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Full length Research Paper

Analysis of genetic variability among plantain cultivars (*Musa paradisiaca L.*) using arbitrarily primed PCR technique

Agoreyo, B. O.[1], Golden, K. D.[2] and Brown, S. E.[2]

[1]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
[2]Department of Basic Medical Sciences, Biochemistry Section and Biotechnology centre, Faculty of Medical Sciences, University of the West Indies, Mona, Jamaica.

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The genetic variability among 6 cultivars of plantain (*Musa paradisiaca* L.) grown in Jamaica and Nigeria was studied, using arbitrarily primed PCR (AP-PCR) technique. The cultivars included Maiden plantains and Horse plantain grown in Jamaica: Bini, Ayo and Igbiya plantains grown in Nigeria. DNA fragment band positions were obtained with fragment sizes ranging from 0.438 to 1.926 kb. The single distance matrix calculations and the generated dendrogram revealed a clustering together of plantain cultivars across sources of propagation. The analysis showed that the plantain cultivars studied, were split into two clusters, One group consisted of Maiden plantains and Horse plantain from Jamaica and the second contained Bini, Ayo and Igbiya of Nigeria. Cultivars Ayo and Igbiya were closest while Horse and igbiya were one of the farthest apart, in genetic relatedness.

Key words: *Musa paradisiaca*, plantain, AP-PCR analysis, genetic diversity.

INTRODUCTION

Plantains are important components of food security in the tropics and they also provide income to the farming community through local trade (Crouch et al., 1998). Morphological description has proven very useful for the identification of the large diversity of plantain cultivars that exist in the tropics (Tezenas du Montcel 1987; Jarret and Gawel, 1995). However, close genetic relationships among cultivars as well as frequent somatic mutations and morphological changes due to environment which have resulted in large number of cultivars, are major obstacles that limit the use of this technique. Consequently, the use of only morphological parameters could result in over- or underestimations of the degree of relatedness among plantain cultivars (Daniells, 1990; Swennen, 1990; Kaemmer et al., 1992).

In addition to the use of morphological description in identifying specific plantain cultivars, various DNA-based marker techniques are also been employed. These techniques can supply additional information not available from the examination of morphological characteristics alone (Jarret and Gawel, 1995; Shaibu et al., 2003). The random amplified polymorphic DNA (RAPD) or arbitrarily primed polymerase chain reaction (AP-PCR) is a DNA-based marker technique that has been successfully used to distinguish diverse *Musa* germplasm (Kaemmer et al., 1992; Howell et al., 1994; Bhat and Jarret, 1995). The present study reports the use of RAPD analysis for the assessment of genetic variability among plantains grown in Jamaica and Nigeria.

*Corresponding author. E-mail: agoreyobo@yahoo.com.

MATERIALS AND METHODS

Plant material

Freshly harvested, mature, green, unripe plantain fruits (*Musa paradisiaca* L.), were obtained from a local market in Benin City, Nigeria and Papine, St. Andrew, Jamaica. The unripe plantain fruits (*Musa paradisiaca* L. cvs. French and Horn) from Nigeria, were sun-dried and air freighted to Jamaica and used for the analysis; while unripe plantain fruits (*Musa paradisiaca* L. cvs. French and Horn) from Jamaica were also used.

Table 1. Plantain cultivars used for the RAPD analysis.

Cultivar	State of origin of sample	Collection series	Genotype	Description/ Synonym
Bini plantain	Nigeria	A	AAB	'French' plantain
Ayo plantain	Nigeria	B	AAB	'Horn' plantain
Igbiya plantain	Nigeria	C	AAB	'Horn' plantain (dwarf mutant)
Maiden plantain	Jamaica	FP	AAB	'French' plantain (curved fingers)
Maiden plantain	Jamaica	F	AAB	'French' plantain (flat fingers)
Horse plantain	Jamaica	H	AAB	'Horn' plantain

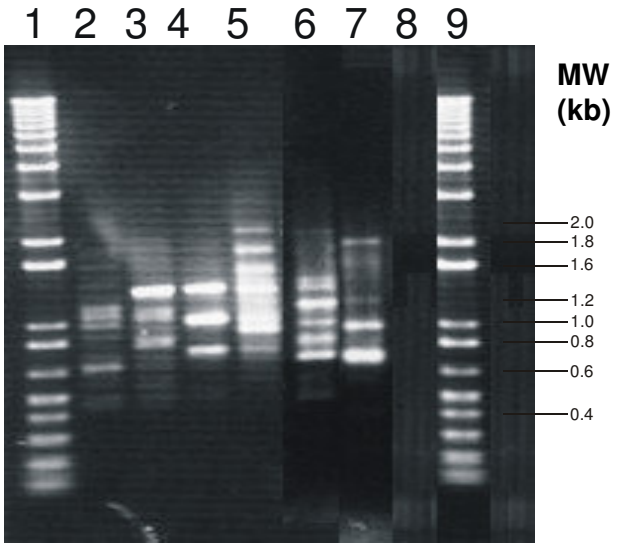

Figure 1. AP – PCR patterns obtained with primer OPC2 (Operon Technologies (Alameda, CA) for plantain cultivars. 1 = Molecular marker, 2 = Maiden Plantain cv. 'French' (curved fingers), Jamaica, 3 = Bini plantain cv. 'French', Nigeria, 4 = Ayo plantain cv. 'Horn', Nigeria, 5 = Igbiya plantain cv. 'Horn' (dwarf mutant), Nigeria, 6 = Maiden Plantain cv. 'French' (flat fingers), Jamaica, 7 = Horse Plantain cv. 'Horn', Jamaica, 8 = negative control, 9 = molecular marker. Positions of molecular weight markers are given in kb.

Extraction of DNA

DNA was isolated from the plantain fruits according to the method of Asemota (1995), while the DNA concentrations were determined spectrophotometrically and diluted to 20 ng μL^{-1}.

Arbitrarily primed PCR analysis

Amplification was carried out in 25 µl reaction volume containing 200 µM nucleotide mix, 1X PCR buffer, 2 mM $MgCl_2$ (Gibco BRL), 10 pmol of random primer (Operon Technologies, Alameda, California), 100 ng plantain template DNA and 1.5 units of Taq DNA polymerase (Life Technologies Inc., Gaithersburg MD). The reaction mixture was overlaid with 1 drop of mineral oil. Amplification was performed in a Perkin Elmer thermal cycler (Applied Biosystems, USA), cycled through the following temperature profile: a first denaturing cycle at 94°C for 2 min to completely denature the DNA template, then 3 cycles at 94°C for 30 seconds, 35°C for 30 s, 72°C for 1 min 30 s. This was followed by 34 cycles at 94°C for 15 s, 35°C for 30 s and 72°C for 1 min 30 s, then a final cycle of 94°C for 15 s, 35°C for 30 s and 72°C 2 min 30 s. The amplified products were separated by electrophoresis on 1.2% agarose gels in TAE buffer (40 mM Tris – acetate, 20 mM sodium acetate, 1 mM EDTA; pH 8.3), stained in ethidium bromide, visualized by ultraviolet transillumination and photographed. An initial screen of the 10 mer oligonucleotide random primers (kits C and G) from Operon Technologies (Alameda, CA) was carried out. One primer (OPC2) of nucleotide sequence 5′ - GTGAGGCGTC - 3′ was found to be suitable for the analysis of plantain cultivars from Jamaica and Nigeria. Experimental reproducibility was estimated by two independent amplifications.

Data analysis

Amplified fragments were scored for band presence (1) or absence (0) using the Labworks computer programme for UVP gel documentation units (Upland, California, USA) and used in the calculation of similarity indices (Weising et al., 1991); where similarity index (S) = 2 x $n_{ab}/n_a + n_b$, with n_a being the total number of bands present in lane a, n_b total number of bands present in lane b and n_{ab} being the number of bands shared by both lane a and b.. A dendrogram was generated using the UPGMA clustering method with the multivariate statistical programme (MVSP, Kovach computing Services, Wales, UK).

RESULTS

A total of 13 different banding patterns were obtained with the OPC2 primer (Operon Technologies (Alameda, CA), with fragment sizes ranging from 0.438 to 1.926 kb (Table 2; Figure 1). The banding patterns were reproducible across extractions. Clustering analysis placed the Nigerian cultivars in a distinct group from the Jamaican cultivars (Figure 2). Analysis of the similarities among the cultivars within each country, showed that in Jamaica there was a 54.5% similarity between the 'French' plan-

Table 2. '0' '1' matrix reflecting presence and absence of RAPD fragments obtained with primer C 2 from DNA of 6 different plantain cultivars from Jamaica and Nigeria.

Plantain RAPD marker	FP	F	H	A	B	C
C2- 1926	0	0	0	0	0	1
C2- 1889	0	0	0	0	0	0
C2- 1816	0	0	1	0	0	0
C2- 1650	1	1	0	0	0	1
C2- 1576	1	0	0	0	0	0
C2- 1433	0	0	0	0	1	1
C2- 1379	0	0	0	1	0	0
C2- 1233	0	0	0	0	0	0
C2- 1190	1	1	1	0	0	0
C2- 1095	0	0	0	1	1	1
C2- 1041	0	0	0	0	0	0
C2- 1012	0	1	1	0	0	0
C2- 965	0	0	0	0	0	0
C2- 850	0	1	0	1	1	1
C2- 776	0	1	1	0	0	0
C2- 650	1	1	0	0	0	0
C2- 500	0	0	0	0	0	0
C2- 438	0	1	0	0	0	0

Table 3. Similarity matrix of 6 plantain cultivars from Jamaica and Nigeria calculated from the presence and absence of products using OPC2 RAPD primer (Operon Technologies (Alameda, CA).

	FP	F	H	A	B	C
FP	100.00					
F	54.50	100.00				
H	25.00	54.50	100.00			
A	0.00	20.0	0.00	100.00		
B	0.00	20.0	0.00	66.70	100.00	
C	22.20	33.30	0.00	50.00	75.00	100.00

FP = Maiden Plantain cv. 'French' (curved fingers), Jamaica
F = Maiden Plantain cv. 'French' (flat fingers), Jamaica
H = Horse Plantain cv. 'Horn', Jamaica
A = Bini plantain cv. 'French', Nigeria
B = Ayo plantain cv. 'Horn', Nigeria
C = Igbiya plantain cv. 'Horn' (dwarf mutant), Nigeria

tains (Table 3). However, there was a 25% similarity between the 'Horn' plantain and the 'French' plantain (curved fingers) and 54.5% between the 'Horn' plantain and the 'French' plantain (flat fingers).

In Nigeria the genetic similarities of the plantains among each other were greater than that of Jamaica. There was a 75% similarity between the 'Horn' plantains. Also, there was a 50% similarity between the 'French' plantain and the 'Horn' plantain (dwarf mutant) and 66.7% similarity between the 'French' plantain and the 'Horn' plantain (Table 3).

There was no similarity between the Horn plantain in Nigeria and the three plantain cultivars in Jamaica (Table 3). Similarities between the two 'French' plantains in Jamaica and the three plantain cultivars in Nigeria were from 20 to 33.3% (Table 3).

DISCUSSION

The large diversity that occurs in plantain, due to genetic variation formed through natural hybridization and soma-

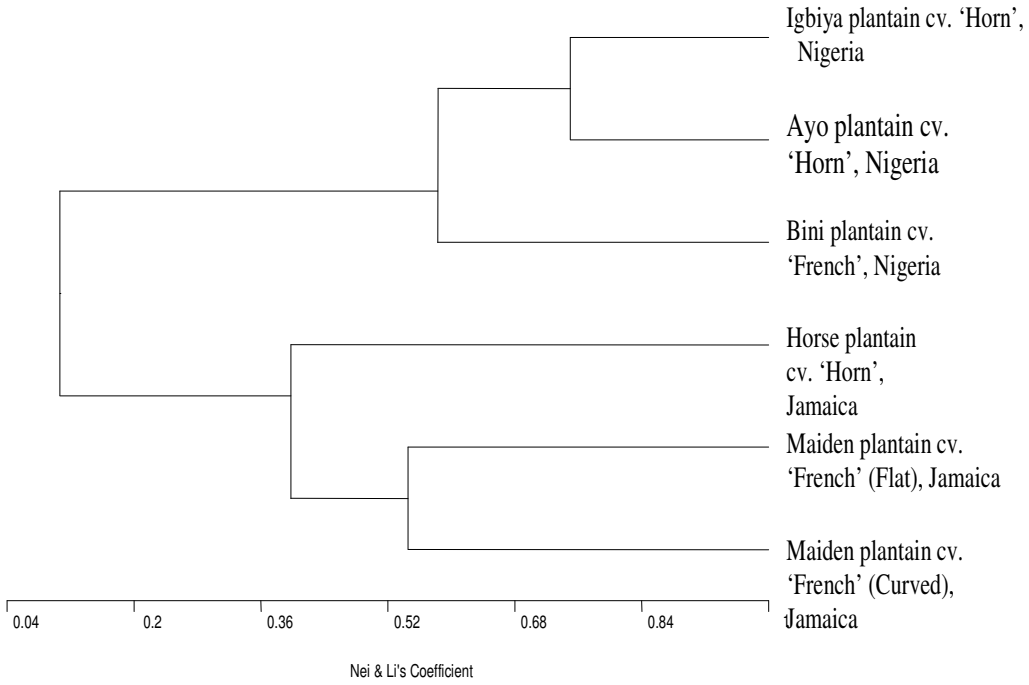

Figure 2. Dendogram based on UPGMA cluster analysis of RAPD from 6 different cultivars of plantain from Jamaica and Nigeria. The analysis was based on 18 informative characters derived from primer OPC2 (Operon Technologies (Alameda, CA). The genetic similarity is given on 0.16 scale.

tic mutation has resulted in a variety of cultivars. In Africa alone, 116 plantain cultivars have been identified and the number of plantain cultivars has been reported to vary from one country to the other (Swennen, 1990; Ogazi, 1996). Identification of this wide variety of cultivars is traditionally based on morphological criteria. However, close genetic relationships among cultivars as well as frequent somatic mutations and morphological changes due to environment are major obstacles for the correct identification of cultivars (Kaemmer et al., 1992). Moreover, plantain cultivars have been named in different dialects resulting in numerous synonyms and homonyms (Shanmugavelu et al., 1992). Scientific techniques that can supply additional information not available from the examination of morphological characteristics alone are therefore necessary for easy and accurate identification of plantain cultivars (Jarret and Gawel, 1995). One method for such analysis is the use of arbitrarily primed PCR technique (AP-PCR technique). AP-PCR markers have been successfully applied in many different plant species to distinguish between cultivars; these plant species include celery (Yang and Quiros, 1993), apple (Koller et al., 1993), rapeseed (Mailer et al., 1994), blackcurrant (Lanham et al., 1995), chrysanthemum (Wolf et al., 1995), yam (Omoregie et al., 2002), and diverse *Musa* germplasm (Kaemmer et al., 1992; Howell et al., 1994; Bhat and Jarret, 1995). AP-PCR technique of indigenous plantain cultivars will also give a more accurate reflection of which cultivars are being cultivated where and how closely, these relate to the names given to these cultivars by farmers in various locations (Crouch et al., 1998).

In the present study the genetic diversity among plantain cultivars grown in Jamaica and Nigeria (Table 1) were revealed by AP-PCR technique. Polymorphisms were distinguished distinctly between the different cultivars (Table 2). This distinction is further demonstrated by UPGMA clustering analysis (Figure 2). These results suggest that AP-PCR technique can unambiguously reveal the diversity and similarities between plantain cultivars grown in Jamaica and Nigeria. Jarret and Gawel (1995) have also reported the use of AP-PCR technique in differentiating and evaluating genetic diversity among Indian banana and plantain cultivars. They also suggested that AP-PCR technique would prove to be of great value as genetic markers in *Musa.* The present results also suggest close similarities between plantain cultivars grown in Nigeria (Table 3). Crouch et al. (1998) have also reported that most plantain landraces in West Africa (which includes Nigeria) have a very high level of genetic similarity to one another, thereby supporting the suggestion that the *Musa* germplasm evolved through somatic mutation of a very small number of progenitors introduced into Africa (Horry et al., 1997). 'Horn' plantain has also been suggested to have been derived from the French plantain by a series of evolutionary changes

(Ogazi, 1996). Ayo and Igbiya of Nigeria ('Horn' plantains) were the closest (Table 3, Figure 2). Igbiya of Nigeria is a dwarf mutant of Ayo, with short fingers that look similar to those of 'French' plantain except that the numbers of hands are very few and the inflorescence is incomplete. The single distance matrix calculations and generated dendrogram revealed that cultivars Horse of Jamaica and igbiya of Nigeria were one of the farthest apart in genetic relatedness (Figure 2).

AP-PCR analysis carried out in the present study on plantain cultivars grown in Jamaica and Nigeria has revealed polymorphisms and similarities that were used in evaluating genetic distances and relatedness between these cultivars. This technique is thus useful for cultivar identification and germplasm characterization for the purpose of *Musa* improvement and for complementing morphological description.

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REFERENCES

Asemota HN (1995). A fast, simple and efficient miniscale method for the preparation of DNA from tissues of yam (*Dioscorea spp.*). Plant Mol. Biol. Rep., 13: 243-247.

Bhat KV, Jarret RL (1995). Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. Genet. Resour. Crop Evol. 42: 107 -118.

Crouch JH, Vuylsteke D, Ortiz R (1998). Perspectives on the application of biotechnology to assist the genetic enhancement of plantain and banana (*Musa* spp.). Elect. J. Biotechnol. 1: 1-18.

Daniells JW (1990). The Cavendish subgroup, distinct and less distinct cultivars. In: Jarret RL (ed.) Identification of Genetic Diversity in the Genus *Musa*. INIBAP, Montpellier, France, pp. 29-35.

Horry JP, Ortiz R, Arnaud E, Crouch JH, Ferris RSB, Jones DR, Mateo N, Picq C, Vuylsteke D (1997). Banana and plantain. In: Fuccillo D, Sears L, Stapleton P (eds) Biodiversity in Trust (Conservation and use of Plant Genetic Resources in CGIAR centres). Cambridge University, London, pp. 67-81.

Howell EC, Newbury HJ, Swennen RL, Withers LA, Ford – Lloyd BV (1994). The use of RAPD for identifying and classifying *Musa* germplasm. Genome, 37: 328-332.

Jarret RL, Gawel N (1995). Molecular markers, genetic diversity and systematics in Musa. In: Gowen, S. (ed.) Bananas and Plantains. Chapman and Hall, London, UK, pp. 66-83.

Kaemmer D, Afza R, Weising K, Kahl G, Novak FJ (1992). Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (*Musa* spp.). Biotechnol. 10: 1030-1035.

Koller B, Lehmann B, McDermott JM, Gessler C (1993). Identification of apple cultivars using RAPD markers. Theor. Appl. Genet. 85: 901-904.

Lanham PG, Brennan RM, Hackett C, McNicol RJ (1995). RAPD fingerprinting of blackcurrant (*Ribes nigrum* L.) cultivars. Theor. Appl. Genet. 90: 166-172.

Mailer RJ, Scarth R, Fristensky B (1994). Discrimination among cultivars of rapeseed (*Brasscia napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theor. Appl. Genet. 87: 697-704.

Ogazi PO (1996). Plantain – Production, Processing, utilization. Paman and Associates Ltd. Imo State, Nigeria, pp. 1-29.

Omoregie SN, Asemota HN, Prasad PD, Osagie AU (2002). Analysis of intraspecific genetic variability among *in vitro* grown *Dioscorea alata* cultivars using arbitrarily primed PCR technique. Biosci. Res. Comm. 14: 555-565.

Shaibu AA, Dimkpa C, Orburia MN (2003). Bio – Diversity in Diploid *Musa acuminata* (AA Group) and their Hybrids using AFLP markers. Afr. J. Agric. Res. 1: 67–72.

Shanmugavelu KG, Aravindakshan K, Sathiamoorthy S (1992). Banana Taxonomy, Breeding and Production Technology. Metropolitan Book Co. PVT. Ltd. New Delhi. Swennen R, Vuylsteke D (1990). Aspects of plantain breeding at IITA. In: Fullerton RA, Stover RH (eds) Sigatoka Leaf Spot Diseases of Bananas, INIBAP, Montpellier, France. pp. 252–266.

Tezenas du Montcel H (1987) Plantain bananas. Macmillan Publisher Ltd, London, UK. pp. 4-6.

Weising K, Ramser J, Kaemmer D, Kahl G, Epplen JT (1991). Oligonucleotide fingerprinting in plants and fungi. In: Burke T, Dolf G, Jeffreys AJ, Wolff R (eds) DNA Fingerprinting: Approaches and Applications. Birkhäuser, Basel, Switzerland, pp. 312-319.

Wolf K, Zietkiewiez E, Hofstr H (1995). Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. Theor. Appl. Genet. 91: 439-447.

Yang X, Quiros C (1993). Identification and classification of celery with RAPD markers. Theor. Appl. Genet. 86: 205-212.