medicine (Teutenica and Knorr, 1985) [17]. With the increasing need of exploring alternate sources of food, it is necessary to accelerate and expand the production of grain amaranth.

Narrow genetic base of this self-pollinating crop (Schulz-Schaeffer *et al*., 1991) [14] has been a main problem in its genetic improvement. Exploiting the gene pool from different geographic collections could increase diversity of the crop and the possibility of improving desirable characters in amaranth.

Genetic diversity of this crop is crucial for selecting promising parent plants for direct cultivation and for hybridization to obtain progenies of high grain productivity and desirable adaptive characters. Genetic divergence for metric traits was to a great extent subjective to an environment. A substitute method of parent selection, employed by the breeders often is interpretation of genetic diversity (Diniz *et al*. 2005) [1]. This may consent to grouping of lines, so lines fit in to extreme groups inter crossed will provide most propitious result along with diminution of time and expanses. Multivariate analysis (D^2 statistics), developed by Mahalanobis (1936) [7], is the most commanding tool to assess genetic divergence in the specified set of genotypes (Murthy & Arunachalam 1966) [8]. Other objectives were to determine grouping pattern of genotypes, identify divergent genotypes for desirable characters, and determine characters accounting for variation among genetic groupings. This research was conducted to examine accessions from different locations for yield improvement and sustainable utilization of grain amaranth.

Materials and Methods
Forty-six eco-geographically distinct genotypes (Table-1.) of Grain amaranth (*Amaranthus hypochondriacus* L.) were taken for interpretation with three replications at Centre for Crop Improvement, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during *Rabi* 2018-19. Field evaluation trial was sown on 24th November, 2018. Genotypes of grain amaranth were grown in RBD (Randomized Block Design) with three replications. The seeds were sown in seed bed with spacing 45 cm × 15 cm and length of row 3.0 m (Two rows per genotype). All agronomic and plant protection measures were done as per schedule.

The observations were recorded as visual assessment and measurement on individual plants (five competitive randomly selected plants per genotype in each replication). Observations were recorded for Days to flowering, Leaf area per plant (cm^2), Days to maturity, Length of inflorescence (cm), Width of inflorescence (cm), Plant height (cm), Seed yield per plant (g), Stem girth (cm), Harvest index (%), Test weight (g/10 ml), Protein content (%).

The data were put through Multivariate analysis (Rao 1952) [11]. The transformation of original mean values was prepared and all possible D^2 values are computed. For formulate group clusters or constellations, quite straight forward decisive factor recommended by Rao (1952) [11], inter-cluster distance was worked out by taking the average of the component genotype after cluster construction. Average intracluster distance and average intercluster distance distance were obtained by using the method suggested by (Singh and Chaudhary, 1977) [15]. The genetic distance (D value) mid the cluster is accomplished by taking the square root of the average D^2 values (Mahalanobis 1936) [7]. The outcome average divergence values were use to deliberation all the possible D^2 values among the members of two clusters measured.

Table 1: Details of amaranth genotypes

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.	Suvarna
16.	SKNA-1406				

Results and discussion
The analysis of variance revealed significant differences among forty-six genotypes for all the eleven traits. This indicated the existence of significant amount of diversity presence among the forty-six genotypes for the characters under studied.

Genetic divergence
On the basis of Mahalanobis D^2 analysis, forty-six genotypes were grouped into twelve clusters based on eleven characters. D^2 statistics computed 1035 pairs by using the data of attributes under studied. The clusters were formed on the basis of average cluster distance, following Tocher's method (Rao, 1952) [11] considering foliage yield and its attributing traits (Table-2). The grouping pattern of the genotypes indicated that the clusters were heterogenous for geographical origin of genotypes.

The results indicate that a maximum number of diverse genotypes (10 genotypes) appeared in cluster II followed by cluster I (9 genotypes) and cluster III (6 genotypes) which indicate genetic divergence has no relationship with geographical divergence. The absence of relationship between genetic as well as geographical diversity designate that the forces genetic drift, spontaneous variation, exchange of genetic stocks are may be in control of genetic diversity (Nagarajan and Prasad, 1980) [9]. Cluster IV (6) and Cluster V has 3 genotypes. Clusters VII, VIII and X have (3 in each) and Clusters VI, IX, XI and XII (1 in each case).

Table 2: The distribution of forty-six amaranth genotypes to various clusters on the basis of D^2 statistic

Clusters	Number of genotypes	Genotypes
I	9	GA-3, RGA-17, KGBA-4, SKNA-908, BGA-20, RGAG-14-3, GA-2, SKNA-1510, SKGPA-61
II	10	SKNA-403, SKNA-1406, RMA-63, SKNA-1503, RGAG-12-22, SKNA-1502, SKNA-808, SKNA-1414, RHGA-13-3, SKNA-1207
III	8	SKNA-1501, BGA-10, KGBA-7, SKGPA-73, SKNA-1305, RMA-62, KGBA-5, RGA-16
IV	6	BGA-4-9, RGA-18, Suvarna, BGA-9, RGA-14, SKNA-1201
V	3	SKNA-401, SKNA-1407, BGA-7
VI	1	RGA-13
VII	2	SKNA-813, SKNA-1403
VIII	2	MGA-502, SKGPA-155
IX	1	BGA-14
X	2	SKGPA-150, SKNA-903
XI	1	SKNA-1313
XII	1	SKNA-1508

Intra and inter cluster distances

The result of intra and inter distance (D) uniting all possible put together of eleven clusters are shown in Table-3. In addition, cluster diagram (Fig. 1) manifests the interrelationship amid clusters. The results acquire through classification of genotypes make use of D^2 statistics come up with a set of groups from which desirable parents may be pick out for further breeding programme with respect to foliage yield allocate characters in general and the trait foliage yield in particular. The universal distance (D) between two populations differs from 0.00(intra cluster) to 29.57(inter cluster) which advisable that there was a considerable diversity subsist in the genotypes studied for seniority of the traits. Range of intra cluster (D) distance between 0.00 to 7.95. Intra-cluster distance was highest in cluster VII (63.27) followed by cluster V (58.82) and cluster II (56.20), suggesting there is a lot of extent for swapping of genes among genotypes within these clusters. The intra-cluster distance was unperceived in cluster VI, IX, XI and XII as these clusters had only one genotype each (Table 3 and Fig. 1). The inter cluster values ranged from 71.00 to 874.76. Concerning to inter cluster distance the cluster X and cluster I were most diverse with each other as distance between exist 874.76 and cluster X genetically divergent with cluster V distance was 819.60. It can be deduced that crossing between these genotypes may result in good recombinants for victorious breeding programme.

Table 3: Average intra (bold) and inter cluster values (D2 and D) (D=$\sqrt{D^2}$)for forty-six genotypes of amaranth

| Clusters	Values	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	D²	56.10	253.05	377.81	459.28	89.00	218.89	122.93	415.20	244.29	874.76	121.75	444.72
	D	7.48	15.90	19.43	21.43	9.43	14.79	11.08	20.37	15.62	29.57	11.03	21.08
II	D²		56.20	85.99	140.03	322.01	79.66	164.36	85.05	78.55	387.14	239.87	226.30
	D		7.49	9.27	11.83	17.94	8.92	12.82	9.22	8.86	19.67	15.48	15.04
III	D²			57.38	71.00	403.68	78.72	282.46	92.40	99.35	301.31	306.45	140.07
	D			7.57	8.42	20.09	8.52	16.80	9.61	9.96	17.35	17.50	11.83
IV	D²				54.54	457.38	103.39	380.47	112.19	166.03	305.49	320.89	115.68
	D				7.38	21.38	10.16	19.50	10.59	12.88	17.47	17.91	10.75
V	D²					58.82	212.13	243.24	483.39	340.32	819.60	93.28	368.67
	D					7.66	14.56	15.59	21.98	18.44	28.62	9.44	19.45
VI	D²						00.00	203.13	126.44	118.39	354.39	127.92	119.12
	D						00.00	14.25	11.24	10.88	18.82	11.37	10.91
VII	D²							36.24	293.52	110.85	726.24	236.91	411.23
	D							6.01	17.13	10.52	26.94	15.39	20.27
VIII	D²								63.27	156.65	286.65	343.29	204.45
	D								7.95	7.52	16.93	18.52	14.29
IX	D²									00.00	529.24	276.41	258.94
	D									00.00	23.00	16.62	16.09
X	D²										54.95	748.32	240.13
	D										7.41	27.35	15.49
XI	D²											00.00	330.59
	D											00.00	18.18
XII	D²												00.00
	D												00.00
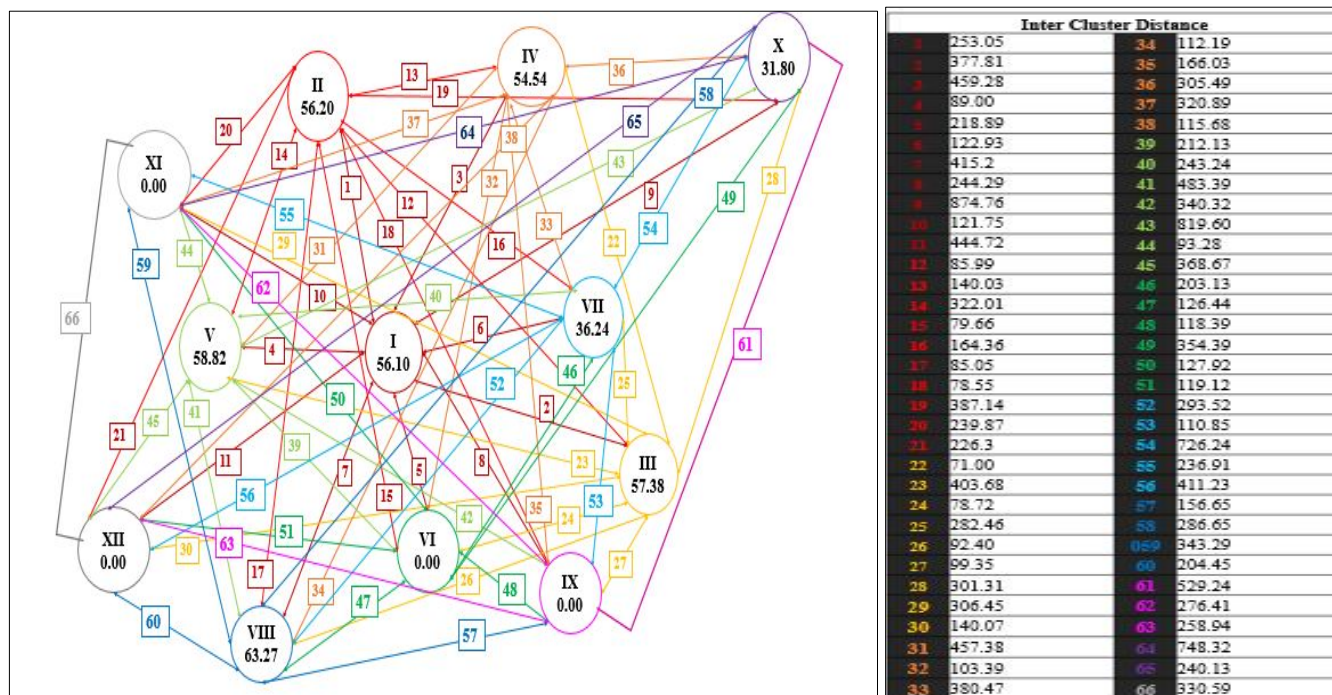
Fig 1: Cluster diagram indicating interrelationship between twelve clusters using D² analysis (D² value) (not to scale)

The results of the inter-cluster distance indicated that presence of considerable diversity in forty-six genotypes included in the present study. This could be the result of selection in different direction by nature and human forces which may suggest that maximum amount of heterosis may be expected in cross combination involving the genotypes of the most divergent clusters. Crossing of genotypes belonging to the same cluster is not expected to yield superior hybrids or desirable segregants because they share common biochemical pathways.

These results are in general accordance with the findings of similar results were reported by Pandey and Singh (2011) [10], Kumar *et al*. (2019) [5] and Tejaswini *et al.* (2018) [16].

Cluster means of several quantitative attributes
The best combination of parents for improvement in various characters can be recommended on the basis of *per se* performance of the genotypes and inter cluster divergence. Based on mean performance of eleven characters (Table-4.), cluster V contained three genotypes, *viz.,* SKNA-401, SKNA1407 and BGA-7 having one of the highest for leaf area per plant (2416.87), width of inflorescence (21.00), plant height (162.22), harvest index (26.22), seed yield per plant (41.18) and medium for days to flowering (57.78), days to maturity (106.67), length of inflorescence (67.62), stem girth (4.24), test weight (8.06). The results of the cluster V take over such characters which are responsible for higher yield and from which ameliorate genotypes could be directly selected or they could be considered as parents to complement the deficit of other parents, present in distant group. So that

for higher yield choice of superior genotypes can safely be done in this cluster. In consequence, the genotypes of this clusters can be used for creating changeability for the above traits. Cluster X was found to be the least yielder. It comprised of two genotypes, *viz.,* SKNA-150, SKNA-903 having lowest days to flowering (39.00), leaf area per plant (615.97), Days to maturity (80.96), width of inflorescence (17.56), plant height (115.05), stem girth (3.13) and lowest seed yield per plant (10.87).

Per cent benefaction of contrasting attributes toward divergence
The per cent benefaction of each character towards divergence is presented in Table- 5. The top characters which contributed more towards genetic divergence were leaf area per plant contributed maximum (39.32%) towards divergence by 407 times followed by seed yield per plant (36.23%) by 375 times, days to maturity (8.88%) by 92 times, protein content (4.44%) by 46 times, stem girth (3.47%) by 36 times, length of inflorescence (2.60%) by 27 times. Test weight (1.64%) by 17 times, plant height (1.44%) by 15 times, width of inflorescence (0.96%) by 10 times, days to flowering (0.77%) by 8 times and harvest index (0.19%) by 2 times. These characters possibly used in selecting genetically diverse parents for hybridization programme to make a use of either maximum heterosis or to accomplish efficient selection in segregating generation. Findings of similar results were reported by Pandey and Singh (2011) [10], Lokesh Kumar and Murthy (2017) [6], Tejaswini *et al.* (2018) [16], Kumar *et al.* (2019) [5].

Table 4: Cluster means for eleven traits in forty-six genotypes of amaranth

Clusters	Days to flowering	Leaf area per plant (cm²)	Days to maturity	Length of inflorescence (cm)	Width of inflorescence (cm)	Plant height (cm)	Stem girth (cm)	Harvest index (%)	Test weight (g/10ml)	Protein content (%)	Seed yield per plant (g)
I	58.11	2345.25	110.56	65.99	20.36	158.79	4.08	23.79	7.82	13.08	37.66
II	55.73	1708.98	105.93	60.75	19.71	149.93	4.02	18.66	7.54	13.07	20.15
III	54.79	1141.55	108.67	58.82	19.41	148.05	3.92	17.98	7.58	13.23	16.68
IV	53.89	778.47	109.28	59.50	19.87	155.73	4.21	18.47	7.31	11.45	17.23

V	57.78	1947.57	106.67	67.62	21.00	162.22	4.24	26.22	8.06	14.55	41.18
VI	48.67	1383.93	109.00	57.40	18.60	157.07	4.67	19.63	7.59	14.83	24.13
VII	47.83	2416.87	112.17	64.60	20.37	134.43	3.87	21.29	7.98	11.43	28.03
VIII	58.17	1381.30	100.50	49.77	18.63	140.23	4.13	17.32	7.46	10.98	16.37
IX	56.00	1675.17	118.33	62.27	19.27	130.60	3.69	17.48	7.56	12.53	19.13
X	39.00	615.97	80.96	47.82	17.56	115.05	3.12	24.91	7.48	14.09	10.87
XI	56.67	1759.27	110.33	69.27	20.53	158.93	5.69	23.54	7.30	13.67	38.40
XII	48.67	634.07	101.33	47.73	20.20	132.53	3.72	19.67	8.48	12.01	22.20

Table 5: Per cent contribution of various traits to the total genetic divergence

Sr. No	Characters	Number of times characters ranked first	Per cent contribution
1	Days to flowering	8	0.77
2	Leaf area per plant (cm^2)	407	39.32
3	Days to maturity	92	8.89
4	Length of inflorescence (cm	27	2.61
5	Width of inflorescence (cm)	10	0.97
6	Plant height (cm)	15	1.45
7	Stem girth (cm)	36	3.48
8	Harvest index (%)	2	0.19
9	Test weight (g/10ml)	17	1.64
10	Protein content (%)	46	4.44
11	Seed yield per plant (g)	375	36.23
	Total	1035	100.00

Conclusion
The D^2 analysis consequently demonstrates to be an extremely constructive technique in isolating diverse groups from the germplasm above study. Considering to inter cluster distance the cluster X and cluster I were most diverse with each other as distance between exist 874.76 and cluster X genetically divergent with cluster V distance was 819.60. It can be deduced that crossing between these genotypes most heterogeneous and these clusters were the best for within group hybridization. The cluster means for the eleven characters under study cluster V contained three genotypes, *viz.,* SKNA-401, SKNA1407 and BGA-7 take over such characters which are responsible for higher yield and from which ameliorate genotypes could be directly selected and utilised for breeding programme.

Acknowledgment

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