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Morphological Assessment of Genetic Variability among Accessions of *Amaranthus hybridus*

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Abstract: *Amaranthus hybridus* is one of the neglected leafy vegetables. It has high levels of lysine and sulphur-containing amino acids which are lacking in many vegetables and cereal grains. Five accessions of the species obtained from National Centre for Genetic Resources and Biotechnology Ibadan, Nigeria, were evaluated in the field for variability in ten quantitative and nine qualitative traits. Completely Randomized Design with five replications per accession was employed. Analyses of variance revealed highly significant differences ($P<0.001$) for leaf width, hypocotyls length, days to 50% flowering, 500 seed weight ($P<0.01$) and leaf length ($P<0.05$). The range, coefficient of variability, phenotypic and genotypic coefficients of variability also revealed high variability for each of the quantitative traits. The highest broad sense heritability (h^2b), GCV, PCV and GA were obtained for days to 50% flowering which was also positively correlated with leaf length and stem diameter. The dendrogram divided the accessions into cluster 1 comprising accessions 3 and 5 and cluster 2 comprising accessions 1, 2, 4. The qualitative traits differed among the accessions with the exception of growth habit, branching index and leaf shape. These variations provide ample opportunities for plant breeders to carry out selection while designing plant breeding programmes for the improvement of the species.

Key words: Morphological · Characterization · Leafy vegetable · Smooth amaranth

INTRODUCTION

Amaranthus hybridus is one of the leafy amaranths which form part of the diet of many indigenous people of Africa and other parts of the world [1, 2]. It belongs to the Family Amaranthaceae. It's common English names include, slender pigweed, green amaranth, smooth amaranth among others [3].

It has been reported that the vegetable amaranths have been largely ignored by the world of science [4]. Many authors actually labeled them as 'neglected crops' [5, 6]. Almost universally, the plants have been scorned as a poor people's resource.

The nutritional endowments of amaranths provide evidence that the plants deserve some scientific attention. Their protein levels are high, 17.4 – 38.3% [4, 7]. Lysine and sulphur-containing amino acids have been found in their leaves. Many vegetables and cereal grains lack these amino acids. Additionally, the leaves are high in carbohydrates, several vitamins including beta-carotene, vitamin c and minerals such as iron, calcium and dietary fibre [7, 8]. Amaranth leaves have been reported to 'contain three times more calcium and three times more vitamin B_3 than spinach leaves as well as twenty times more calcium and seven times more iron than lettuce' [9]. In fact, it has been noted that in spite of a great variety of very nutritious food crops, Africans suffer or die of malnutrition [10].

A great imbalance according to [4] exists between the use of traditional vegetables that have fed Africans for centuries and introduced ones. Many Africans were noted to show more appreciation for the foreign vegetables. The authors further suggested that these neglected vegetables need to be brought out of anonymity because some of them may possess superior genotypes that may help solve some of the world's disturbing food problems.

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Certain aspects of the *A. hybridus* deserve some scientific research and attention. For example, the presence of anti-nutritional factors which interfere with nutrient utilization has been reported in various *Amaranthus* species. Further studies also need to be carried out on the following: pest and disease resistance, delayed flowering, provitamin A and iron bioavailability among others [4] and according to the authors, selection and cross-breeding is one area that could bring rapid advances.

Plant breeders can only achieve success in any crop improvement programme with the knowledge of the extent of genetic variability that exists among accessions of a species [11-13]. The information enables them to choose the accessions to include in a breeding programme.

Four varieties of *A. hypochondriatus* Linn and two varieties of *A. cruentus* L were reported to have been registered and released by the National Crop Varieties and Livestock Breeds Registration and Release committee in Nigeria [14]. None was reported for *A. hybridus*.

A number of authors have advocated the combined use of morphological, biochemical and molecular characterizations to assess a germplasm because they provide complementary information which are necessary for the management and improvement of the germplasm [15, 16]. The aim of this study, therefore, was to assess the morphological variability that exist among five accessions of *A. hybridus*. Both qualitative and quantitative traits were evaluated.

MATERIALS AND METHODS

Experimental Details and Treatments: The seeds of the five accessions were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria while the study was carried out at the Botanical garden of the University of Nigeria, Nsukka. Top soil was collected from the Botanical garden and sieved. Five large broadcast trays labeled 1 to 5 were filled with the sieved top soil and watered. Each accession was sown in each tray on the 20th of April, 2012.

The field work was done in a potted medium to ensure proper watering, since the research was started before the rains. Garden soil, top soil and poultry droppings were mixed respectively in a ratio of 2:2:1 on 27th April, 2012. The mixture was watered and turned everyday till the day of transplanting when it was shared among the pots.

Table 1: Accession codes and colours of seeds used for the study

Accession number	accession code	seed colour
ACC 1	NH 85/82	Brown
ACC 2	NH 84/444	Brown
ACC 3	NH 84/494	Brown
ACC 4	NH 85/193	Black
ACC 5	NG/SA/JAN/09/139	Brown

The five accessions (Table 1 and Fig. 2) were randomly allocated to the plastic pots in a Completely Randomized Design (CRD) and each was replicated five times. The seedlings were transplanted on 5th of May, 2012.

After two weeks of growth, information were obtained from all the plants for the qualitative characters which included growth habit, mature stem colour, leaf pigmentation, leaf shape, leaf margin colour, branching index and petiole pigmentation. Data on flower colour was obtained after six weeks. Then after six weeks of growth, data for the various quantitative traits were also obtained from all the plants. These included plant heights (cm), stem diameters (cm), hypocotyl length (cm), leaf lengths (cm), leaf widths (cm), canopy covers (cm), petiole lengths (cm), length of internodes (cm), days to 50% flowering and 500 seed weight (g). Information on the qualitative trait, colour of the harvested seeds was also obtained after nine weeks of growth.

Statistical Analyses: Many of the statistical analyses for estimating variability were performed. These included calculations of the mean, standard deviation and coefficients of variation for the various quantitative characters. The extent of association among the quantitative characters was also determined using Pearson's correlation coefficient. SPSS 16.0 for windows was used for this. Analysis of variance (ANOVA), genetic coefficient of variability (GCV), phenotypic coefficient of variability (PCV) and heritability (broad sense) were further calculated using the formulae of Mohsin *et al.* [17]. Expected genetic advance was calculated using the formula of Johnson *et al.* [18]. Data from the ten quantitative traits were used to perform a cluster analysis using Ward's method based on squared Euclidean distance. A cophenetic correlation coefficient of the clustering analysis was also performed. The results were displayed in a dendrogram. The analysis was done with the software, InfoStat version 2013e.

RESULTS

Quantitative Characters: There were very highly significant differences among the accessions (Table 2) with regards to leaf width, hypocotyl length, Days to 50% flowering ($P<0.001$), 500 seed weight ($P<0.01$) and leaf length ($P<0.05$). There were no significant differences among the accessions with regards to the other attributes.

Accession 5 (Table 3)had the largest leaf width and it differed significantly from the rest ($P<0.05$). Accession 4 produced the highest leaf length which did not, however, differ significantly from those of accessions 2, 3 and 5 ($P<0.05$). With regards to hypocotyls length, accession 5 again produced the greatest length which did not differ significantly from those of accessions 1 and 3. In terms of days to 50% flowering, accessions 3 and 4 differed significantly from the rest. They also flowered later and the latest was accession 3. Accessions 1, 2 and 5 flowered earlier and the earliest was accession 1. For the trait, 500 seed weight, accessions 5 and 3 produced the highest while the least was produced by accession 2.

It could be inferred from Table 4 that most of the quantitative characters measured exhibited broad variability. Plant height, for example, ranged from 147.25cm to 228.10cm. Days to 50% flowering ranged from 41(Accession 1) to 66 days (Accession 3), an interval of 25days from the earliest to the latest maturing accessions. Relatively high coefficient of variation (CV) was observed for petiole length (23.04%), 500 seed weight (18.56%) and hypocotyls length (17.94%).

Table 5 corroborates the results obtained in Table 4. Here, it could also be seen that plant height and days to 50% flowering had high genotypic and phenotypic coefficients of variability respectively. However, while the broad sense heritability estimate for days to 50% flowering was quite high (79.92%), that of plant height was low (23.75%). Other characters with moderate heritability estimates were hypocotyls length (57.38%); leaf width (56.62%) and 500 seed weight (54.55%). Two characters had negative but low heritability, petiole length (-6.69%) and internodes length (-0.234%). Days to 50% flowering had the highest genetic advance.

Table 2: Analysis of Variance for the Quantitative Characters

Quantitative Traits	Sources Of Variation	Df	MS	VR
Leaf width	Between accessions	4	7.986	7.525***
	Within accessions	20	1.061	
Petiole length	Between accessions	4	9.099	0.686^{NS}
	Within accessions	20	13.257	
Internode length	Between accessions	4	0.845	0.988^{NS}
	Within accessions	20	0.855	
Plant height	Between accessions	4	807.344	2.557^{NS}
	Within accessions	20	315.732	
Stem diameter	Between accessions	4	0.294	2.751^{NS}
	Within accessions	20	0.107	
Leaf length	Between accessions	4	41.642	3.273*
	Within accessions	20	12.722	
Hypocotyl length	Between accessions	4	1.407	7.716***
	Within accessions	20	0.182	
Canopy cover	Between accessions	4	86.337	2.313^{NS}
	Within accessions	20	37.325	
Days to 50% flowering	Between accessions	4	287.540	20.897***
	Within accessions	20	13.760	
500 seed weight	Between accessions	4	0.014	5.667**
	Within accessions	20	0.002	

Table 3: Mean $\pm$ Standard Error (SE) for the significant quantitative traits

Accessions	Leaf Width	Leaf Length	Hypocotyl Length	Days to 50% flowering	500 seed weight
	$X \pm SE$	$X \pm SE$	$X \pm SE$	$X \pm SE$	$X \pm SE$
ACC 1	11.316 ± 0.509^{b}	22.506 ± 2.571^{b}	3.700 ± 0.203^{ab}	45.00 ± 1.789^{c}	0.340 ± 0.025^{b}
ACC 2	10.696 ± 0.384^{b}	26.118 ± 0.788^{ab}	3.160 ± 0.202^{b}	46.80 ± 2.437^{c}	0.280 ± 0.020^{c}
ACC 3	11.926 ± 0.220^{b}	28.780 ± 0.926^{a}	3.360 ± 0.225^{ab}	63.40 ± 1.288^{a}	0.380 ± 0.020^{ab}
ACC 4	11.174 ± 0.686^{b}	29.898 ± 2.099^{a}	2.860 ± 0.181^{b}	53.60 ± 1.208^{b}	0.340 ± 0.025^{b}
ACC 5	13.928 ± 0.314^{a}	25.768 ± 0.479^{ab}	4.240 ± 0.133^{a}	47.00 ± 1.225^{c}	0.420 ± 0.020^{a}
$F\text{-}LSD_{0.05}$	1.359	4.706	0.890	4.894	0.059

1 Means followed by the same lower case letters are not significantly different at 5% level of probability.

Table 4: Estimates of the range, mean, standard deviation and coefficient of variability for the quantitative characters studied

	Range				
Characters	Min.	Max.	X±SE	SD	CV (%)
Leaf width(cm)	8.47	14.76	11.8080 ± 0.298	1.488	12.61
Petiole length(cm)	2.00	19.57	15.387 ± 0.709	3.545	23.04
Internode length (cm)	4.71	8.75	6.492 ± 0.185	0.924	14.23
Plant height (cm)	147.25	228.10	183.514 ± 3.99	19.942	10.87
Stem diameter (cm)	1.21	3.01	2.282 ± 0.074	0.371	16.28
Leaf length (cm)	13.63	33.15	26.614 ± 0.838	4.188	16.74
Hypocotyl length (cm)	2.40	4.70	3.464 ± 0.124	0.622	17.94
Canopy cover (cm)	27.48	58.08	43.502 ± 1.349	6.745	15.50
500 seed weight(g)	0.20	0.50	0.352 ± 0.013	0.065	18.56
Days to 50% Flowering	41.00	66.00	51.160 ± 1.541	7.707	15.06

[1] SD (standard deviation), SE (standard error), CV (coefficient of variability).

Table 5: Estimates of genotypic and phenotypic coefficients of variability, Heritability and Genetic advance for the quantitative characters studied

Characters	GCV	PCV	h^2_B	GA
Leaf width	11.729	20.715	56.623	3.699
Petiole length	- 5.404	80.752	- 6.693	0.414
Internode length	- 0.031	13.14	- 0.234	0.001
Plant height	53.577	225.626	23.746	9.668
Stem diameter	1.639	6.328	25.900	0.315
Leaf length	21.733	69.535	31.255	4.764
Hypocotyl length	7.073	12.327	57.377	2.247
Canopy cover	22.533	108.333	20.800	3.681
500 seed weight	0.682	1.250	54.545	0.211
Days to 50% Flowering	107.029	133.925	79.917	40.061

GCV (Genotypic coefficient of variability), PCV (Phenotypic coefficient of variability) h^2_B (Broad sense heritability in %), GA (Genetic Advance as a percentage of the mean)

Table 6: Linear correlation matrix for quantitative traits of *Amaranthus hybridus*

	LL	LW	PL	IL	PH	Sdia	HL	CC	50F
LW	0.316								
PL	0.138	0.376							
IL	0.554**	0.305	0.164						
PH	0.267	-0.090	-0.159	0.487*					
Sdia	0.593**	0.358	0.115	0.513**	0.222				
HL	-0.339	0.545**	0.332	-0.167	-0.488*	-0.039			
CC	0.371	-0.005	-0.292	0.256	0.595**	0.419*	-0.390		
50F	0.533**	0.013	-0.084	0.254	-0.197	0.542**	-0.302	0.114	
500SW	0.132	0.619**	0.127	-0.177	-0.361	-0.003	0.417*	-0.083	0.016

*, ** = significant at P<0.05 and P<0.01 levels respectively.

KEY: LL (Leaf length), LW (Leaf width), PL (Petiole length), IL (Internode length), PH (Plant height), SDia (Stem diameter), HL (hypocotyls length), CC (Canopy cover), 50F (Days to 50% flowering), 500 SW (500 seed weight).

Table 7: Qualitative characteristics of *A. hybridus* accessions

Accessions	Growth Habit	Branching Index	Mature stem colour	Leaf Pigmentation	Leaf margin colour	Petiole pigmentation	Leaf shape	Flower colour	Mature seed colour
ACC 1 (NH 85/82)	Erect	BAOS	Green stem+ Purple base	Green leaves+ green veins	Purple margin	Green petiole	ovate	Green and purple types	Black, brown yellow types
ACC 2 (NH 84/444)	Erect	BAOS	Purple and Green types	Green leaves+ purple veins & Green leaves+ Green veins	purple & green types	Purple & green types	ovate	Purple-green, green, purple & greenish-purple types	Brown & yellow types
ACC 3 (NH 84/494)	Erect	BAOS	Green stem+ Purple base	Green leaves+ Green veins	Purple margin	Green petiole	ovate	Purple & greenish-Purple types	Brown
ACC 4 (NH 85/193)	Erect	BAOS	Green stem	Green leaves+ Green veins	Green margin	Green petiole	ovate	Green flower	Yellow& Black types
ACC 5 (NG/SA/JAN/09/139)	Erect	BAOS	Green stem+ purple base	Green leaves+ green veins	purple margin	Green petiole	ovate	Purple-green, green, greenish-Purple types	Brown

BAOS (Branches all over stem)

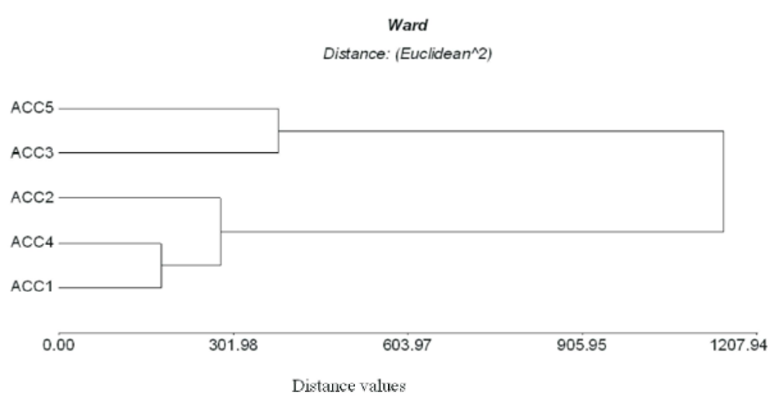
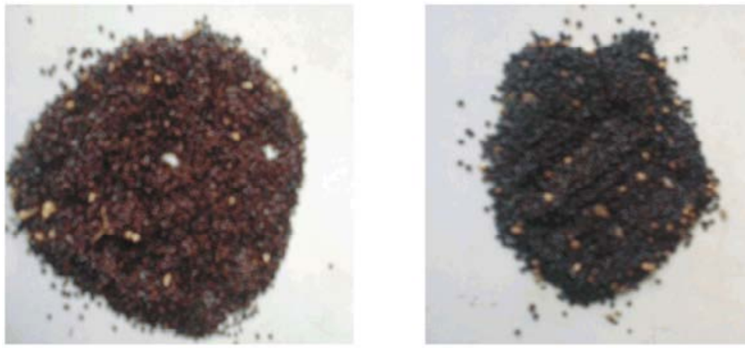


Fig. 1: Dendrogram showing the relationship among the five accessions

Of all the correlation coefficients (Table 6), eleven pairs of characters were positively and highly significantly correlated at $p<0.05$ and $p<0.01$ while one pair was negatively and significantly correlated at $p<0.05$. The highest correlation was between 500 seed weight and leaf width (r= 0.619, coefficient of determination (r^2)= 0.383 or 38%). The 500 seed weight was also moderately correlated with hypocotyls length (r= 0.417, r^2= 0.174 or 17.4%). Other moderate correlation coefficients were between canopy cover and plant height (r= 0.595, r^2= 0.35 or 35%), stem diameter and leaf length (r= 0.593, r^2=0.352 or 35%), internode length and leaf length (r= 0.554, r^2= 0.301 or 30%), days to 50% flowering and stem diameter (r= 0.542, r^2= 0.294 or 29%), days to 50% flowering and leaf length (r= 0.533, r^2= 0.284 or 28%). The only significant but negative correlation was between hypocotyl length and plant height (r= -0.488, r^2= 0.24 or 24%).

The dendrogram in Figure 1 revealed that the accessions formed two distinct clusters. Cluster 1comprised accessions 3 and 5 while cluster 2 comprised accessions 1, 2 and 4. Accessions 3 and 5 had small dissimilarity between them just as accessions 1 and 4. The most dissimilar were accessions 5 and 1. The cophenetic correlation coefficient value of 0.8 was obtained.



Brown **Black**

Fig. 2: Colours of seeds used for the study

Yellow seeds **Brown seeds** **Black seeds**

Fig. 3: Different seed colours harvested from *A hybridus* accessions

Fig. 4: Growth habit of *A. hybridus* accessions

Fig. 5: Petiole, Leaf, Vein and Margin pigmentations in *A. hybridus*

Fig. 6: Different flower colours in *A. hybridus* accessions

Qualitative Characters: A summary of all observations made on the qualitative characters is shown on Table 7 and Figures 3 – 6. All the accessions exhibited erect growth habit, had ovate leaf shape and had branches all over the stem. The other characters like mature stem colour, leaf pigmentation, colour of leaf margin, petiole pigmentation, flower colour and seed colours harvested at maturity differed among the accessions.

DISCUSSION

Evaluation of genetic variability among accessions of a species has been identified as a necessary first step to designing a breeding programme [19, 20]. It enables plant breeders to select the accessions or the particular plant trait to incorporate in a breeding programme. The results of the present study have provided evidence that accessions of *A. hybridus* vary in many traits which is an advantage to any plant breeder. These results agree with the report of Oboh [21) that a great degree of variability exist among the Nigerian accessions of *A. hybridus*.

Days to 50% flowering, for instance, has been shown in this study to vary significantly among the accessions. Early and late flowering accessions were identified. Late flowering has been reported to be an advantage to vegetable amaranths [8] since consumers would have a longer time to harvest the leaves. Early flowering would be more advantageous for grain production.

The variation in days to 50% flowering appear to be truly due to differences in genotype because the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were very high. The heritability in broad sense and the genetic advance (GA) calculated as a percentage of the mean, were also very high. Rajib and Jagafpati [22] explained that high heritability as well as high GA of some traits indicate that there is prevalence of additive gene action in their inheritance. Thus selection for such traits can easily be done. This agrees with the report of Habtamu *et al*. [23] that ‘heritability coupled with genetic gain at specified selection intensity is a reliable tool to guide selection’. Furthermore, Shashi [24] opined that high heritability of characters is an indication that superior genotypes could reliably be selected on the basis of phenotypic performance.

A few other characters studied in this work had moderate broadsense heritabilities. These include hypocotyl length, leaf width and 500 seed weight. These characters, however, had low GCV and PCV as well as GA. These results could be explained with the report of Shashi [24]. The author noted that moderate to high estimates of heritability coupled with low GCV and GA is an indication that such characters were determined by non-additive gene action and for improvement purposes, carrying out selections for such traits will not lead to achievement of breeding aims.

Plant height on the other hand, had the highest PCV but the GCV was moderate. Other characters with high PCVs were Canopy cover, Petiole length and leaf length. Their GCVs, however, as well as broad sense heritabilities and GA were low. Mahmood *et al* [25] explained that in situations where PCV values were much higher than GCVs, it indicates the high involvement of environmental factors or what Habtamu *et al.* [23] called the contribution of non-additive gene effects. Thus, according to these authors, breeding objectives may not be achieved if such traits are selected because of the low proportion of additive gene effect.

Correlation coefficients give an idea of the intensity of associations among characters. Breeding for yield would be very effective when there is positive association among its associated characters [26]. The association between many pairs of characters were positive and highly significant ($P<0.01$).

The highly significant and positive correlation ($P<0.01$) recorded between 500 seed weight and leaf width; 500 seed weight and hypocotyls length; leaf width and hypocotyls length indicate the possibility of increasing the seed yield of *A.hybridus* by indirectly selecting for large leaf width and hypocotyl length. In the same way, large stem diameter and leaf length could serve as morphological markers for the selection of days to 50% flowering. The moderate ‘r^2’ values, however, for the 500 seed weight and its associated characters and days to 50% flowering and its associated characters indicate that apart from these associated characters, other factors could be affecting the 500 seed weight and days to 50% flowering. The highly significant positive association between canopy cover and plant height will be advantageous to the species since greater plant height will expose the canopy to higher light illumination. Zafar *et al* [27] were of the opinion that increasing light penetration into crop canopy may be one of the ways of getting higher grain yield probably as a result of increased photosynthetic rate. For non-significant traits, Taha *et al.* [28] opined that independent and simultaneous selection are required for their improvement.

Cophenetic correlation coefficient is an evaluation measure of the usefulness of the clustering analysis [29]. A value of 0.8 or above is usually considered acceptable [30]. This authenticates the present study where a value of 0.8 was obtained. This shows that there was a good concordance between the actual distances among the accessions and the predicted or dendrogram distances. In otherwords, the accessions truly formed two clusters.

Qualitative characters have also been regarded as being very important for describing a species germplasm. The preference of the consumers, socio-economic scenario and natural selection determine the particular traits that can be found in a society [27]. For instance, most Nigerians prefer to include the green-leaved varieties in their diet while the Chinese prefer the red-leaved variety [9].

In conclusion, this study has actually highlighted the extent of variations (quantitative and qualitative) that exist among the five accessions of *A. hybridus.* It is speculated that such variations or much more could exist among the entire collections of this species being held at NACGRAB. This indicates that plant breeders have ample variations to guide the selection of accessions / quantitative characters to be included in a breeding programme.

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