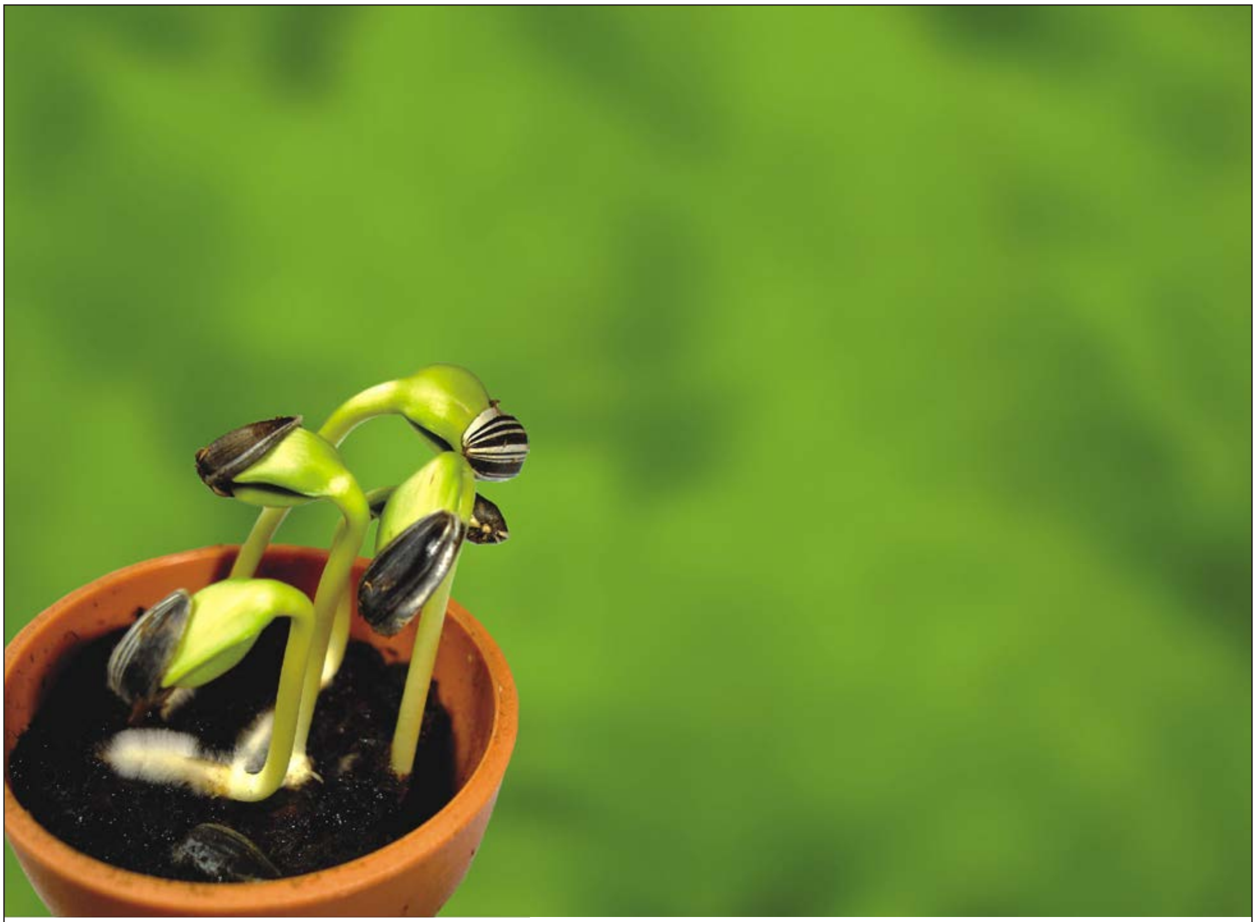

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Multivariate Analysis of the Diversity among Some Nigerian Accessions of *Amaranthus hybridus*

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Abstract: A total of 16 accession of *A. hybridus* from the germplasm of the Nigerian Institute for Horticultural Research (NIHORT) was evaluated in field experiments at the Biological garden of the University of Lagos in 1999. Data was collected on 22 quantitative and visually assessed characters which included leaf length and width, number of days to 1st floral budding, days to 50% flowering, seed length and width and 500-seed weight. Data was analysed using various statistics which included means, standard deviation, correlations and cluster analysis. Results showed a wide range of diversity in most of the quantitative characters assessed with variability in plant height ranging from 74.30-181.00 cm, leaf length from 13.90-29.40 cm and leaf width from 7.00-12.40 cm. The accessions were further classified into 4 district groups with the accessions in each group demonstrating similar characteristics. Accessions within a group were closely placed in the dendogram and showed promise for use in cultivar development. The district groups could serve as breeding lines for the genetic improvement of *A. hybridus* through cross-breeding.

Key words: *A. hybridus*, accessions, multivariate analysis, characters, genetic diversity, dendogram

INTRODUCTION

The genus *Amaranthus* belongs to the tropical family Amaranthaceae. They are short lived annuals and their broad leaves are commonly used as a potherb with the plant producing a significant amount of edible cereal grains. They grow vigorously, resist drought and adapt readily to new environments (Norman, 1992). The major centres of distribution are tropical America, India, Africa and Australia (Randle, 1959). According to Palada and Crossman (1999) several species of *Amaranthus* exist depending on the region in the tropics where they are found. Such species include *A. tricolor* L. which is commonly found in East Asia, *A. cruentus* (L.) *Sauer* common in Africa and *A. dubious* Mart. ee Thell in the Caribbean. *Amaranthus* species consist of grain and weedy types with the common grain types being *A. hypochondriaus*, *A. cruentus* and *A. candatus*. The major weedy types include *A. viridis, A. spinous, A. retroflexus* and *A. hybridus.*

A. hybridus has however shown promise as a leafy vegetable crop and also as a breeding line for grain amaranth. It has the potential of imparting early maturity to grain types through cross-breeding. However, many accession of *A. hybridus* exist and they tend to vary in plant height, leaf, stem, seed and inflorescence colour and grain yield and Ugborogho and Oyelana (1993) also observed these variations and were of the opinion that Amaranths were a taxonomically difficult group because of the ease of intra-and inter specific hybridization which resulted in close morphological resemblance and floral complexes.

Various numerical techniques have been successfully used to classify and measure patterns of genetic diversity in many crops such as black gram (Ghafoor and Ahmad, 2003), pea (Amurrio *et al*., 1995), quinua (Rojas *et al*., 2000), sesame (Rao and Subramanyam, 2003) and amaranths (Varalakshmi, 2004; Wu *et al*., 2000; Xiao *et al*., 2000). Studies on vegetable amaranth showed the presence of wide range of diversity in both agronomic and qualitative traits especially leaf and stem colour (Wu *et al*., 2000; Xiao *et al*., 2000). Xiao *et al*. (2000), further opined that stem and leaf colour were useful indices in the classification of 31 vegetable amaranths out the 17 biological characters used in the evaluation. In the evaluation of 46 accessions of vegetable amaranth Varalakshmi (2004), found the accessions useful in intervarietal hybridization studies based on the wide variability that existed for most agronomic traits evaluated.

In order to develop high yielding leaf and grain amaranths from the Nigerian germplasm a preliminary investigation was designed as there is little or no information on the extent and kind of diversity present in the materials in the germplasm. The study was aimed at assessing the variability within 16 *A. hybridus* accessions within the Nigerian germplasm with a view to identifying promising accessions for future breeding studies.

MATERIALS AND METHODS

Sixteen accessions of *A. hybridus* were selected from the germplasm of the Nigerian Institute for Horticultural Research (NOHORT) based on germination and yield from previous field trials. The list of accessions and their seed colour (Table 1). The seeds were sown on seed beds in the Biological gardens of the University of Lagos in April, 1999. Twenty-eight days after planting, seedlings were transplanted into vegetable beds and planted at a spacing of 60×25 cm as recommended by Norman (1992). Animal (cow) dung was carefully mixed into each vegetable bed before transplanting.

Collection of data was based on 5 randomly selected plants per accessions and 5 measurements for each character trait per plant. A total of 22 characters were recorded in this study. They include leaf length and width, petiole length, internode length, stem diameter, plant height at maturity, plant canopy diameter, number of days to 1st foliage leaf, number of days to 4-leaf stage, number of days to 1st floral budding, number of days to 50% flowering, 500-seed weight, seed length and width. Based on these characters, three ratios leaf length/leaf width, plant height/plant canopy diameter and seed length/seed width ratios were determined. Visually assessed characters include colour of leaves, stem, inflorescence and seeds.

Means, standard deviation and coefficient of variation were used to measure central tendency and dispersion for the various quantitative characters recorded. Estimates of analysis of genetic diversity was carried out using SPSS for Window 11.0. It involved calculating the degree of association among

Table 1: List of accessions used in the study and their seed colour

Accession No.	Accession code	Seed colour
1	NH84/463	Black
2	NH84/ 493-1	Brown
3	NH84/ 444	Black
4	ED87/33	Black
5	NH84/441	Pale yellow
6	NH84/46	Black
7	NH84/190-1	White
8	NH84/445	Brown
9	NH84/494	Pale yellow
10	NH84/443-1	Brown
11	NH84/445-1	Black
12	NH84/448	Black
13	NH84/1019b	Yellow
14	NH84/444-4	Pale yellow
15	NH84/451-k	Pale yellow
16	NH84/190-2	Brown

the different quantitative characters-Pearson's correlation. The accessions were further classified into similar groups by the non-hierarchical k-means technique of cluster analysis with a dendogram (Hair *et al*., 1992).

RESULTS

Most of the quantitative characters measured exhibited broad variability. Plant height for example ranged from 74.30-181.00 cm, while days to 50% flowering had an interval of 23 days from the earliest to the latest maturing accessions. Most of the accessions were of green stem colour (81.25%) although some of these accessions (38.5%) had mixture of green and red colour. Only 1 of the 16 accessions had the stem colour being totally red (Table 2).

Of all the correlation coefficients 17 were positive and highly significantly correlated ($p = 0.01$) (Table 3). Most of the characters that were positively correlated corresponded to characters of morphology. The highest correlation was between seed length and seed breadth ($R = 0.92$).

Table 2: Statistical parameters for quantitative characters and status of qualitative descriptors of the accessions evaluated

Characters	Range	Mean	SD	CV (%)
Leaf length (cm)	13.90-29.40	19.47	3.67	18.85
Leaf width (cm)	7.00-12.40	8.95	1.30	14.53
Petiole length (cm)	8.00-16.90	11.34	2.80	24.69
Internode length (cm)	2.40-6.40	4.13	1.15	27.85
Plant height (cm)	74.30-181.00	123.49	30.07	24.35
Plant canopy (cm)	34.00-94.00	52.54	16.87	32.11
Stem diameter (cm)	0.36-1.78	0.84	0.36	42.86
Days to 1st foliage leaf	3/5			
Days to 4th-leaf stage	7/12			
1st floral buds (days)	74-97	81.38	7.00	8.60
50% flowering (days)	75-98	89.38	6.52	7.29
Seed length (cm)	0.91-1.38	1.17	0.15	12.82
Seed width (cm)	0.75-1.29	1.07	0.17	15.89
500 seed weight (g)	0.11-0.35	0.28	0.07	25.00
Characterization				
Stem colour	Green stem colour had the highest frequency of 81.25%, while 2 of the accessions where light green and one was reddish. Some (38.5%) of the accessions with green had some part of the stem being red in colour.			
Inflorescence colour	Six of the accessions were reddish in colour, while 56.25% of the accessions were a combination of green and red, while one of the accessions had a green inflorescence.			

Table 3: Correlations amongst selected quantitative traits in *A. hybridus*

Character	LL	LW	PL	IL	PH	PC	SD	FFL	4LS	50F	SL	SB	
LW	0.76**												
PL	0.85**	0.79**											
IL	0.46	0.67**	0.66**										
PH	0.64**	0.64**	0.56	0.47									
PC	0.77**	0.61	0.88**	0.66**	0.45								
SD	0.85**	0.62	0.74**	0.36	0.65**	0.68**							
FFL	0.08	-0.15	0.21	0.16	-0.30	0.32	-0.05						
4LS	0.25	0.03	0.20	0.05	-0.24	0.17	0.23	0.33					
FFB	0.19	0.09	0.37	0.15	-0.29	0.47	0.02	0.59	0.15				
50F	-0.08	-0.34	-0.03	-0.48	-0.55	-0.10	-0.07	0.31	0.32	0.40			
SL	0.15	0.30	0.29	0.06	0.28	0.03	0.43	-0.47	-0.14	-0.26	0.19		
SB	0.27	0.32	0.34	-0.13	0.26	0.03	0.50	-0.43	-0.01	-0.18	0.23	0.92**	
500SW	0.21	-0.01	0.09	-0.50	0.23	-0.12	0.33	-0.41	-0.14	-0.20	0.40	0.73**	0.69**

LL = Leaf Length, LW = Leaf Width, PL = Petiole Length, IL = Internode Length, PH = Plant Height, PC = Plant Canopy, SD = Stem Diameter, FFL = Days to 1st Foliage leaf, 4LS = Days to four leaf stage, FFB = days to 1st floral buddings, 5OF = Days to 50% flowering, SL = Seed Length, SB = Seed Width, 500SW = 500 Seed Weight; **: Significant at $p<0.01$

Table 4: Cluster composition of 16 *Amaranthus* accessions

Cluster	No. of accessions	Accession code (Accession No.)
1	1	NH84/443 (3)
2	6	NH84/190-1(7)
		NH84/445 (8)
		NH84/443-1 (10)
		NH84/448 (12)
		NH84/451-k (15)
		NH84/190-2 (16)
3	5	NH84/493-1 (2)
		NH84/441 (5)
		NH84/46 (6)
		NH84/494 (9)
		NH84/445-1 (11)
4	4	NH84/463 (1)
		ED87/33 (4)
		NH84/101913 (13)
		NH84/444-4 (14)

Table 5: Cluster means for some quantitative characters in *A. hybridus* accessions

	Clusters			
Characters	1	2	3	4
No. of accessions	1	6	5	4
Leaf length (cm)	29.40	18.00	20.96	17.33
Leaf width (cm)	12.40	8.43	9.70	7.93
Petiole length (cm)	16.90	10.20	13.04	9.55
Internode length (cm)	5.50	4.17	4.40	3.40
Plant height (cm)	181.00	138.48	128.26	80.78
Plant canopy (cm)	94.00	44.03	59.50	46.65
Stem diameter (cm)	1.78	0.67	1.09	0.55
Days to 1st floral bud	86	77.50	80.60	84.25
Days to 50% flowering	86	86.17	89.40	95.00
Seed length (cm)	1.21	1.17	1.27	1.05
Seed breadth (cm)	1.13	1.02	1.21	0.96
500-seed weight (g)	0.32	0.28	0.29	0.25

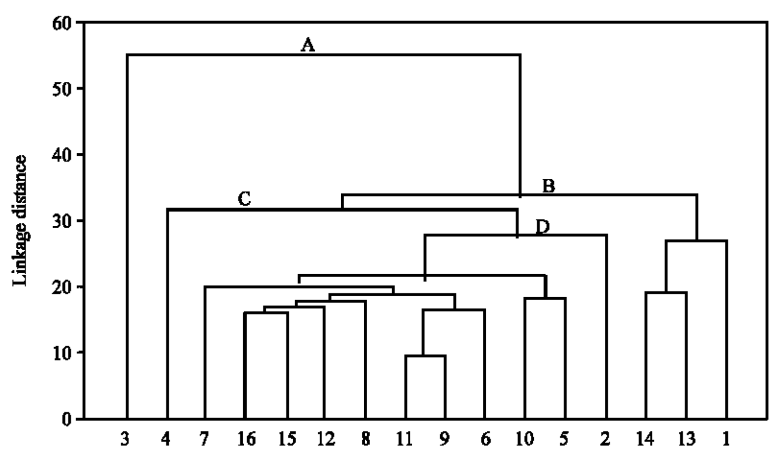

Fig. 1: Cluster analysis dendrogram of the 16 accessions of *A. hybridus*

Five hundred seed weight was also highly correlated with these two traits (R = 0.73 and 0.69), respectively. Generally, days to 50% flowering was negatively correlated with most traits although they were not significant. However, the value of R = -0.55 for plant height with days to 50% flowing was significant at p = 0.05.

Using the k-means non-hierarchical clustering the Amaranth accessions were grouped into 4 clusters (Table 4). There was only 1 accession in cluster 1 and plants in this accession had the highest values for morphological characters such as plant height (181.00 cm), leaf length (29.40 cm), petiole length (16.90 cm) and internode length (5.50 cm) (Table 5). They also flowered earliest in 86 days and it was a black seeded accession. The number of accessions in cluster 2 was six, five in cluster 3 and four in cluster 4. Accessions in cluster 4 had the lowest values for leaf length (17.33 cm) and plant height (80.78 cm). they also had the lowest value for seed characters, while the accessions in cluster 3 had the highest mean values for seed length and width which was 1.27 and 1.21 cm, respectively.

The results of the dendogram were consistent with the results in Table 4 as all accessions were recognized in the dendogram (Fig. 1). It was observed that the accessions coded 4 which was part of the accession in cluster No. 4 (Table 4) was separated from the other accessions in the dendogram.

DISCUSSION

The broad variability observed in leaf and seed morphology of the accessions studied is promising from the viewpoint of the genetic improvement of *A. hybridus* for leaf and grain vegetables. Wu *et al*. (2000) in a study of 229 genotypes from 20 *Amaranthus* species observed wide variability which was useful in cultivar improvement for agronomic traits, such as plant height, seed, stem and leaf colour among genotypes within same species and among different *Amaranthus* species. Similar results were also observed by Xiao *et al*. (2000) and Varalakshmi (2004), in the evaluation of different accessions of vegetable amaranths. Qualitative characters have also been opined to be important for plant description and are mainly influenced by the consumer's preference, socio-economic scenario and natural selection (Zafar *et al*., 2006). They are also useful in separating varieties especially when the range of quantitative characters is limited (Ghafoor and Ahmad, 2003). Such characters in this study are represented by leaf and stem colour which make them acceptable to consumers. Most of the accessions in this study were of green leaf and stem colour which is a desirable qualitative character for selection especially when plant is used as a leaf vegetable.

Correlation measures the intensity of association between variables. In this study, the strong correlation between the leaf morphology variables and also between the seed morphology variables can be exploited directly/indirectly for the genetic improvement of the amaranths along these two lines-leaf and grain. The negative correlation between plant height and days to flowering could also be useful as the farmer can harvest for vegetables for a longer period before harvesting for grain especially if breeding lines could be developed that would combine high leaf and grain yields.

An important aspect of this study is the consistency between the dendogram and the cluster grouping of the accessions. In both cases, the accessions were in 4 distinct groups. This is obvious with the accession coded 3 which was the only accession in cluster 1 and was the most isolated accession in the dendogram. This accession which is quite different from all other accessions exhibited the highest value for leaf and plant height characteristics. Similar closeness was observed for accession coded 7, 8, 12, 15 and 16 which were in cluster 2 and in the group C of the dendogram. Although, in the dendogram, the accession coded 15 and 16 were the closest in the group, they all seem to be a subgroup/relation of the accession coded 7. Members in this group flowered earlier than accession in other groups.

From the results, accessions within each cluster seem to more closely related to each other than accessions in other clusters based on one or two characteristics. Accessions in cluster group 3 which were in group D in the dendogram had relatively high seed size values as measured by seed length and width while those in cluster 4/ Group B could be referred to as plants with small architecture as measured by morphological characters including seed size and weight.

Characterization of germplasm materials helps to ensure an efficient and effective use of such materials. Multivariate analysis provides an effective way of evaluating germplasm materials in order to identify materials that could be further evaluated and utilized at the genetic level. In this study, means, standard deviations, coefficient of variability, correlations, cluster analysis and dendogram have been used to assess the accessions and group them in a more reliable way using quantitative and qualitative characters. The quantitative characters have been useful as they tend to bring accessions that are genetically similar together in the multivariate analysis thus reducing the number of accessions in a breeding programme to a manageable number. Though, this is the fist attempt at classifying these materials along breeding lines, a few accessions can be selected for use directly in hybridization and also intervarietal hybrids can be developed. It is hoped that selection of accession with better mean performance from each group/cluster will give rise to better hybrids with the desired characters in future generations.

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