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Genetic diversity in an African plantain core collection using AFLP and RAPD markers

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Abstract Fifteen AFLP primer pairs (EcoRI+3 and MseI+3) and 60 10-mer RAPD primers were used to detect polymorphisms and assess genetic relationships in a sample of 25 plantains from diverse parts of Western and Central Africa. The discriminatory power of the AFLP technique was greater than that of the RAPD technique, since the former produced markers with greater polymorphic information content (PIC) than the latter. Hence, AFLP analysis appeared to be a morepowerful approach for identifying genetic differences among plantain accessions. In this regard, significant genetic diversity within the plantains was shown by the unweighted pair-group method of arithmetic averages (UPGMA) and the multidimensional principal coordinate (PCO) analyses. The AFLP-derived clusters indicated closer relationships between similar inflorescence types than the RAPD-derived clusters. A small group of cultivars from Cameroon were separated from the bulk of other plantains, suggesting that Cameroon may harbour accessions with useful or rare genes for widening the genetic base of breeding populations derived from the

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plantains. A greater effort should be directed at collecting and characterizing plantain cultivars from Cameroon.

Keywords Plantain · AFLP and RAPD markers · Genetic diversity · Cluster analysis

Introduction

The plantains (Musa spp. subgroup AAB) are triploid (2n = 3x = 33) giant perennial herbs with a characteristic orange yellow starchy flesh when ripe and a similarly coloured compound petal (Swennen and Vuylsteke 1987). The fruits are usually boiled, roasted or fried before consumption. Plantains are natural hybrids of two diploid species, Musa acuminata Colla and Musa balbisiana Colla, which contributed the A and B genomes, respectively. Plantains originated in South East Asia but are so predominant in the humid lowlands of west and central Africa that this region is now considered as the secondary centre of diversification (Swennen and Rosales 1994; Simmonds 1995). This diversity resulted from an accumulation of somatic mutations and was fostered by human activities during the long history of the cultivation of the crop in this region (De Langhe 1961, 1964a). Commensurate with the geographical dispersion of the plantains, many local names and synonyms exist among the accessions, due to the indiscriminate assignment of names by different language groups in different countries and also within the same country (Rossel 1998). Farmers have often given the same name to several similar-looking cultivars. For example, in Nigeria, several plantains are referred to as 'Ntanga' (which means plantains), without consideration of their morphological differences.

Germplasm characterization and classification provide useful information for the genetic improvement of crops (Ortiz 1997). In this regard, Swennen and Vuylsteke (1987) identified 25 plantain cultivars as representative of the total variability in West Africa, based on morphology, agronomic characters and geographic distribution. This group was designated as the core collection of the West African plantains, with differences in pseudostem height, bunch-type, bunch orientation, fruit-apex shape and fruit curvature, but no variation in any of the remaining traits examined (Swennen and Vuylsteke 1987). Morphological traits in plantains are influenced by genotype \times environment interactions making them unstable and variable over time and locations, which limits their use in taxonomy (Ortiz 1995). Moreover, morphological characteristics are usually determined by a small number of genes that may not represent the total genetic diversity within the genome (Brown-Guedira et al. 2000). Therefore, taxonomic groupings based on morphological descriptors may not accurately describe the relationships among accessions. With the advent of molecular markers, especially PCRbased DNA markers such as random amplified polymorphic DNA (RAPD) (Williams et al. 1990) and amplified fragment length polymorphic (AFLP) (Zabeau and Vos 1993), it is now possible to survey a large number of loci and ascribe unambiguous taxonomic-genetic relationships among accessions. RAPD studies in Musa have been used to determine genetic diversity and relationships (Bhat and Jarret 1995; Kaemmer et al. 1997; Crouch et al. 2000; Pillay et al. 2001) and for genome identification (Howell et al. 1994; Pillay et al. 2000).

The core plantain collection of Swennen and Vuylsteke (1987) has been used for the genetic improvement of this crop. Whether this core collection is representative of the genetic diversity of the plantains has not been assessed. The objectives of this study were to: (1) assess the molecular diversity of the core plantain collection, based on AFLP and RAPD analyses, and (2) compare the effectiveness of the RAPD and AFLP techniques in determining genetic relationships in the plantains.

Materials and methods

Plant materials

Twenty five plantain cultivars (Table 1) including 23 from the core collection of Swennen and Vuylsteke (1987) were used in this study. The sample included 'Nazika', a cultivar from the Congo with many hands and small fingers, and 'Baka' from Gabon that is regarded as a synonym of 'Ovang' from Cameroon. The east African Highland Banana, 'Isha' (AAA genome), was used as the outgroup taxon. These plants are a part of the germplasm collection maintained by the International Institute of Tropical Agriculture at the Onne station, in South Eastern Nigeria.

DNA extraction and marker analysis

Genomic DNA was extracted from each accession as previously described (Ude et al. 2002). The AFLP analyses were also performed according to Ude et al. (2002) using the following 15 primer pairs (*Eco*RI+3 and *Msel*+3) from the GIBCO BRL commercial AFLP kit: AAGCTA, AAGCTC, ACACAA, ACACTT, ACGCTG, ACGCAC, AGCCAC, AGCCAC, ACCCAG, AGCCAA, AGCCTT, AGCCTC, AGCCTC, AGCCTG, AGCCAG, ACACTC and ACACAC. RAPD analysis was carried out with 60 decamer primers from kits A, B, C and D from Operon Technologies Inc., Alameda, Calif. RAPD reactions and PCR procedures were carried out as described by Pillay et al. (2000).

 Table 1 Cultivars, countries of origin, inflorescence types and plant stature of the plantains used in the study

Cultivar	Country	Inflorescence type	Plant stature					
Ovang	Cameroon	French	Giant					
Bobby Lannap	Cameroon	French	Medium					
Big Ebanga	Cameroon	False Horn	Medium					
Njock Kon	Cameroon	French	Dwarf Giant					
Batard	Cameroon	French Horn	Medium					
Nazika	Congo	French	Giant					
Baka	Gabon	Horn	Small					
Osoaboaso	Ghana	French Horn	Small					
Asamiensa	Ghana	Horn	Medium					
Agbagba	Nigeria	False Horn	Medium					
Mimi Abue	Nigeria	False Horn	Giant					
Obubit Ukom	Nigeria	False Horn	Small					
Kiogo	Nigeria	False Horn	Medium					
Ukom	Nigeria	False Horn	-					
Ngok Egome	Nigeria	False Horn	Medium					
Orishele	Nigeria	False Horn	Medium					
Egjoga	Nigeria	French	Medium					
Ntanga 5	Nigeria	French	Giant					
Agbagba FR	Nigeria	French	Medium					
Ntanga 2	Nigeria	French	Giant					
Obino L'Ewai	Nigeria	French	Medium					
Akpakpak	Nigeria	French	Medium					
Obubit Ntanga 1	Nigeria	French	Medium					
Ihitism	Nigeria	Horn	Medium					
Ubok Iba	Nigeria	Horn	Medium					
Isha	East African	French	Small					
(AAA genome)	banana							

Data scoring and analysis

A band was considered as polymorphic if it was present in at least one genotype and absent in the others. A data matrix was generated in which each band was scored as "1" if present and as "0" if absent. The relative discriminatory value of a locus was estimated by its polymorphic information content (PIC), which measures the information content as a function of a marker system's ability to distinguish between genotypes (Weir 1990). It is calculated as follows: PIC = $1-\Sigma pi^2$, where pi is the allele frequency for the ith allele. The number of alleles refers to the number of scored AFLP and RAPD fragments. The frequency of an allele was obtained by dividing the number of accessions where it was found by the total number of accessions.

The NTSYS-pc software package (Version 2.02f, Rohlf 1998) was used to calculate genetic similarities between pairs of genotypes based on simple-matching (SM) coefficients. The Mantel test of significance (Mantel 1967) was used to compare the similarity matrices produced by the AFLP and RAPD techniques. The unweighted pair-group method of arithmetic averages (UPG-MA) and the multidimensional principal coordinate (PCO) analyses were used to portray relationships among the plantain cultivars. Thereafter, the co-phenetic correlation (*r*-value) coefficient (Rohlf 1998) was used to test for association between the clusters in the dendrograms and the similarity index matrix.

Results

AFLP analysis

The 15 AFLP primer pairs produced 78 polymorphic bands among the 25 plantains. The number of polymorphic bands per primer pair ranged from 1 to 11 with a mean of 4.87, while PIC values ranged from 0.1 to 0.5

with a mean of 0.24 (see Table 3). Twenty percent (16 bands) of the 78 polymorphic AFLP bands showed PIC scores >0.30.

Genetic distance (GD) values between the plantains ranged from 6.3 to 52% with a mean of 24.4% (Tables 2 and 3). The accessions 'Egjoga' and 'Ntanga 5' were the closest in this study with a GD of 6.3%, followed by 'Agbagba FR' and 'Ntanga 2' with a GD of 6.4%. The four accessions and 'Akpakpak' constituted a group of closely related French plantains with an average genetic distance of 8.5%. All these accessions were assigned to the same cluster by UPGMA analysis (Fig. 1). Other members of this cluster were 'Asamiensa', 'Kiogo', 'Mimi Abue', 'Nazika', 'Ngok Egome', 'Obino l'Ewai', 'Obubit Ntanga 1' 'Obubit Ukom' and 'Orishele'. Kiogo appeared to be quite isolated from other accessions of this cluster (Fig. 1). The UPGMA analysis also produced two smaller clusters with four accessions each: one comprised 'Bobby Tannap', 'Ihitism', 'Njock Kon' and 'Ovang', while the other contained 'Batard', 'Big Ebanga', 'Osoaboaso' and 'Ubok Iba' (Fig. 1). Finally, a fourth cluster with only two accessions, 'Agbagba' and 'Baka', was found (Fig. 1). The accession 'Obino l'Ewai' has been extensively used as a parent in crosses to develop hybrids with resistance to black Sigatoka (Vuylsteke et al. 1993). Interestingly, that accession clustered closely with 'Nazika' and 'Mimi Abue', despite differences in inflorescence morphotype. Quite surprising was the relatively large (31.7%) genetic distance between 'Agbagba' (False horn) and its somaclonally derived French mutant 'Agbagba FR'. Similarly, high GD values ranging from 27% to 32% were found between 'Agbagba' and the French plantains clustering with 'Agbagba FR' (e.g. Akpakpak, Egjoga, Ntanga2 and Ntanga5). In general, genetic distances ranged from 6.4% to 40.5% with a mean of 22% among the French plantains, and from 10% to 34% with a mean of 21% amongst the False horn plantains (Table 3). The genetic distance between 'Baka' and 'Ovang' was 52%, although these accessions were considered synonyms by Swennen and Vuylsteke (1987).

The scatter-plot produced from principal co-ordinate analysis (Fig. 2) also distinguished four major groups in the plantains. Principal co-ordinates 1, 2 and 3 explained 45% of the total variation with each co-ordinate contributing 22.5%, 12.9% and 9.5% of the variation, respectively.

The UPGMA and PCO analyses produced similar genetic clusters of the plantain cultivars. However, 'Baka' and 'Agbagba' that showed closer relationship to 'Bobby Tannap', 'Ihitism', 'Njok Kon' and 'Ovang' in the UPGMA dendrogram (Fig. 1) displayed closer affinity with 'Batard', 'Big Ebanga', 'Osoaboaso' and 'Ubok Iba' in the PCO two-dimensional scatter-plot (Fig. 2).

RAPD analysis

Sixty eight polymorphic bands corresponding to an average of 1.13 bands per RAPD primer were observed.



Fig. 1 UPGMA phenogram of 25 West African plantain cultivars using 78 polymorphic AFLP markers



Fig. 2 Principal co-ordinate map of 25 West African plantains using 78 polymorphic AFLP markers

The mean PIC per RAPD polymorphic band was 0.15 with a range of 0.1 to 0.5. Only 4% of the 68 bands showed PIC scores \geq 0.30. Genetic distances between the accessions ranged from 0 to 50% with a mean of 15.4%, indicating little genetic differences among the cultivars (Table 2). With the exception of 'Obino 1'Ewai', all accessions clustered at 80 to 100% similarity whereas clustering occurred at 64% to 100% with the AFLP data.

Cophenetic correlation and Mantel test

The co-phenetic correlation coefficient (*r*-value) for AFLP and RAPD data was 0.94 and 0.96, respectively, suggesting a very good fit between the dendrogram clusters and the similarity matrices from which they were derived. The Mantel test for comparison of the AFLP-based and RAPD-based similarity matrices showed no correlation [r = -0.11 (P = 0.05)].

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Table 2 Genetic distances (%) between pairs of West African plantain cultivars derived from AFLP data

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	10							10.45	13.24	8.82	19.12	46.27	5.88	11.76	14.71	8.82	12.5	7.35	19.4	16.67	00.1	I4.29
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en pairs of West African plantain cultivars derived from RAPD data autad (0%) betwee Table 3 Genetic distant

Discussion

Comparison of the AFLP and RAPD data

Several studies have compared the effectiveness of different DNA-based marker systems to determine relationships in crop plants. AFLP generally reveals more polymorphisms than RAPD and is a more reliable and robust genetic molecular-marker assay (Lin et al. 1996; O'Neill et al. 1997; Ude et al. 1999). However, Virk et al. (2000) reported that the potential usefulness of molecular techniques in identifying genetic relationships vary from plant to plant because of the uniqueness of different genomes.

In this study, a comparison of the PIC values and the number of fragments that had a PIC ≥ 0.30 between the AFLP and RAPD data clearly showed that the AFLP technique has a higher potential to produce markers with a stronger discriminatory power. With very few exceptions (e.g. Obino l'ewai) the genetic-distance values between any two genotypes in the AFLP-derived dissimilarity matrix are higher than those in the RAPD-derived matrix (Table 2). The PCO analysis also showed greater separation of clusters with the AFLP data (Fig. 2) than with the RAPD data (Fig. 3). This observation is corroborated by the report of Newbury et al. (2000) that the RAPD technique showed very limited polymorphism in a sample of 15 West African plantain landraces that were morphologically diverse.

The co-phenetic correlation values showed that the genetic clusters accurately represented the estimates of genetic similarity. However, the Mantel test product-moment correlation value (r = -0.11, P = 0.05) showed that there was no relationship between the AFLP and RAPD similarity matrices. It appears that the discriminatory power of the RAPD technique was reduced by: (1) the fewer fragments produced, and (2) the smaller percentage of informative fragments since only three fragments had PIC values ≥ 0.3 . Perhaps, the use of more RAPD primers may uncover a greater level of polymorphisms in the plantains.

The combination of the genetic similarity estimates of both techniques is expected to decrease the effect of their independent inaccuracies (Schut et al. 1997). However, the combination of our AFLP and RAPD data showed a bias of genetic clustering towards the AFLP-based clusters (Figs. 5, 6). 'Obino l'Ewai' remained separated in the combined data set as it appeared in the RAPD data (Fig. 3, 5). A likely explanation for this placement of 'Obino l'Ewai' is difficult with the present available data.

In general, our result is consistent with previous reports (Crouch et al. 2000) that AFLP is more powerful than RAPD. Hence we have relied more on the AFLP data for explanation of genetic relationships between the plantain accessions studied.



Fig. 3 UPGMA-based phenogram of 25 West African plaintains using 68 polymorphic RAPD markers. Three clusters were identified with Obino l' Ewai isolated from the clusters

Genetic diversity

The range of genetic distances obtained with the AFLP and RAPD data indicates that there is significant DNA diversity within the putative core collection of West African plantains. This diversity correlates well with the wide range of morphological variability described for these plants (Swennen and Vuylsteke 1987). The AGD of 15.4% for the RAPD data compares well with the AGD of 14.1% obtained by Crouch et al. (2000) for a larger sample of 76 accessions, including the 25 accessions used in this study. Our data indicate that the core collection reflects the genetic variation existing in the plantains. This collection represents about 22% of the plantain cultivars in the germplasm bank at the International Institute of Tropical Agriculture. Using quantitative morphological descriptors, Ortiz et al. (1998) suggested that a core collection of 10% of the available Musa germplasm stored in vitro may contain most of the genetic diversity in Musa. Our estimate for the size of a core collection is higher.

The DNA markers used in this study were unable to clearly differentiate the plantains into their distinct morphogroups based on inflorescence characteristics and pseudostem stature. Similarly no geographical patterns of clustering were observed. However, unlike the RAPD data (Fig. 4) the AFLP data (Fig. 1) showed more clustering of accessions on the basis of inflorescence types. For example, clusters 1 and 3 (Fig. 1) were composed entirely of accessions with degenerate inflorescence types, and included 'Baka' (horn) and 'Agbagba' (False horn) in one group and the French horn types 'Batard' and 'Osoaboaso', 'Big Ebanga' (False horn) and 'Ubok Iba' (Horn) in the other. Similarly, with the exception of 'Ihitism', cluster 2 was composed of French horn types. There was also a distinct subgroup of French types made up of 'Egjoga', 'Ntanga 5', 'Agbagba FR', 'Ntanga 2' and 'Akpakpak' (Fig. 2). These observations suggest that the AFLP technique may be more useful in identifying molecular markers for inflorescence types in



Fig. 4 Principal co-ordinate map of 25 West African plantains using 68 polymorphic RAPD markers



Fig. 5 UPGMA-based dendrogram of 25 plaintain cultivars using 78 AFLP and 68 RAPD polymorphic markers



Fig. 6 Principal co-ordinate scatter plot of 25 plantain cultivars using 78 AFLP and 68 RAPD polymorphic markers

the plantains. Inflorescence morphology was used to cluster plantains (Ortiz et al. 1998). Morphological diversity in the West African plantains apparently arose from somatic mutations in an hypervariable region of the genome from a very limited number of botanically different clonal sources (De Langhe 1961, 1964b; Vuylsteke et al. 1991; Lebot et al. 1999). Similarly, it was hypothesized that inflorescence morphology was regulated by a hypervariable region or hot spot (Vuylsteke et al. 1991). In this study, the test clones included 'Agbagba', a False horn plantain and its somaclonal variant with the French inflorescence, 'Agbagba FR'. Estimates of dissimilarity between 'Agbagba' and its somaclone based on AFLP and RAPD analyses were 32 and 18, respectively. Previous RAPD work showed a genetic distance of 22% between 'Agbagba' and 'Agbagba FR' (Crouch et al. 2000). These estimates are comparable, with a genetic distance of 25% between the giant Cavendish banana and its dwarf somaclone (Engelborghs and Swennen 1999). The cultivars 'Akpakpak', 'Egjoga', 'Ntanga 2' and 'Ntanga 5' are all French plantains that clustered very closely with 'Agbagba-FR', with a narrow average genetic distance of 8.5%. Surprisingly, all of them were equidistant from the 'Agbagba' landrace maintaining a genetic distance from it that is similar to that recorded between 'Agbagba FR' and 'Agbagba' (Table 2). It is tempting to suggest that this cluster may represent a 'somaclone complex' that arose from a somatic mutation in the inflorescence region of a single cultivar, perhaps 'Agbagba', at different times and different geographic locations. It is also possible that the same mutant was taken to different places and given different names in local dialects. These somaclones may have been selected by farmers in different locations.

A small genetically diverse group of cultivars, comprising 'Bobby Tannap', 'Ihitism', 'Njok Kon' and 'Ovang', were separated from the bulk of other plantains (Fig. 2). With the exception of 'Ihitism' that was collected in Nigeria, the other cultivars are from Cameroon. This may suggest that the cultivars from Cameroon are a potential source of useful or rare genes for widening the genetic base of breeding populations derived from the plantains. Thus, a greater effort should be directed at collecting and characterizing plantain cultivars in this region.

In conclusion this study showed that the AFLP technique is a more powerful tool than RAPD for assaying genetic polymorphisms, genetic relationships and cultivar identification among the West African plantain. Our study also showed that the core collection of plantains identified by Swennen and Vuylsteke (1987) is a good representation of the genetic diversity in these plants. The clear separation of the cultivars from Cameroon from those of other parts of West Africa suggests a genetic uniqueness of the Cameroon cultivars. These plants should be exploited for improvement of the plantains. We recommend further collections and characterization of plantains from Cameroon.

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