Theor Appl Genet (2000) 101:1056–1065

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Comparative analysis of phenotypic and genotypic diversity among plantain landraces (*Musa* spp., AAB group)

Received: 8 January 2000 / Accepted: 2 March 2000

Abstract Genetic diversity amongst 76 plantain landraces has been studied using RAPD analysis at two levels of intensity and compared with groupings based on phenotypic indices and morphotype. There was a good correlation (R^2=0.78) between estimates of genetic diversity based on 76 RAPD bands and 164 RAPD bands. However, there was a poor correlation between RAPD-based estimates of genetic diversity and a phenotypic index based on agronomic characters. There was also a poor correlation between RAPD analyses and morphotype group (based on bunch type and stature). These results suggest that the traditional designations of plantain landraces based on morphotype do not provide a true reflection of overall genetic divergence. Similarly, classification systems using phenotypic indices based on agronomic characters may not provide accurate taxonomic differentiation. The level of genetic divergence within morphogroups based on bunch type suggests that True Horn plantains are derived from False Horn plantains which in turn are derived from French plantains. Genetic divergence was found to be generally quite low within the plantain landrace genepool, which is consistent with the proposed evolution of this germplasm through somatic mutation of a relatively small number of introductions. However, putative synonyms/duplicates have been shown to be genetically distinct. In contrast, a group of 12 landraces have been identified that are highly distinct from one another (showing 20–35% dissimilarity). Fertile members of this group may be useful for generating genetically diverse 2x and 4x breeding populations that can be used in breeding secondary triploid hybrid plantain varieties.

Key words Plantain · *Musa* · RAPD · Phenotype · Breeding

Communicated by P.M.A. Tigerstedt

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Introduction

The genus *Musa* L. (family Musaceae, order Zingiberales) contains a wide range of triploid (2n=3x=33) cultivars and landraces of plantains and bananas that evolved from interspecific and intraspecific hybrids of the two wild diploid species, *M. acuminata* Colla. and *M. balbisiana* Colla., which provide the A and B genomes, respectively (Simmonds and Shepherd 1955). Plantains are giant perennial herbs of considerable importance to the agriculture of tropical humid forest regions in Africa, Central and South America and Asia (Robinson 1996; FAO 1999). Although the *Musa* genus originates in Asia (Simmonds 1996), there is a wide array of variability of plantains and bananas in Africa. In particular, the humid forest zone of West and Central Africa is considered a secondary centre of plantain diversification (De Langhe 1961, 1964), where a complex germplasm has evolved through somatic mutation (Simmonds 1996) and somaclonal variation (Vuylsteke 1998).

Considerable morphological variation is observed amongst plantain landraces, particularly in inflorescence characters (Ortiz 1997a), some of which have been used to sub-divide this germplasm (Tezenas du Montcel et al. 1983; Swennen and Vuylsteke 1987). Four broad groups have been identified on the basis of inflorescence morphology: French, French Horn, False Horn and Horn (see Tezenas du Montcel 1987). Plantains have also been subdivided on the basis of pseudostem height: giant, medium, small (De Langhe 1964). Plant size depends on the

Table 1 List of plantain landraces screened in this study showing bunch and stature type, source (not necessarily origin) and reported putative synonyms

Landrace	*Bunch type*	*Stature type*	*Source*	*Putative synonym*[a]
Abomienu	False Horn	Small	Ghana	
Agbagba	False Horn	Medium	Nigeria	
Agbagba (French Rev.)[b]	French	Medium	Nigeria	
Amou	French	Medium	Cameroon	
Apem Omniabu	French	Medium	Ghana	
Asamiensa	True Horn	Medium	Ghana	
Atali Kiogo	False Horn	Medium	Nigeria	
Batard	French Horn	Giant	Cameroon	
Big Ebanga	False Horn	Giant	Cameroon	
Bisé Egome 2[c]	Variable	Medium	Nigeria	
Bo-Ahie Abue	False Horn	Giant	Nigeria	
Bobby Tannap	French	Medium	Cameroon	
Bungaoisan	French	Medium	Philippines	Ntanga 4
Congo 2	French	Medium	Congo	
Currare	False Horn	Medium	Honduras	
Currare Enano	False Horn	Small	Honduras	
Daluyao	True Horn	Medium	Philippines	
Diby 2 off-type	French Horn	Medium	Cote d'Ivoire	
Didiede	False Horn	Medium	Cote d'Ivoire	
Dwarf False Horn 1–46	False Horn	Small	Honduras	
Dwarf French	French	Small	Honduras	
Eba Oboikpa	False Horn	Medium	Nigeria	
Egjoga	French	Medium	Nigeria	
Elar Icon	True Horn	Medium	Gabon	
Essang	False Horn	Medium	Gabon	
French Rouge	French	Medium	Cameroon	
Gabon 2	French	Medium	Gabon	
Horse Plantain	True Horn	Medium	Caribbean	
Kar Ngou	French	Medium	Cameroon	
Kiogo	False Horn	Medium	Nigeria	
Kwa	French	Medium	Cameroon	
Lifongo Loko	French	Medium	Cameroon	
Lysoka	True Horn	Medium	Cameroon	
Madre del Platanar	French	Medium	Cote d'Ivoire	
Mbi Egome 1	French	Small	Nigeria	
Mbirinyong	False Horn	Medium	Nigeria	
Mbirinyong G.M.	False Horn	Medium	Nigeria	
Mbouroukou 3	False Horn	Medium	Cameroon	
Mimi Abue	False Horn	Giant	Nigeria	
Moungeli	French	Giant	Congo	
Moutouka 2	False Horn	Medium	Gabon	
Msisa	East African	Medium	East Africa	
Mulolou	French	Medium	Gabon	
Mzuzu	True Horn	Medium	Burundi	Nothing but Green
Nazika	French	Giant	Congo	
Niangafelo	False Horn	Medium	Cote d'Ivoire	
Njock Kon	French	Giant	Cameroon	
Nothing but Green	True Horn	Medium	Cameroon	Mzuzu
Nothing but Red	True Horn	Medium	Cameroon	
Nselouka	French	Medium	Cote d'Ivoire	
Ntanga 2	French	Giant	Nigeria	
Ntanga 3	French	Medium	Nigeria	
Ntanga 4	French	Medium	Nigeria	Bungaoisan
Ntanga 5	French	Giant	Nigeria	
Ntie	French	Giant	Cameroon	
Obino l'Ewai	French	Medium	Nigeria	
Obubit Ntanga 1	French	Medium	Nigeria	
Obubit Ukom	False Horn	Medium	Nigeria	
Okela	French	Giant	Cameroon	
Okoyo Ukom	False Horn	Medium	Nigeria	
Orishele	False Horn	Medium	Nigeria	
Osoaboaso	French Horn	Small	Ghana	
Ovang	French	Giant	Cameroon	
Plantain 2	French	Medium	Cameroon	
Purple Plantain	French	Medium	Honduras	
Red Plantain	False Horn	Medium	Nigeria	

Table 1 (Continued)

Landrace	*Bunch type*	*Stature type*	*Source*	*Putative synonym*[a]
Red Plantain Hembra	French	Medium	Honduras	
Red Ukom	False Horn	Giant	Nigeria	
Three Hands Planty	True Horn	Medium	Cameroon	
Tshambunu	East African	Medium	East Africa	
Ubok Iba	True Horn	Medium	Nigeria	
Walungu 8	East African	Medium	East Africa	
Wine Plantain	French	Medium	Honduras	
Zue Ekon	French	Medium	Nigeria	
85.03	French	Medium	Nigeria	
75.19 s	French	Medium	Nigeria	

[a] Putative synonyms as reported by Swennen (1990)
[b] Mutant accession generated through somaclonal variation
[c] Exhibits a high frequency of somatic mutation affecting bunch morphotype

number of leaves produced prior to flowering. Giant plantains have more than 38 leaves, while small plantains produce less than 32 leaves (Swennen et al. 1995).

Plantains are almost completely sterile and develop fruit through parthenocarpy. Nevertheless, promising plantain-derived tetraploid hybrids have been generated in several breeding programmes across the world (Sathiamoorthy and Balamohan 1993; Vuylsteke et al. 1993; Dantas et al. 1995; Vuylsteke et al. 1995; Rowe and Rosales 1996). Although these hybrids produce high-yielding bunches in comparison to landraces, they exhibit only a fraction of the yield potential of this crop (de Vries et al. 1967). Further genetic gains will require effective utilization of *Musa* genetic resources (Ortiz 1997b). Despite the presence of over one hundred plantain landraces in the *Musa* collection at IITA, the breeding of this crop has concentrated on the use of only a few landraces, primarily due to the relatively high seed set of these genotypes (Vuylsteke et al. 1997). Clearly, if certain high-yielding genotypes are shown to be genetically diverse, this would warrant efforts to achieve hybridizations using these genotypes. This may be a critical step in the generation of genetically diverse tetraploid and diploid hybrids which could be used for the breeding of high-yielding secondary triploid hybrids (Ortiz 1997b).

Random amplified polymorphic DNA (RAPD) analysis has been used for several studies of genetic diversity in *Musa* germplasm (Kaemmer et al. 1992; Howell et al. 1994; Bhat and Jarret 1995). However, these reports focus on diverse germplasm across the genus rather than intensive analysis of a large number of accessions from within a single group. Putative duplicate accessions have already been assigned within the IITA plantain landrace collection. However, genetic diversity may be present despite the absence of morphological variation in the few descriptors used for this purpose. Conversely, accessions observed to exhibit morphological differences may be genetically uniform across much of the genome. In the latter case, molecular characterization may allow the development of a core collection which would simplify the management and enhance the utilization of *Musa* genetic resources.

RAPD analysis is sensitive to changes in conditions during polymerase chain reaction (PCR) amplification. However, we have found, in common with others (Munthali et al. 1992; Sobral and Honeycutt 1993; Micheli et al. 1994; Penner 1996; Rafalski et al. 1996), that reliable RAPD data can be generated through precise standardization and strict control of appropriate protocols, replication of ambiguous assays and stringent interpretation of results.

The objective of the study reported here was to screen a large proportion of the plantain germplasm in order to provide an accurate classification of this germplasm on which to base selections for breeding purposes and in particular for defining putative heterotic groups. The implications of the findings from this study for plantain evolution, classification and breeding are discussed.

Materials and methods

Plant material

Plantain landraces were collected in Nigeria, Ghana, Cote d'Ivoire, Cameroon, Congo, Gabon and Burundi or introduced from East Africa, Asia and Latin America (Vuylsteke et al. 1997). A random selection of 76 accessions (listed in Table 1) from the total collection of 105 landraces were screened in this study. These accessions are maintained at IITA Onne Station with other *Musa* germplasm as the designated regional reference collection (INIBAP/IBPGR, 1990). Field-grown plants in this collection were used for the isolation of DNA for RAPD analysis as described previously (Crouch et al. 1998). Site characterization data and crop management details relating to this collection have been described previously (Ortiz 1997a). Analysis of phenotypic diversity amongst this germplasm has also been reported elsewhere (Swennen et al. 1995; Ortiz 1997a; Osuji et al. 1997; Ortiz et al. 1998).

RAPD analysis

Random decamer primers A1-A19 and B1-B19 were obtained from Operon (Alameda, Calif.). A random selection of 38 plantain landraces were screened with 27 primers, and then all 76 landraces were screened with the most reliably polymorphic 11 primers. The PCR contained 40–80 ng of template DNA, 0.4 µ*M* primer, 1/10 vol of reaction buffer, 2.5 m*M* $MgCl_2$, 0.2 m*M* dNTP and 1 U *Taq* DNA polymerase (Boehringer) in a total volume of 10–25 µl.

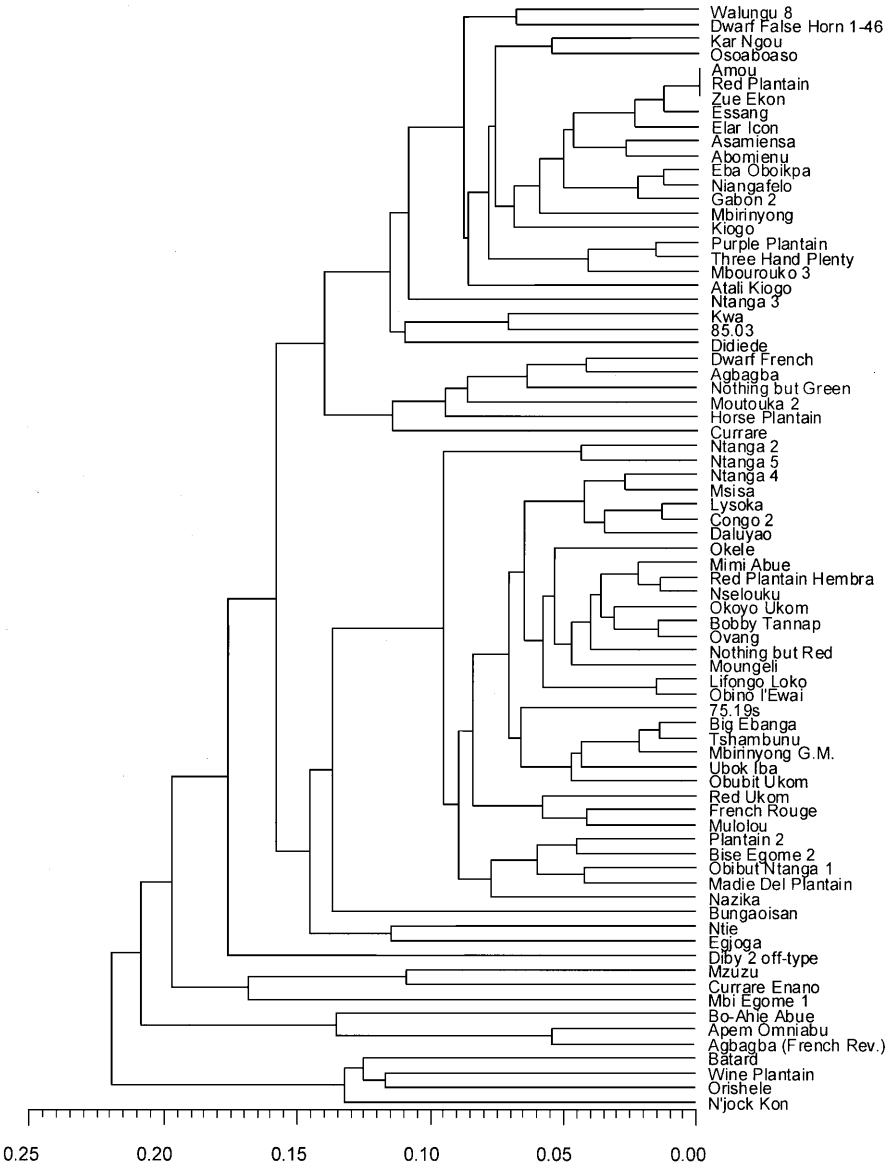

Fig. 1 Dendogram of genetic relationship amongst plantain landraces (*Musa* spp., AAB group) based on analysis of 76 RADP bands

The amplification cycle consisted of an initial 3-min denaturation of all reaction components at 94°C followed by 40 cycles of 45 s at 94°C, 30 s at 36°C, 30 s at 50°C and 60 s at 72°C and terminated with 7 min at 72°C. All reactions were carried out using a Perkin-Elmer model 9600 thermocycler. A 10- to 15-µl fraction of the amplified product was separated on 16 g l^{-1} agarose containing 0.3 ml ethidium bromide in 1×TBE buffer at 5 V/cm for approximately 3–4 h and photographed using UV illumination. RAPD assays generating weak or ambiguous amplification products were repeated up to three times to confirm the consistency of these markers. Amplification products were scored as present (1) or absent (0).

Data analysis

From the two sets of RAPD profiles, a distance matrix was generated comprising pairwise distance indices determined as 1–Jaccard's index (Jaccard 1908). The matrices were then subjected to cluster analysis using the simple average linkage method to generate dendrograms of genetic relatedness (only shown for the dataset representing 76 plantain landraces). All analyses were carried out with the aid of SAS software using the SAS procedures IML, CLUSTER and TREE (SAS 1994). A comparison of estimates of genetic similarity based on different RAPD analysis datasets and phenotypic characterization was carried out by calculating linear regressions or Spearman's rank coefficient of correlation (Zar 1996). Comparison of single RAPD marker bands with bunch and stature morphotype was carried out using a programme developed by Fretter and Mithen (John Innes Centre, unpublished). This programme calculates the mean of morphotype scores from landraces possessing a particular RAPD fragment (x) and the mean of scores from genotypes without that fragment (y). The hypothesis that x and y are not significantly different from each other is then tested using a Student's t-test.

Results

RAPD analysis of plantain landraces

A total of 76 RAPD bands were scored across 76 plantain landraces, of which 24 (32%) were monomorphic across all accessions. In addition, 17 RAPD bands (22%)

Table 2 Comparison of estimates of the genetic dissimilarity between 37 plantain landraces and Walungu 8 based on 76 RAPD marker bands and 164 RAPD marker bands (R^2=0.78; *P*=0.001)

Landrace	Percentage dissimilarity from Walungu 8 based on:	
	76 RAPD bands	164 RAPD bands
Walungu 8	0.00	0.00
Dwarf False Horn 1–46	6.90	5.23
Red Plantain	6.90	8.00
Amou	6.90	10.32
Zue Ekon	6.90	13.38
Eba Oboikpa	8.20	9.62
Mbourouko 3	8.20	11.88
Essang	8.20	13.38
Osoaboaso	8.30	6.49
Kiogo	8.30	7.19
85.03	8.50	7.84
Gabon 2	8.70	9.40
Niangafelo	9.50	7.64
Elar Icon	9.50	8.92
Abomienu	9.50	12.03
Didiede	9.90	8.72
Kar Ngou	10.80	15.72
Purple Plantain	11.10	11.02
Three Hand Plenty	11.10	11.04
Atali Kiogo	11.30	10.07
Agbagba	12.20	15.19
Asamiensa	12.20	16.45
Mbirinyong G.M.	12.30	8.39
Ntanga 3	12.30	10.26
Dwarf French	13.50	20.00
Kwa	14.90	19.35
Diby 2 off-type	15.30	12.21
Mbi Egome 1	15.30	15.79
Currare	15.50	17.97
Mzuzu	15.50	18.27
Wine Plantain	16.10	13.68
Nothing but Green	17.60	13.91
Horse Plantain	17.80	21.05
Moutouka 2	18.70	20.83
Njock Kon	21.60	23.03
Batard	23.90	23.48
Currare Enano	24.30	26.85
Orishele	26.70	24.18

were monomorphic across 95% of the germplasm, while a further 22 RAPD bands (29%) were monomorphic across 80% of the plantain landraces. Only 3 primers (A12, A18, B1) generated an amplification product in less than 50% of the accessions tested. Accessions possessing these relatively rare RAPD bands belonged to all morphogroups. These data suggest that all the plantain landraces tested are closely allied and that it is unlikely that any accessions harbouring unique alleles will be readily found.

The analysis of 76 RAPD bands distinguished virtually all 76 accessions (Fig. 1), with only 3 accessions appearing indistinguishable. A random selection of 38 accessions (including Amou, Red Plantain and Zue Ekon) was subjected to additional screening, thereby providing a total of 164 RAPD bands (dendrogram not shown). Diversity analysis based on this more extensive dataset differentiated these 3 plantain landraces. In general, there was an acceptable correlation between estimates of diversity based on datasets of 76 and 164 RAPD bands. For example, when comparing the relationship of 37 landraces with Walungu 8 (Table 2), there was a significant (*P*=0.001) correlation between the estimates of genetic diversity based on the two datasets (R^2=0.78). For 6 accessions (Amou, Dwarf French, Essang, Kar Ngou, Mbourouko 3 and Zue Ekon) there is a large disparity between estimates of diversity based on the two datasets. This may suggest that these landraces have undergone particular differentiation in limited regions of the genome.

In general, the plantain germplasm appears closely related, with the average dissimilarity between landraces being 14.1%. This high level of similarity amongst individuals within this genepool was expected based on the putative evolution of all these landraces through somatic mutation of a limited number of clonal introductions (De Langhe 1964). However, 17 landraces were estimated to be at least 30% dissimilar to 1 or more other landraces. Twelve of these landraces appear to be particularly diverse from the majority of the plantain germplasm screened in this study (Table 3).

The average dissimilarity between landraces within morphogroups based on bunch type varied substantially, with the dissimilarity amongst the East African plantains being the lowest (9.0%). Dissimilarity amongst the True

Table 3 Dissimilarity matrix showing the percentage dissimilarity between the 12 most diverse plantain landraces screened in this study

Moutouka 2 *(G1)*	–											
Mbi Egome 1 *(G2)*	23.0	–										
Batard *(G3)*	15.2	22.4	–									
Kwa *(G4)*	17.6	19.2	24.3	–								
Currare Enano *(G5)*	14.5	18.8	21.5	20.8	–							
Biby 2 Off–type *(G6)*	20.6	22.2	31.0	14.1	23.9	–						
Orishele *(G7)*	09.0	23.9	12.9	23.3	20.6	26.4	–					
Njock Kon *(G8)*	11.6	23.6	14.1	25.3	20.3	21.1	12.1	–				
Egjoga *(G9)*	30.4	26.9	28.6	30.0	28.8	25.8	37.7	39.9	–			
Bungaoisan *(G10)*	26.7	28.4	32.4	27.6	30.1	26.0	34.7	27.4	20.3	–		
Apem Omniabu *(G11)*	28.1	32.3	27.9	32.8	29.0	32.3	33.3	28.6	14.0	19.7	–	
Agbagba French Rev. (*G12)*	26.0	32.0	25.4	31.6	31.9	30.1	31.9	26.8	18.0	19.1	5.5	–
	G1	*G2*	*G3*	*G4*	*G5*	*G6*	*G7*	*G8*	*G9*	*G10*	*G11*	*G12*

Table 4 Comparison of estimates of diversity based on 76 RAPD bands and 15 phenotypic descriptors for comparisons between the French plantain landraces used extensively in breeding 4*x* plantain hybrids and representatives of the True Horn and False Horn groups. Correlation between phenotypic- and genotypic-based estimates of diversity were tested by linear regression of absolute dissimilarities (R^2) and Spearman's correlation coefficient of dissimilarity ranks (r_s)

	Asamiensa True Horn		Agbagba 3 False Horn		Mbi Egome 1 French		Bobby Tannap French		Obino l' Ewai French	
	Phenotype	RAPD	*Phenotype*	RAPD	*Phenotype*	RAPD	*Phenotype*	RAPD	*Phenotype*	RAPD
Abomienu	23.3	2.8	10.8	8.1	15.6	16.2	18.0	13.2	17.5	13.3
Agbagba 3	16.5	8.2	0.0	0.0	18.2	13.9	17.7	15.8	18.4	16.0
Amou	31.9	5.5	23.0	5.5	9.7	13.7	10.8	13.2	8.0	15.8
Apen Onniaba	25.6	26.2	17.3	23.4	8.0	32.3	8.8	18.8	4.3	21.9
Asamiensa	0.0	0.0	16.5	8.2	28.1	16.4	29.5	15.8	26.6	16.0
Batard	26.0	23.9	19.4	18.8	24.0	22.4	23.6	25.0	22.5	22.9
Big Ebanga	21.5	9.5	13.0	9.5	26.3	17.6	27.6	6.9	26.0	6.9
Bise Egome 2	25.0	14.7	12.8	14.7	11.4	20.3	12.3	4.2	12.9	7.0
Bobby Tannap	29.5	15.8	17.7	15.8	7.6	21.3	0.0	0.0	5.9	5.6
Eba Oboikpa	15.2	6.9	7.0	9.5	24.5	17.6	21.5	14.5	23.4	17.1
Egjoga	26.9	20.9	15.2	20.9	6.3	26.9	3.3	16.7	5.9	19.7
Madre del Platanar	24.6	13.3	13.5	13.3	12.5	18.9	13.7	5.6	13.8	5.6
Mbi Egome 1	28.1	16.4	18.2	13.9	0.0	0.0	7.6	21.3	6.8	16.7
Mbirinyong	24.0	8.3	9.8	11.0	15.1	16.7	14.7	18.4	15.3	21.1
Mbirinyong G.M.	23.3	10.8	8.7	8.2	18.4	16.4	18.6	8.2	20.0	8.3
Mimi Abue	25.1	12.2	17.0	12.2	26.4	17.8	25.4	4.2	24.4	4.3
NJock Kon	46.8	20.3	44.4	15.3	36.1	23.6	32.6	22.7	30.2	20.6
Nseuloka	29.6	12.0	17.6	12.0	8.3	20.0	5.4	4.2	8.2	4.2
Ntanga-2	42.7	14.9	38.4	14.9	31.0	20.6	27.9	9.7	26.4	9.9
Ntanga-3	24.4	11.0	19.1	16.0	6.8	19.2	9.0	18.4	4.6	21.1
Ntanga-4	26.4	13.3	17.7	13.3	3.2	18.9	7.0	5.6	5.1	8.3
Ntanga-5	44.2	13.7	39.1	16.2	25.9	21.9	26.6	11.1	24.1	11.3
Obino l' Ewai	26.6	16.0	18.4	16.0	6.8	16.7	5.9	5.6	0.0	0.0
Obubit Ukom	19.6	13.5	3.6	11.0	18.5	19.2	18.4	5.6	19.9	8.5
Okoyo Ukom	18.0	12.2	4.9	12.2	21.6	17.8	21.4	4.2	21.9	7.0
Orishele	21.2	23.0	8.6	18.1	17.6	23.9	16.4	27.6	16.2	25.7
Osoaboaso	26.6	6.9	12.5	6.9	10.6	17.8	13.8	17.1	14.5	19.7
Ovang	36.9	12.5	32.7	13.9	26.9	19.7	23.9	1.5	22.4	4.4
Plantain No. 2	29.2	18.3	18.9	16.9	7.7	22.9	6.6	7.5	4.6	10.5
75.19 S	26.2	8.3	14.3	11.0	7.6	21.6	6.0	11.0	7.1	11.1
R^2	0.17*		0.14*		0.02		0.02		0.01	
r_s	0.45*		0.52*		0.05		0.17		0.06	

* P=0.05

Horn plantains (11.8%) was significantly lower (P=0.05) than that amongst the False Horn plantains (13.3%), whilst the dissimilarity amongst the French plantains (14.5%) was significantly higher than that amongst both the False and True Horn plantains (P=0.001 and P=0.0001, respectively). A similar comparison of diversity amongst morphotype groups based on stature and geographical source groups showed no significant differences.

Based on phenotypic evaluation, it has been proposed that Nothing but Green is a synonym of Mzuzu and, similarly, that Ntanga 4 is a synonym of Bungaoisan (Swennen 1990). However, genetic analysis based on 76 RAPD marker bands estimated genetic dissimilarities

of 13.8% and 15.3%, respectively, between these pairs of putative duplicates.

Comparison of RAPD-based and phenotypic diversity indices

A phenotype-based classification of plantain landraces has been reported using 15 descriptors (Ortiz et al. 1998). We compared the estimates of pair-wise dissimilarity based on these phenotypic characters (which were not presented in Ortiz et al. 1998) with RAPD-based estimates of dissimilarity (for all common landraces). Table 4 presents this comparison for the three French plantain landraces used extensively in IITA breeding programmes (Vuylsteke et al. 1997) plus representatives of the True Horn and False Horn groups. The overall association between RAPD-based and phenotype-based estimates of diversity was tested using simple regression analysis of actual values (R^2=0.02, s) and Spearman's correlation coefficient of ranked values (r_s=0.12, s). Both approaches indicated that there is no significant (P=0.05) relationship between the two indices.

Dissimilarity estimates based on these phenotypic descriptors suggest that a number of landraces are highly divergent from the three French plantains used extensively in plantain breeding. However, the RAPD analysis does not support the high dissimilarity of these pair-wise comparisons and suggests that within the landraces analysed there are relatively few highly divergent landraces (Table 4).

RAPD markers associated with bunch and stature morphogroups

DNA markers have been identified for the somaclonal mutants causing dwarfism in Cavendish dessert bananas using RAPD analysis (Damasco et al. 1996) and in plantains using amplified fragment length polymorphism (AFLP) analysis (Engelborghs et al. 1999). Similarly, in this study we have compared polymorphisms at individual RAPD markers with variation in bunch type and stature amongst the 76 plantain landraces. Several primers generated RAPD-band markers significantly associated (P=0.01) with bunch type (A10, A18 and B10) or stature (A7). However, in all cases, although the RAPD band of interest was observed in virtually all members of a specific morphotype, this RAPD band was also present in a small proportion of the other morphogroups. For this reason, we do not believe that any of these markers are intimately associated with genomic regions affecting these phenotypes. The RAPD marker reported to distinguish dwarf mutants in Cavendish bananas (Damasco et al. 1996) was generated from a primer not used in the present study (J04).

Discussion

Genetic relationships among plantain landraces

RAPD analysis has been reported to effectively distinguish plantain landraces from other *Musa* germplasm (Howell et al. 1994). In this study, we have shown that RAPD analysis can also detect sufficient polymorphism to distinguish 76 plantain landraces from each other (representing a major proportion of the currently available plantain landrace germplasm). Furthermore, two pairs of putative synonyms (Table 1) were also distinguished by the analysis based on 76 RAPD bands. Thus, despite the apparent phenotypic similarity of these pairs of landraces, they clearly harbour some degree of genetic differences which may be worthwhile conserving.

In this study we have screened a random subset of half the germplasm at two levels of intensity. Analysis of 76 RAPD bands represents approximately 1 marker per chromosome arm, while 164 RAPD bands equates to nearly 5 markers per chromosome. Comparative analysis of the two datasets shows that there is a significant correlation (R^2=0.78; P=0.001) between the two estimates of genetic diversity. Furthermore, the analysis based on 76 RAPD bands identifies 8 of the 10 landraces that were found to be most distinct from Walungu 8 based on 164 RAPD bands (amongst the 38 accessions tested). Thus, relatively limited RAPD screening is adequate for rapidly identifying the most diverse accessions amongst the plantain landrace germplasm, which can then be introduced in plantain breeding programmes. However, more intensive analysis may be required for resolving questions of duplication and on just what to base the selection of a core collection. Nevertheless, even based on the current analysis, it appears clear that for the generation of a core collection in *Musa*, the selection of representatives from each morphogroup may not lead to an effective representation of the genetic diversity present in the plantain germplasm.

The RAPD analysis presented in this study suggests that the level of dissimilarity amongst morphogroups based on bunch type varies considerable. On this basis French plantains appear to be a significantly more diverse group than False Horn plantains, which in turn appear to be a more diverse group than the True Horn plantains. Standard evolutionary thought would predict that True Horn plantains evolved from False Horn plantains which were derived from French plantains. This is based on the theory that perfect flowers are primitive, monoecious plants are intermediate and unisexual plants are the most advanced (Cronquist 1988). Similarly, new structures are considered to arise through the modification of old ones. Thus, unisexual plants are derived typically through the evolution of vestigial male organs or, in the case of False Horn plantains, through deciduous male parts. Assuming more archaic groups have had more evolutionary time for diversification, the RAPD analysis presented in this study would tend to suggest that True Horn plantains are derived from False

Table 5 Summary of datasets used in previously reported analyses of phenotypic diversity amongst plantain landrace germplasm

	Ortiz 1997	*Osuji et al. 1997*	*Ortiz et al. 1998*	*Swennen et al. 1995*
Number of characters	43	15	13	9
Number of landraces	52	80	78	24
Correlation with genomic group	Poor	Good	Good	Good
Vegetative traits				
Leaf orientation	X			
Pseudostem pigments	X			
Pseudostem wax	X			
Pseudostem colour	X			
Pseudostem bloches	X			
Lamina base shape	X			
Petiole canal	X			
Petiole margin clasp	X			
Plant juice colour	X			
Sucker orientation	X			
Plant height		X	X	X
Plant girth		X	X	X
Ht tallest sucker (flowering)		X	X	
Httallest sucker (harvest)		X	X	
Number of leaves		X		X
Leaf length/width ratio			X	X
Inflorescence traits				
Female inflorescence angle	X			
Peduncle hairiness	X			
Fused pedicles	X			
Fruit base insertion	X			
Bunch compactness	X			
Neutral flower persistence	X			
Rachis curve	X			
Male axis	X			
Male axis angle	X			
Male axis bract scars	X			
Male bract external colour	X			
Male bract textures	X			
Male bract persistence	X			
Male bract shape	X			
Male bract apex	X			
Male bud imbrication	X			
Male bract behaviour	X			
Male bract internal colour	X			
Male bract top colour	X			
Days to flowering		X	X	X
Days to harvest		X	X	
Number of neutral flowers		X		
Bunch weight		X	X	X
Number of hands per bunch		X	X	X
Number of fruits per bunch		X	X	X
Number fruits per hand		X	X	
Male flower traits				
Free tepal corrugation	X			
Style and staminode persistence	X			
Free tepal texture	X			
Stigma colour	X			
Ovary colour	X			
Fertility	X			
Fruit traits				
Two row arrangement	X			
Orientation	X			
Shape	X			
Apex	X			
Cross section shape	X			
Unripe peel colour	X			
Unripe pulp colour	X			
Ripe pulp colour	X			
Average fruit weight		X	X	X
Average fruit length		X	X	
Average fruit circumference		X		
Average fruit girth			X	

Horn plantains which are derived from French plantains. Genetic analysis of male and hermaphrodite organs in plantain hybrid populations also supports this direction of evolutionary development (Ortiz 1996). In contrast, the absence of significant differences in mean dissimilarity amongst morphogroups based on stature may suggest that variation in this character evolved independently in all bunch-type groups. This is plausible as it appears that variation in stature has a simple genetic basis (Ortiz and Vuylsteke 1995).

Correlation between genetic and morphological diversity indices

Classification of plantains based on morphological descriptors has provided data which supports (Swennen et al. 1995; Osuji et al. 1997; Ortiz et al. 1998) or conflicts (Ortiz 1997a) with the morphotype grouping of plantain landraces based on bunch type and height (De Langhe 1964). Studies reporting a positive correlation are characterized by the use of a relatively small number (10–15) of agronomic characters (Table 5). In contrast, a poor correlation is reported when a relatively large number (43) of agronomically neutral botanical traits is used (Ortiz 1997a).

Similarly, good correlations can be obtained between molecular marker diversity and morphotype group when a small number of genotypes are screened (Engelborghs et al. 1999). However, in this study we present RAPD analysis of a large proportion of the available plantain landrace germplasm screened with a relatively large number of genetic markers. In general, there is a poor correlation between RAPD-based dissimilarity estimates and phenotype index or morphotype group (Table 4). This suggests that the use of limited datasets (in terms of the number of variables or genotypes) may lead to misleadingly simplistic interpretations.

It is highly probable that certain somatic mutations affecting agronomic characters have been selected by farmers during the evolution of plantains in their secondary centre of diversity (De Langhe 1964). This may have resulted in some coordinated drift of genomic regions directly affecting agronomic characters. However, it is apparent from the present study that agronomically neutral somatic mutations have contributed to considerable random drift in other genomic regions. As a consequence, we observe that members of each morphogroup possess diverse genetic backgrounds (based on the RAPD analysis reported here) related to regions of the genome most likely not associated with traits affecting bunch type and stature (but quite possibly including taxonomic traits such as those assessed by Ortiz 1997a). The observation of somaclonal mutants from micropropagated plantain populations may provide further insight into the potential pattern and extent of genetic variation generated through somatic mutation. In this respect, the current study offers only one relevant pair-wise comparison: Agbagba (False Horn) and its selected somaclonal variant exhibiting a French bunch type (Agbagba French Reversion). The RAPD-based estimate of dissimilarity between these 2 accessions is 0.219. This clearly supports the hypothesis that somaclonal variation contributes random mutation across the genome.

Implications for the breeding of plantain hybrids

Twelve plantain landraces amongst the germplasm tested have been identified in this study as being particular diverse (Table 3). Pairwise comparisons between these landraces are frequently in excess of 20% dissimilarity. Although some of these landraces have already been used extensively in plantain breeding (Mbi Egome and Agbagba FR), most others have not. With respect to the Horn plantains (Moutouka, Batard, Currare Enano, Biby 2, Orishele) this is due to the sterility of these accessions. Of the remaining accessions, Kwa and Bungaoisan are known to exhibit usable levels of fertility (Vuylsteke et al. 1993) and could, therefore, easily be introduced into plantain breeding schemes. Njock Kon, Egjoga and Apem Omniabu have failed to generate seeds in standard test crosses with the wild accession *M. acuminata* subsp. *burmannucoides* Calcutta 4 (AA genome) (Vuylsteke et al. 1993). However, it may still be possible to derive progeny from these landraces through crosses with other diploid genotypes. On the basis of the diversity analysis presented here, we would propose that it is of value to pursue the use of these accessions in plantain breeding, in particular to introduce the maximum level of diversity into plantain-derived 2x and 4x breeding populations for the breeding of secondary triploid hybrids (Ortiz 1997b).

In memoriam The authors wish to dedicate this article in the memory of Dirk R. Vuylsteke (1958–2000) who recently and tragically died in the course of his work. Dirk devoted his life to agricultural development in Africa as a biotechnologist, breeder and team leader at IITA. His innovative ideas, open-minded style, hard work and commitment to the African small landholder will always be a source of inspiration for his colleagues in the CGIAR and the new generation of scientists joining international agricultural research.

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