



International Journal of Botany

ISSN: 1811-9700

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Assessment of Genetic Interspecies Relationships among Five Selected *Amaranthus* Species Using Phenotypic and RAPD Markers

¹A.E. Tony-Odigie, ¹K.O. Adekoya, ²S.C.O. Makinde, ¹B.O. Oboh, ¹L.A. Ogunkanmi and ³M.A. Fowora

¹Department of Cell Biology and Genetics, University of Lagos, Lagos, Nigeria

²Department of Botany, Lagos State University, Lagos, Nigeria

³Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria

Abstract: The genus, *Amaranthus*, is a typical annual flowering plant valued as vegetables, grains and ornamentals. It exhibits a high degree of interspecies variability and as such enhances biodiversity. Two common grain types (*A. caudatus* and *A. cruentus*) and three major weedy types (*A. hybridus*, *A. spinosus* and *A. viridis*) were studied. Phenotypic traits were determined using FAO descriptors for both qualitative and quantitative traits. Extracted DNA samples were amplified through the PCR technique using four RAPD primers (OPA-02, OPA-04, OPB-08 and OPE-01). Statistical analyses for morphological and molecular data were done using the NTSYS Version 2.02j software. Qualitative morphological characters did not significantly discriminate among the five species except for floral morphology. Quantitative characters, however, exhibited wide interspecies variation. Morphological cluster analysis showed that the five species were entirely distinct with a similarity coefficient of 0 except for *A. cruentus* and *A. hybridus* which shared an even very low coefficient of 0.093. The RAPD primers generated a total of 150 bands in the size range of 250-2000 bp. Molecular cluster analysis showed that all the species studied shared a similarity coefficient of 0.57 and some individuals within each species were clustered with individuals from other species. For example, most of the randomly selected plants from *A. viridis* were clustered with plants selected from *A. spinosus* at a similarity coefficient up to 0.81. This close relationship between *A. viridis* and *A. spinosus* may be of medicinal importance for both humans and animals especially since *A. viridis* is more appealing for consumption. The use of RAPD molecular marker systems in *Amaranthus* spp. should be advanced so as to impel specific linkage among genes controlling important traits.

Key words: *Amaranthus*, interspecies relationship, genetic variation, phenotypic markers

INTRODUCTION

Amaranthus originates from the Greek words: a -not, maraino-to wither and anthos-flower. *Amaranthus* are commonly called amaranths or pigweeds and are characteristically annual flowering plants. They possess small flowers that are arranged in dense clusters. Their stems and leaves are deeply pigmented and their fruits are dry, indehiscent and one-seeded (Costea and DeMason, 2001). The genus *Amaranthus* belongs to a relatively large family of dicotyledonous flowering plants, Amaranthaceae which has about 65 genera and 900 species (Costea *et al.*, 2003). Within the genus, approximately 70 species have been recognized with inflorescences bearing colors such as green, purple, red or gold.

Amaranthus, like Quinoa, is known as a “pseudograin” or “pseudocereal” because the flavor, appearance and cooking of many species exhibit

similarities to grains (Gajdosova, 2002). The seeds can also be ground into flour. They have high protein and provide a good source of dietary fiber and dietary minerals like iron, magnesium, phosphorus, copper and especially manganese (Akubugwo *et al.*, 2007). The leaves are used both in cooking and salads as a nutritious leaf vegetable. Some *Amaranthus* species, especially *A. caudatus*, have the most impressive tassel flower which makes it a plant of choice for ornamentals. Their flowers have been used as a symbol of immortality since the time of Ancient Greece with the Greek name “amarantos” meaning “one that does not wither” or “unfading” (Wegerle and Zeller, 1995).

Amaranthus species consist of grain and weedy types with the common grain types being *A. hypochondriacus*, *A. cruentus* and *A. caudatus* and the major weedy types include *A. viridis*, *A. spinosus*, *A. retroflexus* and *A. hybridus* (Oboh, 2007). The genus *Amaranthus* has a high degree of variability both within

and among species. Some species are, in fact, thought to be hybridizations of other species (Brenan, 1981; Grubben and Denton, 2004; Agong, 2006). Historically, taxonomic separation of *Amaranthus* species has been based on differences in floral characteristics but new methods using molecular biology techniques are being employed.

In Nigeria however, little work has been done to assess the interspecies relationships among these species at the molecular level. The bulk of literatures are based on agronomical and physiological characteristics which are often influenced by the environment. This makes them unsuitable for correct assessment of the genetic diversity. Ogunkanmi *et al.* (2010) working on jute plants, showed that this limitation can be largely overcome by the use of molecular markers, which are unlimited in number and are not influenced by environmental factors, growth stages and agronomic practices. Arif *et al.* (2010) stated that Sequence-based analyses sometimes fail to distinguish between species because of the significant similarity between their DNA sequences in the amplified region. They reported that RAPD primers are able to distinguish taxa below the species level because RAPD analysis reflects both coding and non-coding regions of the genome. RAPD markers offer quick screening of different regions of the genome for genetic polymorphism and is applied widely, particularly in plant molecular biology for the detection of genetic variation, construction of linkage maps in plants and animals and in bulk segregant analysis for identifying markers linked to target genes (Huang *et al.*, 2003).

The present study was carried out to determine the genetic interspecies relationships among five selected *Amaranthus* species including grain (*A. caudatus* and *A. cruentus*) and weedy (*A. hybridus*, *A. spinosus* and *A. viridis*) types using RAPD markers.

MATERIALS AND METHODS

Seed collection: Seeds of five *Amaranthus* species (2 grainy species: *A. caudatus* and *A. cruentus* and 3 weedy species: *A. hybridus*, *A. spinosus* and *A. viridis*) were collected from the Department of Botany, Lagos State University, Lagos, Nigeria. The seeds of each species were sown about 1-2 cm deep in pots containing soil and organic matter. The pots were 19 cm high with a diameter of 20 cm. The species were separated apart in the same screen house in the biological garden of the University of Lagos, Lagos, Nigeria. The planting date was noted and thinning occurred when the first true leaves of the plants were fully expanded, leaving three plants per pot. The plants were watered daily and

monitored till flowering to distinguish clearly the different species by their various flower types as this is the main descriptor that distinguishes the amaranths.

Phenotypic data: Phenotypic data were recorded on 5 randomly selected plants per species using both qualitative and quantitative traits. The qualitative traits recorded include leaf venation, leaf margin, leaf shape, leaf apex, leaf base, leaf color, leaf type, nature of stipule, stem habit, type of branching, stem color, seed color, petiole color and also, type and color of inflorescence. The quantitative traits recorded include number of days to germination, highest growth after 12 days, number of days to flowering, 500-seed weight and at onset of flowering: stem diameter, number of leaves per plant, number of branches per plant, leaf width, leaf length, petiole length and plant height.

DNA extraction: DNA was extracted from the leaves of the 5 randomly selected plants per species using a modified protocol (Eppendorf tube centrifuge) based on procedure by Dellaporta *et al.* (1983). Fresh young leaves (0.3 g) were ground properly with mortar and pestle in 800 μ L of extraction buffer preheated at 65°C. The properly ground paste was poured into an Eppendorf tube and 100 μ L of 20%SDS (sodium dodecyl sulphate) were added and incubated at 65°C. The tube was then cooled to room temperature for approximately 4 min after which 300 μ L of ice-cold 5 M potassium acetate was added and mixed. The tube was incubated on ice for 30 min and thereafter centrifuged at 12,000 rpm for 10 min. The supernatant was transferred to two tubes per sample and 1 mL of ice-cold isopropanol was added and mixed for appearance of DNA strands. The isolated DNA is suitable for further molecular analysis. The protocol overcomes the problem of co-precipitation with contaminating agents. The concentration and purity of the DNA was checked by reading the absorbance at 260 nm and the ratio of absorbance at 260/280 nm using a Nanodrop spectrophotometer.

PCR amplification using RAPD primers: The DNA samples were subjected to PCR amplification with seven RAPD primers: OPA-02, OPA-04, OPB-06, OPB-08, OPC-10, OPD-14 and OPE-01 (Table 1). The reaction was carried out in a 25 μ L reaction cocktail consisting of 1 μ L of template DNA, 5 μ L of 1x PCR buffer, 2 μ L of 1.5 mM Magnesium Chloride, 0.5 μ L of 200 μ M of each dNTP, 0.5 μ L of 20 pmol of RAPD primer, 0.2 μ L of 1U *Taq* DNA polymerase (Fermentas) and the remaining 15.8 μ L was made up of ultrapure water. The PCR amplification was carried out in an Eppendorf Mastercycler gradient with an

initial denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 30 sec, 36°C annealing for 1 min and 72°C for 1 min. This was followed by a final extension at 72°C for 10 min. The PCR products were analyzed by separating on a 1% agarose gel and visualized under UV light and photographed. One kibar pair DNA ladder was used as DNA molecular weight standard to estimate the molecular weights of the amplified products.

Statistical analysis: Quantitative morphological data were subjected to analysis of variance using SPSS 15.0 for Windows, release 15.0.0. Student-Newman-Keuls and Duncan's multiple comparison tests were used to test for significant differences among the species. The values generated from morphological characters were also used to compute pair wise distance (similarity) matrices using sequential, hierarchical and nested (SAHN) clustering option of the Numerical Taxonomy System of Multivariate Program (NTSYS) software package, Version 2.02j (Rohlf, 1993). The program generated a dendrogram which grouped the test lines on the basis of Nei genetic distance (Nei, 1972) using Unweighted pair group method with arithmetic average (UPGMA) cluster analysis. Statistical packages from Excel were used in calculating the mean and standard deviation. The fragment sizes of PCR amplified products were estimated from the gel by comparison with standard molecular weight marker (1 kbp DNA ladder). For each decamer primer, a matrix of all the bands present in the different DNAs was generated using '1' when the band was present and '0' when the band was absent. Statistical analyses for the data were also done using the NTSYS Version 2.02j software.

Table 1: List and sequence of RAPD primers used for the amplification of DNA from the five species of *Amaranthus*

Primer code	Nucleotide sequence 5' to 3'
OPA-02	TGCCGAGCTG
OPA-04	AATCGGGCTG
OPB-06	TGCTCTGCCC
OPB-08	GTCCACACGG
OPC-10	TGTCTGGGTG
OPD-14	CTTCCCAAG
OPE-01	CCCAAGGTCC

Table 2: Qualitative characters of *Amaranthus* species

Species	Habit	Stem morphology			Inflorescence	
		Branching	Color	Seed color	Color	Type
<i>A. caudatus</i>	Tall, prostrate	Less branched	Red	Cream, yellow, dark brown	Cream, light red, dark red	Racemose
<i>A. cruentus</i>	Tall, erect	Less branched	Light green with red coloration	Black	Dark pink	Racemose
<i>A. hybridus</i>	Tall, erect	Multi-branched	Green	Dark brown	Bright green	Cymose
<i>A. spinosus</i>	Erect	Highly multi-branched	Light green with red coloration	Black, brown	Green	Cymose
<i>A. viridis</i>	Tall, erect	Fairly branched	Green, red	Brown, dark brown, orange	Light green	Racemose

RESULTS

Morphology: Qualitative characters such as leaf and stem morphology did not significantly discriminate among the five species studied. The stems of *A. caudatus* however, were different from the stems of other species in having a pronounced prostrate habit. Floral morphology discriminated among the species and most of the floral phenotypic characters were observed and recorded at the onset of inflorescence (Table 2). For quantitative characters, *A. cruentus* generally had the most significant highest values while *A. spinosus* generally had the least significant values for most of the characters observed, for example, number of days to flowering, leaf width and plant height (Table 3). There was no significant difference among the species for leaf length, stem diameter and petiole length except for *A. cruentus* which has no petiole. For number of branches per plant, only *A. cruentus* was significantly different from the other species. It was observed that there was no significant difference between *A. caudatus* and *A. viridis* for most of the quantitative characters recorded.

Morphological data analysis: The means of the quantitative parameters observed for each species were used to generate a UPGMA dendrogram (Fig. 1). It showed that the five species were very distinct except for *A. cruentus* and *A. hybridus* which were clustered together at an even very low similarity coefficient of 0.093.

RAPD-PCR: Out of the seven decamer primers used, four produced polymorphic patterns and three of these primers gave RAPD profiles for at least one sample in each species. OPA-02 gave 36 bands (Fig. 2a), OPA-04 gave 23 bands (Fig. 2b), OPB-08 gave 13 bands (Fig. 2c), while OPE-01 gave 78 bands (Fig. 2d). The primers generated a total of 150 bands in the size range of 250-2000 bp. The seven primers which were tested had G+C contents of 60-70% and the primers that generated higher numbers of bands had G+C contents of 70% (OPA-02 and OPE-01). However, OPB-06 also had a G+C content of 70% but gave no amplification product and therefore generated no band.

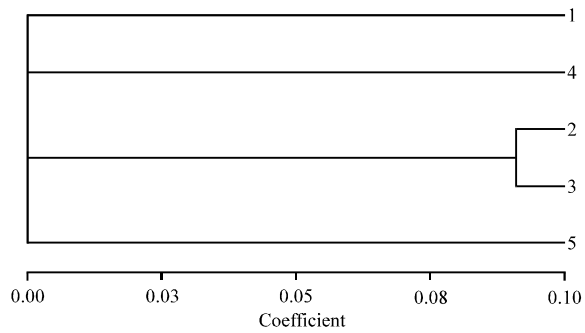


Fig. 1: UPGMA dendrogram showing interspecies relationship among the studied *Amaranthus* spp. based on quantitative morphological parameters. 1: *A. caudatus*, 2: *A. cruentus*, 3: *A. hybridus*, 4: *A. spinosus* and 5: *A. viridis*

Molecular data analysis: To yield more accurate and reliable results, the RAPD profiles of the four primers were compounded together to generate a UPGMA dendrogram shown in Fig. 3. All the plants studied shared a genetic similarity of 0.57. Four major clusters were observed at a similarity coefficient of 0.65.

A. viridis plant 1 was clustered with *A. hybridus* plant 5 at a coefficient of 0.75 in the first major clades, in fact, plant 4 was clustered with *A. hybridus* cluster; plants 2 and 4 were in the second major cluster but in different plant 4 at a very high coefficient of 0.9123; plant 3 was in the third major cluster; while plant 5 was alone in the fourth major cluster at the coefficient of 0.65.

A. cruentus plants 1, 3 and 4 were grouped in the first major cluster at a coefficient of 0.77 while plants 2 and 5 were grouped in the third major cluster at a high

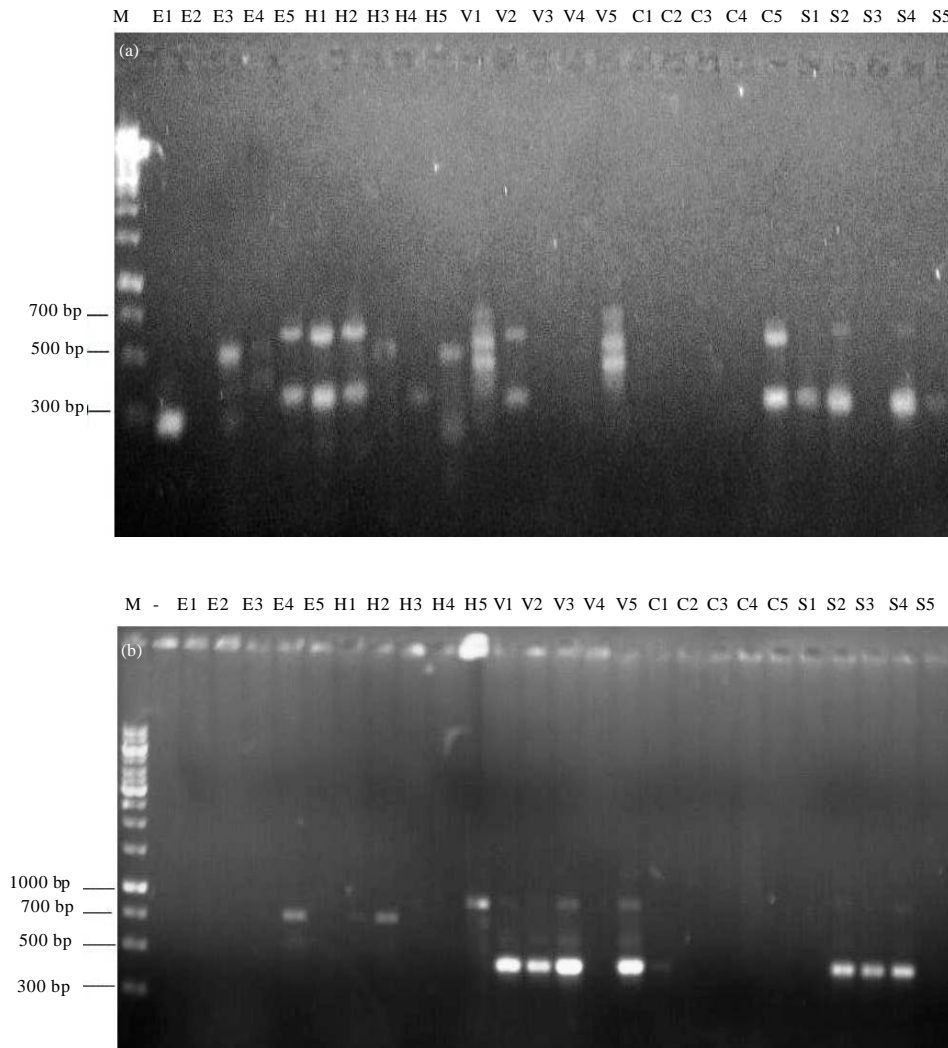


Fig. 2: Continue

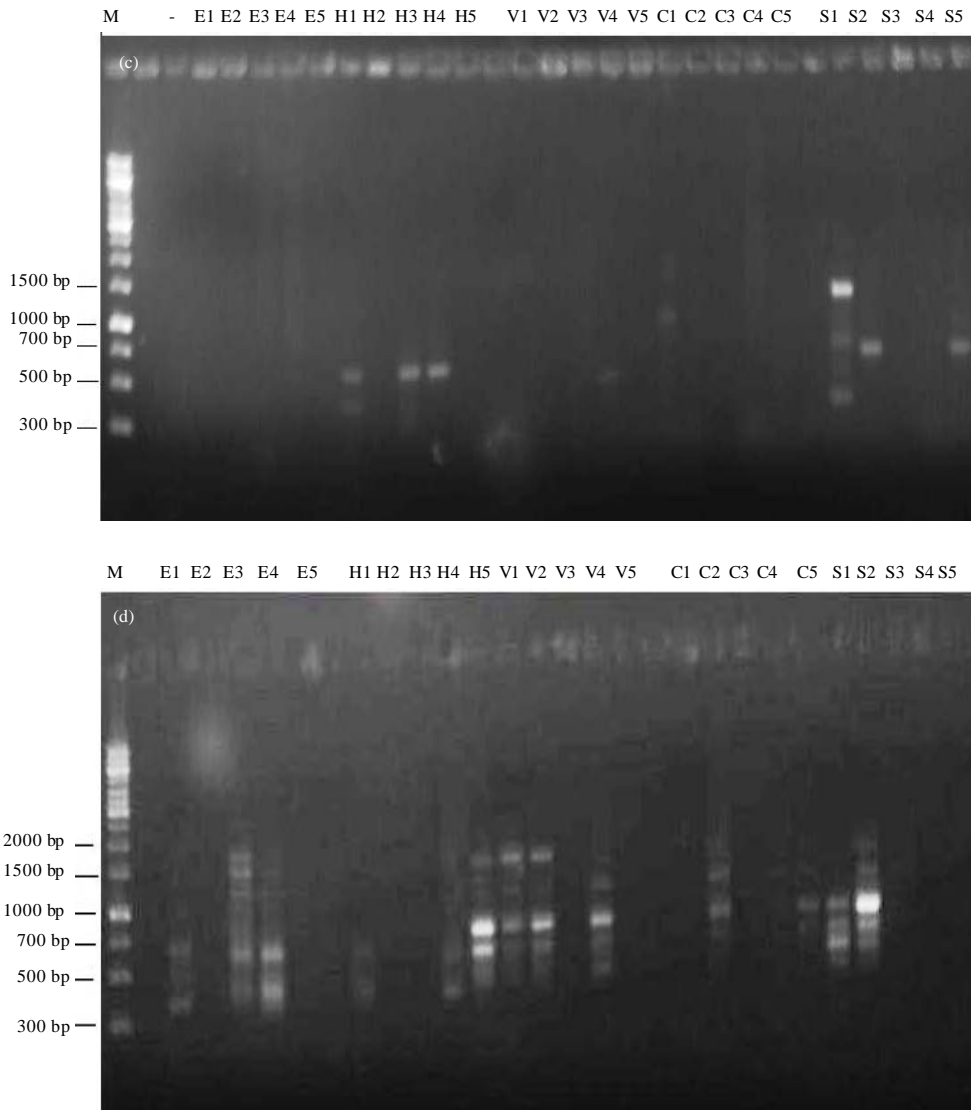


Fig. 2(a-d): RAPD profile using primer (a) OPA-02, (b) OPA-04, (c) OPB-08 and (d) OPE-01, Lane M: Primer marker, 1 kbp DNA ladder, Lanes E1-E5: *A. cruentus* plants 1-5, Lanes H1-H5: *A. hybridus* plants 1-5, Lanes V1-V5: *A. viridis* plants 1-5, Lanes C1-C5: *A. caudatus* plants 1-5, Lanes S1-S5: *A. spinosus* plants 1-5, were all selected at random

coefficient of 0.88; plant 2 was also in the third major cluster but interestingly clustered with *A. caudatus* plant 3 at an extremely high similarity coefficient of 1.00.

A. hybridus plants 1 and 3 were related at 0.697 in the third major cluster; plant 2 was clustered with *A. cruentus* plant 5 at a very high coefficient of 0.934 in the third major cluster.

Amaranthus caudatus plants 1, 3 and 4 were related at 0.79 in the third major cluster while plants 2 and 5 were related at 0.78 in the second major cluster

with plant 5 in the same clade with *Amaranthus spinosus* plant 2 at a high coefficient of 0.87.

A. spinosus plants 1 and 2 were related at 0.702 in the second major cluster while plants 3, 4 and 5 were related at a high coefficient of 0.88 in the third major cluster.

These results show the high degree of variability among and also within *Amaranthus* species even at the molecular level. This provides the amaranths the potential capacity of enhancing biodiversity especially within the cereal food supply.

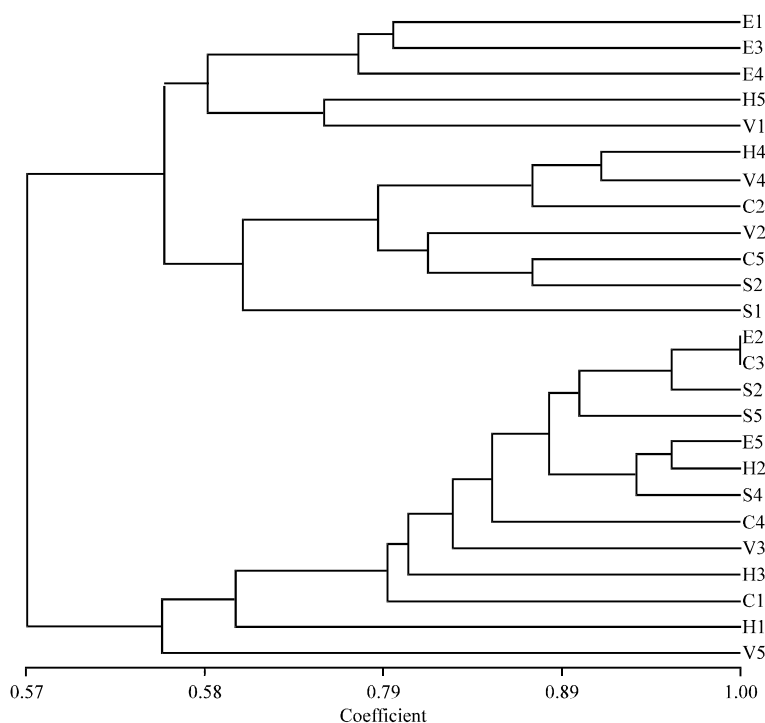


Fig. 3: UPGMA dendrogram showing molecular relationships among *Amaranthus* spp. for primers OPA-02, OPA-04, OPB-08 and OPE-01. E1-E5: *A. cruentus* plants 1-5, H1-H5: *A. hybridus* plants 1-5, V1-V5: *A. viridis* plants 1-5, C1-C5: *A. caudatus* plants 1-5 and S1-S5: *A. spinosus* plants 1-5 selected at random

DISCUSSION

Morphological study: For qualitative characters, only floral characters significantly distinguished the five species. As is also found in the literature, members of *Amaranthus* species are usually confounded with one another and are majorly distinguished by their flowers. The grain amaranths, especially *A. cruentus*, gave the highest values for most of the quantitative parameters observed while the weedy amaranths, especially *A. spinosus*, gave the lowest values (Table 3). The number of leaves/plant and number of branches/plant at onset of flowering were highest in *A. cruentus* with mean values of 157.60 and 59.20, respectively followed by *A. spinosus* with values of 138.60 and 18.60, respectively. This indicates that these plants have more potential for use as leafy vegetables. However, due to the very small size of the leaves of *A. spinosus* coupled with the fact that it.

The UPGMA dendrogram (Fig. 1) generated from the means of the quantitative parameters observed for each species showed that the five species were unrelated. *A. cruentus* and *A. hybridus* were clustered together but at an even very low coefficient of 0.093. This extremely high phenotypic variation is not farfetched since most of

the parameters used to generate the data were collated at the onset of flowering when the species can be easily distinguished.

Molecular study: Unlike results from morphological study, all the plants studied shared a genetic similarity of 0.57. This is expected since they are all members of the same genus *Amaranthus* and should therefore share some degree of genetic similarity. Interestingly, (Ray and Roy, 2009) working on the genetic diversity and relationships among 6 *Amaranthus* species from 8 phytogeographic regions of the Indo-Gangetic plains calculated a mean genetic similarity coefficient of 0.56 using RAPD marker. A similarity coefficient of 0.57 means that there is about 43% genetic variation among all the *Amaranthus* species studied.

The molecular data from this study also showed that some individuals within each species were confounded with individuals from other species even at the molecular level (Fig. 3). Gupta and Gudu (1991) stated that most of the species of *Amaranthus* are predominantly self-pollinated, although outcrossing is also possible. *A. cruentus* plant 2 seemed to share a 100% genetic similarity with *A. caudatus* plant 3 (Lanoue *et al.*, 1996) found *A. caudatus* and *A. cruentus*

Table 3: Means of quantitative characterization of *Amaranthus* species

Parameters	<i>A. caudatus</i>	<i>A. cruentus</i>	<i>A. hybridus</i>	<i>A. spinosus</i>	<i>A. viridis</i>
Germination rate (days)	2.20±0.22	2.60±0.27	3.80±0.42	4.40±0.27	3.40±0.27
Days to flowering	88.80±9.22	127.20±5.39	53.60±0.84	26.00±0.5	99.40±7.04
Stern diameter (cm)*	0.95±0.20	1.21±0.13	0.84±0.13	0.76±0.04	0.80±0.12
No. of leaves/plant*	28.8±6.20	157.60±13.60	48.60±12.63	138.60±12.51	80.40±21.72
No. of branches/plant *	28.80±6.20	59.20±7.20	21.60±4.54	18.60±0.91	26.40±9.54
Leaf width (cm)*	5.10±1.27	4.65±1.66	4.65±1.03	1.90±0.85	4.80±1.70
Leaf length (cm)*	24.95±5.27	10.25±4.77	9.75±5.27	3.25±1.31	10.35±3.85
Plant height (cm)*	101.34±19.65	113.30±12.99	55.14±10.14	19.10±1.23	68.34±15.01
Petiole length (cm)*	7.65±3.57	**	4.95±2.02	1.90±0.49	5.35±2.29
500-seed weight (g)	0.41	0.46	0.27	0.19	0.31

*At onset of flowering, ***A. cruentus* leaves have no petiole

to be more closely related to each other and to their putative progenitor *A. hybridus* using restriction-site variation of PCR-amplified chloroplast and nuclear DNA. *A. cruentus* plants 1, 3 and 4 shared a 68% genetic similarity with *A. hybridus* plant 5; plants 2 and 5 shared 88% genetic similarity with *A. hybridus* plant 2 and plant 5 shared 95.6% genetic similarity with *A. hybridus* plant 2. Also, *A. caudatus* plants 1, 3 and 4 shared a 79% genetic similarity with *A. hybridus* plants 2 and 3; and plants 2 and 5 also shared a genetic similarity of 79% with *A. hybridus* plant 4. This genetic similarity between the two grain amaranths (*A. caudatus* and *A. cruentus*) and *A. hybridus* strongly support the monophyletic origin of grain amaranths. (Xu and Sun, 2001) also reported significant similarities between these grain amaranths and *A. hybridus* using several molecular systems including internal transcribed spacer, amplified fragment length polymorphism and double-primer fluorescent intersimple sequence repeat markers.

The data also show that *A. viridis* plants 2 and 4 were related with *A. spinosus* plant 2 at a high similarity coefficient of 0.79; plant 2 was indeed clustered with *A. spinosus* plant 2 at 0.81 and they were all in the same clade with *A. spinosus* plant 1 at 0.71. Also, *A. spinosus* plants 3, 4 and 5 were in the same clade with *A. viridis* plant 3 at 0.83. This close relationship between *A. viridis* and *A. spinosus* may be of medicinal value. Several numerous medicinal benefits of *A. spinosus* have already been identified but one major problem associated with the consumption of this species for both humans and animals has been the numerous spines it possesses. (Jansen, 2004) stated that "*A. spinosus* is acquiring the status of a famine food." *A. viridis* might have potential as a better substitute. Interestingly (Khan *et al.*, 2011) reported that these two species are very identical in terms of therapeutic properties and dosage.

The trends of species relationships amongst the amaranths determined by this study are consistent with their cytogenetic and evolutionary relationships that have already been established. The results of this study

support the hypothesis of a single primary domestication, with subsequent spreading and differentiation of other domesticates. This complies with the available molecular data to date on the evolutionary origin of grain amaranths (Xu and Sun, 2001; Ray and Roy, 2009).

CONCLUSION

The use of RAPD molecular marker systems in *Amaranthus* spp. should be advanced so as to impel specific linkage between RAPD markers and genes controlling important traits. These molecular tools will greatly assist researchers in the identification of new and different sources of diversity which may provide breeders a handful of information with which they could use to decide what genotypes to cross so as to make new genetic combinations. This would improve plant quality and yield *Amaranthus*, because of the high degrees of interspecies variability, may enhance biodiversity within cereal food supply and deliver essential ingredients both as a grain and vegetable crop.

ACKNOWLEDGMENTS

The authors would like to thank Department of Cell Biology and Genetics, University of Lagos, for the provision of logistics and space. Department of Botany, Lagos State University, for the provision of seeds of *Amaranthus*. Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, for the provision of some molecular tools.

REFERENCES

- Agong, S.G., 2006. *Amaranthus caudatus* L. In: PROTA 1: Cereals and Pulses, Brink, M. and G. Belay (Eds.). PROTA, Wageningen, The Netherlands.
- Akubugwo, I.E., N.A. Obasi, G.C. Chinyere and A.E. Ugbogu, 2007. Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo, Nigeria. Afr. J. Biotechnol., 6: 2833-2839.

- Arif, I.A., M.A. Bakir, H.A. Khan, A.H. Al Farhan and A.A. Al Homaidan *et al.*, 2010. Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. *Genet. Mol. Res.*, 9: 2191-2198.
- Brenan, J.P.M., 1981. The genus *Amaranthus* in Southern Africa. *S. Afr. J. Bot.*, 47: 451-492.
- Costea, M. and D.A. DeMason, 2001. Stem morphology and anatomy in *Amaranthus* L. (Amaranthaceae) a-Taxonomic significance. *J. Torrey Bot. Soc.*, 128: 254-281.
- Costea, M., S.E. Weaver and F.J. Tardif, 2003. The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. *Can. J. Plant Sci.*, 84: 631-668.
- Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA miniprep: Version II. *Plant Mol. Biol. Rep.*, 1: 19-21.
- Gajdosova, A., 2002. Improvement of selected *Amaranthus* cultivars by means of mutation techniques and biotechnological approaches. Report of the Second Research Coordination Meeting of FAO/IAEA CRP Genetic Improvement of Under-Utilized and Neglected Crops in LIFDCs Through Irradiation and Related Techniques, San Jose, Costa Rica, pp: 27-28.
- Grubben, G.J.H. and O.A. Denton, 2004. *Plant Resources of Tropical Africa 2: Vegetables*. Backhuys Publishers, Netherlands, Pages: 667.
- Gupta, V.K. and S. Gudu, 1991. Interspecific hybrids and possible phylogenetic relations in grain amaranths. *Euphytica*, 52: 33-38.
- Huang, M.C., Y.M. Horng, H.L. Huang, Y.L. Sin and M.J. Chen, 2003. RAPD fingerprinting for the species identification of animals. *Asian-Aust. J. Anim. Sci.*, 16: 1406-1410.
- Jansen, P.C.M., 2004. *Amaranthus spinosus* L. In: PROTA 2: Vegetables/Legumes, Grubben, G.J.H. and O.A. Denton (Eds.). PROTA, Wageningen, The Netherlands.
- Khan, M., S. Musharaf, M. Ibrar and F. Hussain, 2011. Pharmacognostic evaluation of the *Amaranthus viridis* L. *Res. Pharm. Biotechnol.*, 3: 11-16.
- Lanoue, K.Z., P.G. Wolf, S. Browning and E.E. Hood, 1996. Phylogenetic analysis of restriction-site variation in wild and cultivated *Amaranthus* species (Amaranthaceae). *Theor. Applied Genet.*, 93: 722-732.
- Nei, M., 1972. Genetic distance between populations. *Am. Naturalist*, 106: 283-292.
- Oboh, B., 2007. Multivariate analysis of the diversity among some Nigerian accessions of *Amaranthus hybridus*. *Int. J. Plant Breed. Genet.*, 1: 89-94.
- Ogunkanmi, L.A., W.O. Okunowo, O.O. Oyelakin, B.O. Oboh, O.O. Adesina, K.O. Adekoya and O.T. Ogundipe, 2010. Assessment of genetic relationships between two species of jute plants using phenotypic and RAPD markers. *Int. J. Bot.*, 6: 107-111.
- Ray, T. and S.C. Roy, 2009. Genetic diversity of *Amaranthus* species from the Indo-Gangetic plains revealed by RAPD analysis leading to the development of ecotype-specific SCAR marker. *J. Hered.*, 100: 338-347.
- Rohlf, F.J., 1993. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.02J. Applied Biostatistics, New York, USA.
- Wegerle, N. and F.J. Zeller, 1995. Grain amaranth (*Amaranthus* ssp.): cultivation, breeding and properties of an Old Indian plant. *J. Agron. Crop Sci.*, 174: 63-72.
- Xu, F. and M. Sun, 2001. Comparative analysis of phylogenetic relationships of grain *Amaranthus* and their wild relatives (*Amaranthus*, Amaranthaceae) using internal transcribed spacer, amplified fragment length polymorphism and double-primer fluorescent inter simple sequence repeat markers. *Mol. Phylogenet. Evol.*, 21: 372-387.