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Diversity Analysis of Fleshy Leaf Type *Amaranthus* for Semi-arid Ecosystems

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ABSTRACT

The lack of fleshy leaf type varieties in *Amaranthus* is the major reason for poor consumer acceptance as the existing genotypes wilts within 2-3 h of harvest. To search for a fleshy leafy type, 28 accessions comprising *A. tricolor*, *A. cruentus*, *A. hybridus* and *A. dubius* were collected and examined for 22 descriptors based on morphological characters and nutritional values. There was significant variability in the gene pool, evident by a high variation as per cent of mean or coefficient of variation (CV). Principal component analysis revealed that plant height, number of branches/plant, internode length, number of leaves/plant, leaf length, leaf width, petiole length, node at which a flower appeared, plant weight and leaf weight exhibited 94% of the total variability. Hierarchical cluster analysis classified the accessions into 12 cluster groups at 0.78 Euclidean distances: clusters I, II, III and V were major clusters having 14, 2, 2 and 2 accessions, respectively. Yield/plot had a significant positive correlation with leaf length (r = 0.44), leaf width (r = 0.51), stem weight (r = 0.98), leaf weight (r = 0.94), number of leaves/plant and shoot weight (r = 0.98). Accession IC-469646 had fleshy, broad leaves with high yield potential coupled with higher Ca, Vitamin-C and Fe content.

Keywords: amaranthus, consumer preference, fleshy leaf type, iron content

INTRODUCTION

Malnutrition is estimated to affect over 925 million people in the world, which is about 13.6 per cent of total population and the growth rate is predicted to increase year by year (FAO 2010). Worldwide, nearly two-thirds of deaths of children are associated with nutritional deficiencies (Caballero 2002; Kennedy *et al.* 2003), in which iron deficiency is the greatest cause for malnutrition (Mason *et al.* 2001; Ruel 2001), which is estimated to be more than 2 billion people over 30% of world population (Stoltzfus *et al.* 2010). The main cause for malnutrition is the lack of knowledge on the correct choice of food, reduced dietary diversity (Abukutsa Onyango 2003), lack of suitable varieties (Kimiywe *et al.* 2007) and poor bioavailability (Hunwell and Egli 2010). Countries that use indigenous leafy vegetable in diets with high consumption are less likely to be affected by malnutrition (Weinberger and Msuya 2004; Onyango *et al.* 2005), particularly Fe deficiency (Johns and Sthapit 2004).

Amaranthus is an important under-exploited leafy vegetable and is often described as "poor man's vegetable" (Peter 2003) enriched with minerals, especially Fe, proteins, vitamins and essential amino acids (Okafor 1983; Rangarajan and Kelly 1994; Segura-Nieto *et al.* 1994). *Amaranths* foliage is used as a vegetable, animal feed (Brenner *et al.* 2000), for grain and pigments but is also considered to be a weed (Granjero Colín *et al.* 1994; Kigel 1994).

In addition to its huge nutraceutical value (Sathyanarayana *et al*. 2008), genetic variability in *Amaranths* has made it exceptionally adaptable to a wide range of environments (Putnam 1990; Covas 1994; Kulakow and Hauptli 1994; Berti *et al.* 1996). In India, from region to region, there is wide variation in consumer acceptability of *Amaranths* based on leaf size, colour, yield and quality. For example, consumers in southern India prefer *A. polygonoides* with smaller leaves than in the western part, where the larger-leaved *A. tricolor* is preferred. *Amaranthus* genotypes with small leaves are still very common in rural areas than in urban areas, but this might be due to a lack of choice of varieties. There are no reports on the levels of consumption of *Amaranthus* indicates very poor intake in the diet, which is due to the lack of a fleshy leaf type that can withstand shrinkage after harvest is supported by the findings of Onyango and Imungi (2007) that the lowest edible portion (38.9%) and greatest spoilage (5.5%) per day observed in amaranths as compared to other leafy vegetables. There are three main problems. Firstly, the leaves of existing varieties wilt or shrink within 2-3 h of harvest causing the leaves to have a poor appeal and thus less preferred by consumers. Secondly, the rate of Fe absorption by plants from soil depends on many factors such as soil type, genetic make up of the plant, root length, Fe availability, etc. Plants require 10 nM of Fe in the soil solution for normal growth and development, although its concentration does not reach values higher than 100 pM (Hell and Stephan 2003) in calcareous soils (prevalence of bicarbonate ion) of semi-arid ecosystems, which limits the absorption and availability of Fe in the plant (Jeong and Conolly 2009). Hence, assessing the magnitude of genetic variation for different characters, to predict the association of characters for high Fe absorption, and to isolate fleshy leaf *Amaranthus* genotypes having high yield potential for semi-arid ecosystems is essential.

MATERIALS AND METHODS

Plant materials

The experimental site is located at 22° 41′ 33″ N and 73° 33′ 22″S at 110-115 m above sea level. Annual precipitation is 750 mm,

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Table 1 Location of the *Amaranthus* accessions included in the study.

Accessions	Species	Location	Latitude	Longitude	Leaf shape	Leaf color
IC- 469558	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Oblong	Dark green
IC- 469530	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Broad ovate	Dark green
IC- 469521	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Broad ovate	Green
IC- 469708	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Obavate	Green
IC- 469645	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Obavate	Dark green
IC- 469601	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Ovate	Green
IC- 469692	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Ovate	Green
IC- 469658	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Broad ovate	Green
IC- 469646	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Ovate	Green
IC- 469620	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Ovate	Green
IC- 469546	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Broad ovate	Dark green
IC- 469624	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Ovate	Dark green
IC- 469605	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Ovate	Dark green
IC- 469705	*A. dubius*	Thirchur	10º 31" N	76º 16" E	Ovate	Dark green
IC- 469703	*A. dubius*	Thirchur	10º 31" N	76º 16" E	Ovate	Dark green
IC- 469677	*A. dubius*	Thirchur	10º 31" N	76º 16" E	Broad ovate	Dark green
IC- 469679	*A. hybridus*	Thirchur	10º 31" N	76º 16" E	Ovate	Green
IC- 469678	*A. hybridus*	Thirchur	10º 31" N	76º 16" E	Ovate	Dark green
IC- 469674	*A. hybridus*	Thirchur	10º 31" N	76º 16" E	Ovate	Dark green
IC- 469676	*A. cruentus*	Thirchur	10º 31" N	76º 16" E	Oblong	Green
IC- 469545	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Broad ovate	Green
Arka Arunima	*A. tricolor*	Bangalore	13º 05" N	77º 23" E	Broad ovate	Purplish green
Arka Suguna	*A. tricolor*	Bangalore	13º 05" N	77º 23" E	Broad ovate	Green
IC- 469528	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Triangular	Dark green
Kannara local	*A. tricolor*	Thirchur	10º 31" N	76º 16" E	Broad ovate	Greenish purple
Panchmahal local-2	*A. tricolor*	Vejalpur	22º 40" N	73º 33" E	Ovate	Green
Panchmahal local	*A. tricolor*	Vejalpur	22º 40" N	73º 33" E	Broad ovate	Dark green
Dharwad collection	*A. tricolor*	Dharwad	15º 07" N	74º 51" E	Ovate	Green

mainly from July to September with an average 35 rainy days/year. The annual maximum and minimum temperature ranges from 42 to 43°C in May and from 6 to 7°C in January, respectively. The annual potential evapo-transpiration ranges from 1500 to 1600 mm. Seeds of 22 accessions (IC = indigenous collections), 2 released varieties (Arka Suguna, Arka Arunima) and 4 land races (Kannara, Panchmahal-1, Panchmahals-2, Dharwad) comprising four species (*A. hybridus*, *A. cruentus* (L.), *A. dubius* (Mart. Ex. Thell.), and *A. tricolor* L.) collected from diversified ecosystems (subtropical, humid and arid) (**Table 1**) were used for this study. The former two species are grain type in which the seeds produced are used as edible part after its boiling, while the latter two are leafy type in which the leaves are primarily used as edible parts. The experiment was laid out in a randomized complete block design with three replications per accession and was conducted over two consecutive seasons from 15th July, 2007 to 15th October, 2007 and repeated during the same season of the year 2008. As *Amaranthus* seeds are very smaller in size (weighing 0.3 to 0.9 g/1000 seeds), they were mixed with sand in a 1: 10 ratio and sown in seed beds (2.0 × 3.0 m plots) and covered with finely powdered farm yard manure and first irrigation was given immediately. The seeds germinated at 4 days after sowing (DAS) and seedlings were thinned out at when 10 cm tall and spaced at 60 cm × 25 cm of inter row and inter plant distances, respectively. Fertilizer (50: 50: 20 kg N, P and K/ha) was applied as basal dose, in which the N was applied in 2 split doses. The first half as basal (before sowing) and the second half as top dressing at 8 weeks after sowing of the crop according to Veeragavathatham *et al.* (1998). The crop was harvested at 40 DAS by clipping shoots from the ground level; fresh shoot yield was recorded (Alfaro *et al.* 1987).

Assessment of yield and quality

Each plot contained 40 seedlings. Data was collected from 10 randomly selected plants per accession per season. The following 22 characters were studied, including 4 quality parameters: plant height (cm) (PH), number of branches/plant (NBP), internode length (cm) (IL), number of leaves/plant (NLP), node at which flower appeared (NFA), average leaf length (cm) (ALL), average leaf width (cm) (ALWT), average petiole length (cm) (APL), average plant weight (g) (APW), average leaf weight (g) (ALW), average root weight (g) (ARW), average petiole weight (g) (APTW), average stem weight (g) (ASW), average shoot weight (g) (ASHW), yield/plot (g) (YP), shoot to plant ratio (SHPR), root to shoot ratio (RSHR) and leaf to shoot ratio (LSHR). Quality parameters such as protein, Ca, Fe and vitamins were analyzed using fresh leaf samples collected at 35-40 DAS. The protein content (mg/100 g) (PC) was analyzed using the Kjeltec auto distillation method (Sadasivam and Manickam 1992), calcium content (mg/100 g) (CAC) was analyzed using a Elico flame photometer (model No. CL361), Fe content (mg/100 g) (FEC) was analyzed using Gorusch (1959) and (Jeffrey *et al*. 1989) methods and vitamin C content (mg/100 g) was analyzed by a calorimetric method proposed by Sadasivam and Manickam (1992), in which 0.5 g of leaf sample was ground using a pestle and mortar in 25-50 ml 4.0% oxalic acid solution and centrifuged to collect supernatant liquid. To a prepared aliquot (10 ml), bromine water was added; the extract turns orange yellow in the presence of excess bromine, measured at 540 nm to estimate vitamin C content.

Statistical analysis

The pooled data of both seasons was used to compute descriptive statistics viz. mean, range, standard deviation and coefficient of variation (CV) for all morphometric traits. Hierarchical cluster analysis, which adopted Ward's minimum variance method (Ward 1963), was applied over all 22 traits resulting in a dendogram. Principal component analysis (PCA) was performed using SAS software (SAS 1999) to identify potential traits contributing to variability. Statistica 6.0 was employed for statistical analysis of the data.

RESULTS

Genetic variation

In the present study, the level of variation found for leaf, floral, yield and quality parameters in the existing collection showed a greater potential to develop a genotype having broader leaves, higher yield and with high Fe (**Table 2**). Among the leaf characters, NLP ranged from 10.3 (IC-469530) to 272.6 (IC-469679) with a mean value of 58.2, although IC-469679 had the highest NLP even though it had very small leaves. IC-469624 had the shortest leaves

Table 2 Summary of the analysis of variance for 22 descriptors evaluated in 28 accessions evaluated over two years.

Parameters	Mean	Range	Variance	CV	SD	SE
Plant height	25.7	5.9-53.5	198.4	54.6	14.0	2.6
Number of branches per plant	5.7	3.3-11.2	4.5	36.8	2.1	0.401
Internode length	2.3	0.53-6.21	2.0	59.4	1.4	0.268
Number of leaves per plant	58.3	10.3-272.6	2580.0	87.0	50.7	9.599
Node at which flower appeared	4.0	1.3-8.1	2.7	40.7	1.6	0.312
Average leaf length	7.5	3.7-12.5	5.0	29.4	2.2	0.422
Average leaf width	4.7	2.6-9.3	2.2	32.0	1.5	0.284
Average petiole length	3.3	1.8-5.7	0.7	25.0	0.8	0.158
Average plant weight	44.5	14.3-142	861.2	65.8	29.3	5.5
Average leaf weight	11.3	5.1-27.7	47.5	60.8	6.8	1.3
Average root weight	4.9	2.1-13.3	5.6	48.0	2.3	0.449
Average petiole weight	1.7	0.552-4.3	0.8	52.1	0.9	0.176
Average stem weight	26.6	5.3-98.3	410.1	76.0	20.2	3.8
Average shoot weight	39.6	11.8-128.7	734.8	68.4	27.1	5.1
Shoot to plant ratio	0.893	0.844-0.94	0.005	2.7	0.024	0.004
Root to shoot ratio	0.119	0.062-0.19	0.009	26.1	0.031	0.005
Leaf to shoot ratio	1.0	0.197-4.0	0.5	73.4	0.736	0.139
Protein content	2.7	1.1-3.8	0.3	21.9	0.607	0.114
Calcium content	214.6	122.3-322.1	4094.6	29.8	63.9	12.0
Fe content	0.307	0.185-0.521	0.007	27.7	0.083	0.015
Vitamin-C content	75.4	16-150.5	1105.9	44.0	33.2	6.2
Yield per plot	2.0	0.74-6.4	1.7	64.7	1.3	0.253

CV - coefficient of variation, SD - standard deviation, SE - standard error

and least leaf width (3.7 and 2.6 cm) compared to Arka Suguna, which showed the highest values (12.5 cm and 9.3 cm, respectively) with the mean value of the gene pool being 7.61 cm and 4.73 cm, respectively. ASHW ranged from 11.87 to 128.7 g with a mean value of 42.67 g among the accessions. Quality parameters such as Ca, Fe and vitamin C content showed wide variation, where Ca ranged from 122.3 (IC-469692) to 322.1 mg/100 g (IC-469545) with a mean value of 198.5 mg. The lowest Fe content among the accessions was recorded in Arka Arunima (0.185) versus 0.236 in IC-469646 (0.521). The lowest vitamin C content was observed in IC-499546 (16 mg/100 g) compared to IC-469545, which recorded the highest value (150.5 mg/100 g). Great variability was evident from a high CV observed for most traits. A very high CV (> 60%) was observed for NLP, APW, ALW, ASHW, ASW, LSHR and YP. Quality parameters like vitamin C content (< 60%) showed a higher CV, with Fe content registering an intermediate level (< 40%) indicating that these characters can also be used to improve high-yielding genotypes. Among the accessions, the highest total leaf yield/plot was recorded in Arka Suguna (5.66 Kg) followed by Arka Arunima; however, IC-469646 showed the highest protein (3.8 g/100 g) and Fe (0.521 mg/100 g) contents in addition to high yield potential under semi-arid conditions.

Principal component analysis

The identification of a principal component is based on the correlation between different morphometric descriptors, their eigen values and eigenvectors of the principal components (**Table 3**). PCA identified the minimum number of descriptors, which explained the maximum variation out of the total variance in the present study. The first 10/22 characters explained 94.0% of total variability. Among these descriptors, the highest variance (34.2%) was recorded in PH, followed by NBP (12.6%), both of which collectively contributed most from all the variables to morphological diversity of the collection.

Correlation study

PH had significant positive correlation with NLP and LSHR (**Table 4**). NBP showed a positive significant relation with NLP, NFA and CAC, despite its negative correlation with IL. IL had significant positive correlation with NLP although it exhibited a negative correlation with NFA indicating that

Table 3 The first 10 principal components showing Eigen values and their contributions to the variability among accessions of *Amaranthus*.

Characters	Eigen values	Percent of variance	Cumulative percent of variance
Plant height	7.521	34.2	34.2
Number of branches per plant	2.766	12.6	46.8
Internode length	2.600	11.8	58.6
Number of leaves per plant	2.073	9.4	68.0
Average leaf length	1.799	8.2	76.2
Average leaf width	1.436	6.5	82.9
Average petiole length	0.916	4.2	86.9
Node at which flower appeared	0.604	2.7	89.6
Average plant weight	0.494	2.2	91.6
Average leaf weight	0.457	2.1	94.0

Sample size: 28 genotypes from two seasons

the higher NLP and NFA at later nodes were ideal characters for leafy vegetables as the leaf production is ceased after the initiation of flowering in the earlier node which drastically reduces leaf yield. The magnitude of the correlation index showed that a plant type with flower initiation at the node which appears at later stage of growth with higher number of branches could produce higher calcium and vitamin C content in leaves.

The economically important leaf characters such as ALL and ALWT showed a significantly positive correlation with APTL, APW, ARW, APTW, ASW, ASHW and YP. However, the ALL showed a non-significant positive correlation with ALW than ALWT, which indicated the need for focusing on the selection of a broader leafy type for achieving high yield than on the selection of a narrow leafy type for semi-arid conditions. APTL and APTW played a tremendous role in maintaining the fleshiness and turgidity of the leaf which are generally lacking in *Amaranthus*. APTL was significantly correlated with NFA and APTW was significantly positively correlated with leaf Fe content. ALW had a positive significant correlation with ALL, ALWT, ARW, APW, ASW, ASHW, YP and SHPR. The high-yielding genotypes could be selected through ALL, ALW, ARW, APW, ALW, ASW and ASHW. This indicates the priority of selection pressure towards plant vigour having a broader leaf with a long petiole for not only obtaining high yield, but also to have fleshy leaves with a high Fe content under semi-arid conditions.

Table 4 Correlation coefficients among various quantitative traits of *Amaranthus* accessions collected from diversified areas of India.

Character	PH	NLP	ALL	ALWT	APTW	ALW	ARW	APW	ASW	ASHW	LSHR	CAC	FEC
PH	1												
NLP	0.45*	1											
ALL	-0.34	-0.08	1										
ALWT	-0.12	0.03	0.90**	1									
APTW	-0.12	0.14	0.44*	0.51**	1								
ALW	-0.07	0.16	0.36	0.40*	0.93**	1							
ARW	-0.14	0.08	0.50*	0.57**	0.96**	0.86**	1						
APW	-0.02	0.09	0.45*	0.45*	0.69*	0.72*	0.68*	1					
ASW	-0.13	0.13	0.44*	0.52**	0.99**	0.87**	0.95**	0.63**	1				
ASHW	-0.11	0.14	0.44*	0.51**	0.98**	0.93**	0.95**	0.69**	0.99**	1			
LSHR	0.44*	0.07	-0.42*	-0.35	-0.23	-0.09	-0.19	0	-0.28	-0.23	1		
CAC	-0.17	-0.02	0.04	0.06	0.21	0.03	0.2	0.01	0.27	0.21	-0.04	1	
FEC	0.07	-0.14	0.25	0.22	0.15	0.13	0.2	0.46*	0.13	0.15	0.09	-0.07	1
YP	-0.1	0.15	0.44*	0.51**	0.97**	0.94**	0.95**	0.70**	0.98**	0.98**	0.17	0.2	0.15

*significant at 1% level and ** significant at 5% level

Table 5 Cluster mean values of different characters of *Amaranthus* accessions collected from diversified areas of India.

Clusters/Characters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
PH	22.3	23.6	28.1	47.3	28.2	34.9	10.6	22.8	22.2	23.5	45.4	42.5
NLP	36.6	40.5	48.4	129.3	55.9	43.3	32.8	98.4	27.7	96.3	117.3	272.6
ALL	7.1	7.3	7.5	5.8	8.1	5.9	5.2	12.5	8.3	9.3	7.7	5.6
ALWT	4.3	4.5	4.6	4.2	5.4	3.5	2.8	9.3	5.1	5.9	3.9	4.1
APW	25.7	30.6	36.6	29.5	48.2	72.6	96.5	142.5	45.2	43.5	56.9	42.5
ALW	6.6	7.6	9.1	8.2	12.9	14	25.4	27.8	11.2	8.2	21.2	11.6
ARW	3.6	4.2	4.6	2.9	5.4	6.1	8.3	13.4	4.3	5.4	5.3	4.1
APTW	1.1	1.5	1.8	1.9	2.4	2.2	1.7	2.7	2.1	2.7	2.4	1.2
ASW	14.5	17.3	21.1	16.5	27.7	50.4	61.1	98.3	28.3	27.3	28.1	25.8
ASHW	22	26.4	32	26.6	42.8	66.6	88.2	128.7	41.2	38.2	51.7	38.5
YP	1.2	1.4	1.7	1.4	2.2	3.3	4.5	6.5	2.1	2.2	2.8	2.3
LSHR	1.1	1.2	1.1	1.5	1.3	0.4	0.7	0.6	0.5	0.6	2.1	0.9
CAC	227.7	233.7	247.8	213.5	213.7	176.4	273.6	312.4	165.8	128	125.8	213.3
FEC	0.294	0.402	0.356	0.215	0.369	0.378	0.185	0.321	0.262	0.411	0.289	0.237
VCC	91.9	89.3	94.4	71.4	74.3	63.9	67.3	44.4	16	38	44.3	85.8

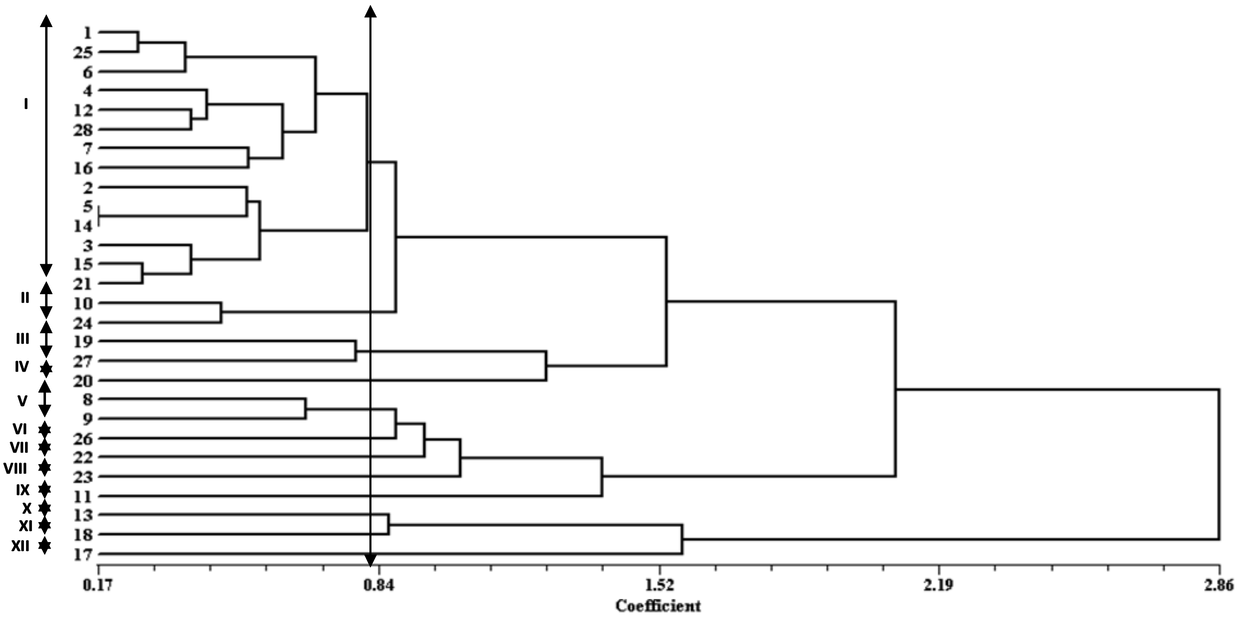

Fig. 1 Dendrogram generated by Ward's method of cluster analysis using SAS software showing major clusters of *Amaranthus* germplasm.

Cluster analysis

The locations of collection ecosystems were diverse: humid (Trichur), subtropical (Bangalore) and semi-arid (Vejalpur and Dharwad). To clearly visualize the genetic relationship among accessions, hierarchical clustering by UPGMA was performed. The cluster analysis primarily separated accessions according to geographical origin and breeding history. Hierarchical cluster analysis using Ward's method classified the accessions into 12 groups at 0.78 Euclidean distances (**Fig. 1**). Clusters I, II, III and V were major clusters having 14, 2, 2 and 2 accessions, respectively. The remaining clusters had one accession each. In general, the accessions clustered together based on their geographical origin and species. Cluster I included accessions collected from Trichur representing a humid ecosystem only. Clusters II, IV, V, IX, X, XI and XII also had accessions from Trichur whereas clusters III and VII had accessions collected from two different locations. Cluster VIII had accessions from Bangalore representing a subtropical ecosystem only. The most distinct cluster was cluster III, which had accessions 19 and 27 (IC-469674 and Panchmahal local-1, respectively) collected from two diversified locations representing a humid (Trichur) and semi-arid ecosystem (Vejalpur) and belonging to two different species. In cluster III, accession 17 (IC-469679) belonged to cluster XII collected from other location (Trichur), forming a distinct subgroup.

Considering different *Amaranthus* species, cluster I had both *A. tricolo*r and *A. dubius*. Cluster III had both *A. tricolor* and *A. hybridus* whereas *A. cruentus* grouped separately as the only accession in cluster IV. Distinct morphological groups among the accessions were observed among the clusters (**Table 5**). The highest NLP was observed in Cluster IV irrespective of leaf size. Cluster VIII showed the highest value for ALL, ALWT, APTL, NBP, ALW, ARW, ASW, ASHW, YP and CAC. The highest FEC content and SHPR were observed in cluster VI. Cluster XI registered the higher value for PC, ALW and NLP. The highest VCC content was exhibited by clusters I and III.

DISCUSSION

Traditional vegetables are not only better adapted to a particular environment but also supply high quality nutrition for a larger population (Akindahunsi and Salawu 2005; Orech *et al*. 2005). However, due to the onset of the market economy and modernization of agriculture, to a large extent, the concentration shifted to conserving the genetic resources of exotic vegetables rather than indigenous (or) traditional vegetables which, threatened the extinction of traditional vegetables as they have to compete for the attention of consumers with the much more popular exotic vegetables (Onyango and Imungi 2007). The leafy vegetables are highly perishable due to high moisture content and susceptible to rapid depreciation of nutritive values soon after harvest. Therefore, leafy vegetables have to be stored if not consumed immediately. Hence, the leaves loose their color and freshness within a day and their acceptance for consumption decreases (Oyebanji *et al.* 2006). There are a lack of desirable genotypes with turgid leaves, slow shrinkage or wilt for long shelf life after harvest, which is the long-term goal of *Amaranthus* breeding. Hence, assessing the existing variability for fleshy, larger leaves with a turgid petiole enriched with higher micronutrients in *Amaranthus* is highly desirable. In the present study, the higher CV (>60%) observed for NLP ASW, LSR, ASHW, APW, YP and ALW is an indicator of a greater level of variability in *Amaranthus*. A low CV (<40%) was observed for APL, ALL, RSHR, FEC and PC. These results show that greater genetic variability is present in the *Amaranths* germplasm and that there are greater possibilities for crop improvement. Similar to the present findings, the genotypic CV values ranged from 6.80 to 28.25% for different yield-related parameters in *A. tricolor*; in addition, the heritability estimate values were high for all parameters, ranging from 0.89 to 0.98% (Shukla and Singh 2000; Shukla *et al*. 2006). Leaf and stem color were also used as indices to classify leafy *Amaranthus* accessions based on 17 biological parameters (Wu *et al.* 2000; Xiao *et al.* 2000). Among the accessions studied, IC-469646 registered high yield (3.79 kg), having a long petiole (7.72 cm), broad and succulent leaf (12 cm × 7.6 cm) coupled with high protein (38 mg) and high Fe (0.521 mg) contents. Zafar *et al.* (2006) noted high variability in quality characters in *Amaranthus*. High variability in leaf yield and quality of amaranths would influence the preference of consumers; therefore its scope for natural selection of the best genotype would be enhanced.

All economically important descriptors such as PH, NBP, IL, NLP, ALL and ALWT exhibited Eigen values > 1, indicating the existence of large variation. However, no single qualitative character was highly influenced by the growth environment due to which the variability in the expression of quality parameters among genotypes was low. However, both quantitative and qualitative characters appeared together in the PCA of *Pelargonium sidoides* (Lewu *et al.* 2007), indicating the lack of variation in qualitative characters such as FEC, PC and VCC of *Amaranthus*.

Correlation studies predict the descriptor that should be given priority while selecting the genotypes for different purposes. In the present study, the taller genotypes produced more leaves that led to a higher leaf to shoot ratio; however, PH did not predict the high-yielding potential of genotypes due to its non-significant correlation (r = -0.01). Further, the negative relation of PH with ALW indicated that a greater number of leaves/plant (r = 0.45) exhibited much smaller leaves. Hence, selection of a genotype for greater leaf weight by selecting for broader leaves would be advisable for higher shoot and plant weight; consequently, selecting for high yield rather than for smaller leaves would result in a poor leaf to stem ratio (> 1). The broader leafy genotypes (*A. hybridus*) were less likely to wilt or shrinkage immediately after harvest compared to the genotypes with smaller leaves (*A. cruentus*) (Onyango and Imungi 2007). Oboh (2007) also reported a similar result in *A. hybridus* accessions for yield; however, in that study, there was a negative correlation of leaf weight with plant height (r = -0.07). This contradiction in the present study is due the inclusion of more species (*A. tricolor*, *A. cruentus*, *A. dubius* and *A. hybridus*) which has a different weighting for plant height and leaf size compared to *A. hybridus* accessions alone. Considering NBP, branching is a major descriptor that differentiates grain and leafy types at an early stage of crop growth. Two types of branching were observed in the germplasm: i) Long branches > 0.5 cm in diameter with sparse leaves; ii) Short branches < 0.5 cm in diameter with dense foliage. The grain type had the former type of branching while the leafy types had the latter branching type. Hence, the breeding approach prioritized the characters that resulted in higher ALL, ALWT, ARW, APW, ALW, ASW and ASHW, with more possibility of obtaining a high-yielding genotype. Varalashmi and Reddy (1997) found similar findings in *Amaranthus* in which the yield of greens were positively and significantly associated with plant height, leaf length and breadth, stem girth, stem weight and leaf weight. Higher shoot weight was positively correlated with ALL (r = 0.44), ALWT (r = 0.51), APW (r = 0.98), ALW (r = 0.93), ARW (r = 0.95), APTW (r = 0.98), ASW (r = 0.99) and YP (r = 0.98). Shukla *et al.* (2010a) also reported similar results in *A. tricolor*, in which plant height had a significant positive association with stem diameter, number of leaves/plant and leaf size. Considering the quality parameters, those genotypes with higher calcium and vitamin C content could be isolated if they produced more NBP and initiated flowers at later nodes indicating that late flowering genotypes are superior for larger, fleshy leaves with higher leaf weight and higher Fe content. Our findings support those of Shukla *et al.* (2010a), who also noted that the number of branches/plant had a negative association with all the qualitative characters, except for protein content, in *A. tricolor.* Brown and Jones (1976) also classified crop plants into Fe-efficient and Fe-inefficient based on their Fe absorption capacity from alkaline soil; Fe efficiency was determined by the morphological, physiological and biochemical changes in the shoot and root systems of *Phaseolus vulgaris* L. (Welch *et al.* 2000) and soybean (Kolb *et al.* 2003).

Cluster analysis clearly showed the geographic grouping of accessions, but also exhibited the co-occurrence of species in single clusters (**Fig. 2**). Although the majority of accessions collected from Trichur segregated into cluster I, indicating similarity in adaptation, few other Trichur accessions grouped in clusters II, IV, V, IX, X, XI and XII in which the accessions differed significantly from those in cluster I. Accessions in cluster I had higher calcium and Vitamin C contents, higher NLP with higher ALW, moderate PH, and higher ALL, APW and NBP indicating the diversity that existed in Trichur. This might be due to the highest phenotypic dispersion among germplasm found growing in this location. The cluster showing distinct mean values for different morphological characters such as ALL (12.5 cm) and ALWT (9.3 cm) and CAC (312.2 mg/100 g) in cluster VIII, FEC (0.411 mg/100 g) in cluster X and VCC

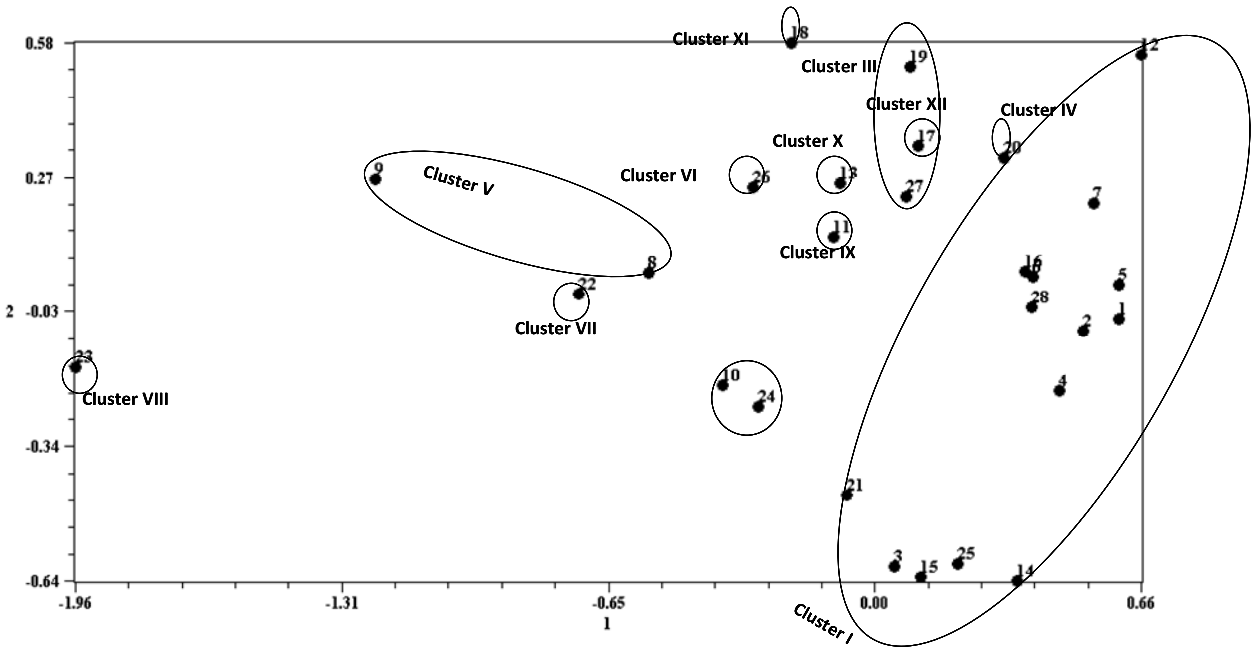

Fig. 2 Clustering of accessions of *Amaranthus* germplasm collection using Gower's method.

(94.4 mg/100 g) in cluster III compared to other clusters may be explained by the adaptation of germplasm to natural selection at the particular location been driven by high selection pressure for a particular character (Omolaja *et al.* 2000; Vilaro *et al.* 2004). Cluster VIII had accessions collected from Bangalore only. Similar parallelism between genetic diversity and geographic distribution was observed in garlic (*Allium sativum* L.) from Nepal (Panthee *et al.* 2006) in which there were three major clusters with no relation with geographical location; however, each cluster had alot of overlap among the accessions due to the exchange of planting materials between locations. The reason for the existence of a relationship between clusters and geographical regions could be due to the restricted movement of planting material from one region to another. This could also be explained by the fact that the quantitative clustering obtained from each location is related to the similarity of adaptation (Shukla *et al.* 2010). Lewu *et al.* (2007) also reported that plant materials (*Pelargoinum sidodes* DC) collected from the same location were found to cluster into a pair of accessions, implying a similarity of adaptation among 30 accessions studied.

Cluster I had *A. dubius* and *A. tricolor* accessions clustered together while cluster III had *A. trioclor* and *A. hybridus* accessions. Such clustering is due to the common parentage of all these three species, phenotypic differences or similarity between the accessions in terms of leaf size or other morphological characters. The phenotypic differences in many accessions of *A. hybridus* tend to vary in plant height, leaf, stem, seed and inflorescence colour. Intra-interspecific hybridization among the species is caused by the nature of *A. hybridus* species as it is also a breeding line in grain type (Ugborogho and Oyelana 1993). Lee *et al.* (2008) also reported that the close morphological resemblance, floral complexes in *Amaranthus* is due to self pollination and a varying amount of out crossing, while frequent interspecific and inter-varietal hybridization have brought wider variation, a reason for the tremendous diversity related to their wider adaptability to different geographic locations.

Cluster IV had one *A. cruentus* accession, as it belongs to a grain type with a prominent and dissimilar morphological descriptor allowing it to be isolated from the other group with leafy type accessions. Gupta and Gudu (1991) reported that *A. cruentus* and *A. hypochondriacus* grouped together in grain *Amaranthus* as they are genetically more similar as they are characterized by the production of grains. Khoshoo and Pal (1972) also reported that a hybrid of *A. hybridus* and *A. hypochondriacus* clustered with the latter species, indicating that the hybrids naturally produce grains with characters similar to the parent *A. hypochondriacus* and unlike the parent *A. hybridus*, which does not produce grains. The low genetic distances between the hybrids of these parents compared to other accessions showed that the hybrids are not strongly genetically differentiated. Ray and Roy (2008) also reported the existence of variation in the genetic diversity of different populations such as hybrids, breeding lines and clustering of population of the same species.

Identification of a genotype with nutritional quality is more discriminative than the use of total yield alone. In recent times, developing micronutrient-enriched plant food through different breeding methods is a powerful intervention tool to target the most vulnerable people through biofortification without negatively impacting crop productivity on soil with poor micronutrient content (Welch and Graham 2004). In the present study, the accessions Arka Suguna and Arka Arunima exhibited higher yield potential than IC-469646, which had higher Ca and Fe content and higher yield in a semi-arid ecosystem where calcareous soil is predominant, indicating that IC-469646 is an efficient genotype for Fe absorption. The utilization of these quality parameters such as Fe and Ca content in the determination of genetic diversity of *Amaranthus* could prove to be a novel technique for establishing breeding lines or hybrids through identification of genotypes for future utilization in *Amaranthus* breeding programs.

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