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Origin and phylogeny of Guinea yams as revealed by RFLP analysis of chloroplast DNA and nuclear ribosomal DNA

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Summary. The origin and phylogeny of the Guinea yams, consisting of the white yam (*Dioscorea rotundata* Poir.) and the yellow yam (*D. cayenensis* Lamk.), has been investigated. Fourteen cultivars of Guinea yams were sampled with 12 accessions from seven wild yam species. A total of 26 accessions were surveyed for restriction fragment length polymorphisms (RFLP) in chloroplast DNA (cpDNA) and nuclear ribosomal DNA (rDNA) using seven restriction endonucleases and various heterologous probes. Chloroplast DNA probes covering 80% of the total chloroplast genome revealed nine restriction site changes and one length mutation among the cpDNAs of Guinea yams and their wild relatives. The estimated numbers of nucleotide substitutions per site (*d*) among these cpDNAs were very low (0.0005–0.0027), indicating a rather recent divergence of this group. On the basis of these ten mutations, five chloroplast genome types (A–E) were recognized. It was revealed that two cultivated species (*D. rotundata* and *D. cayenensis*) display the same chloroplast genome type, type A, as the three wild species *D. praehensilis*, *D. liebrechtsiana* and *D. abyssinica*. Chloroplast genome types B, C, D and E were found in *D. minutiflora*, *D. burkilliana*, *D. smilacifolia* and *D. togoensis*, respectively. Maximum parsimony analysis produced a hypothetical phylogeny of three primary lineages among cpDNAs of Guinea yams and their relatives: the genome type A lineage, the genome type B lineage and one lineage including genome types C, D and E.

Using rDNA clones of rice and taro as probes, we detected ribosomal DNA variation, presumably at the intergenic spacer region, in Guinea yams and their wild relatives. The survey of rDNA together with that of cpDNA indicates that *D. rotundata* (white yam) was domesticated from either *D. abyssinica*, *D. liebrechtsiana* or *D. praehensilis* or their hybrid, and that *D. cayenensis* (yellow yam) is derived from hybridization between a male plant of either *D. burkilliana*, *D. minutiflora* or *D. smilacifolia* and a female plant of either *D. rotundata*, *D. abyssinica*, *D. liebrechtsiana* or *D. praehensilis*. We propose that the previous nomenclature of white yam should be retained, *D. rotundata* Poir. *nomen nudum*, and that yellow yam should be treated as a variety of *D. rotundata*, denoted as *D. rotundata* var. × 'cayenensis'.

Key words: Guinea yams – *Dioscorea* – Chloroplast DNA – Nuclear ribosomal DNA – Phylogeny

Introduction

Africa produces more than 90% of the world yam crop (FAO 1988). Among the several yams cultivated in Africa, the Guinea yams of West Africa account for the largest share. Guinea yams have long been cultivated in the so-called "Yam Zone" of Africa (Coursey 1967), namely, the region between the middle of the Ivory Coast and eastern Cameroon, and between the Gulf of Guinea and 12° North. The interrelationships between humans and Guinea yams in the region have developed into an unusual agro-cultural complex called "la civilization de l'igname (the civilization of yam)" (Miège 1954).

Despite the economic and cultural importance of Guinea yams in the region, very little is known about their origin and phylogeny. Enormous morphological polymorphism, high plasticity and predominant vegeta-

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Table 1. Sources of plant materials used for DNA analysis

DNA no	Species	Locality	Source
Cultivated species			
1	*D. rotundata*	Nigeria	IITA TDr747
2	*D. rotundata*	Nigeria	IITA 87/00220
3	*D. rotundata*	Nigeria	IITA IYT808
4	*D. rotundata*	Togo	IITA 1280 Kudayele
5	*D. rotundata*	Nigeria	IITA Chikakwudu (1)
6	*D. rotundata*	–	IITA Tchaba
7	*D. rotundata*	Togo	IITA Allasora
8	*D. rotundata/ cayenensis*	Nigeria	IITA TDr131 (Abi)
9	*D. rotundata/ cayenensis*	Nigeria	IITA Aiman (2)
10	*D. cayenensis*	Togo	IITA 90/00967
11	*D. cayenensis*	Ivory Coast	IITA IC-14
12	*D. cayenensis*	Ghana	IITA GH-52
13	*D. cayenensis*	Benin	IITA BE-89
14	*D. cayenensis*	Nigeria	IITA TDc760
Wild species			
15	*D. praehensilis*	Ibadan, Nigeria	IITA RT106
16	*D. praehensilis*	Igbobor, Nigeria	IITA RT139
17	*D. liebrecht-siana*	Ofos, Nigeria	IITA RT140
18	*D. burkilliana*	Ibadan, Nigeria	IITA RT150
19	*D. minutiflora*	Omo, Nigeria	IITA RT138
20	*D. minutiflora*	Omo, Nigeria	IITA RT143
21	*D. smilacifolia*	Omo, Nigeria	IITA RT118
22	*D. smilacifolia*	Onne, Nigeria	IITA RT134
23	*D. abyssinica*	Mokwa, Nigeria	IITA RT8
24	*D. abyssinica*	Mokwa, Nigeria	IITA RT9
25	*D. togoensis*	Ibadan, Nigeria	IITA RT151
26	*D. togoensis*	Jebba, Nigeria	IITA RT1

–: Unknown
For DNAs 1–5 and 7–14, the precise locality of origin in the respective country is unknown

tive reproduction have so hindered critical biosystematic study that the taxonomy has long been controversial (see Miège 1982). Guinea yams consist of two phenotypically distinguishable types, namely, white yam with white-fleshed tubers and a 6- to 8-month growth period, and yellow yam with yellow-fleshed tubers and an 8- to 12-month growth period. Both of these types are true cultigens. White yam is cultivated in both savanna and rain-forest zones, whereas yellow yam is confined to the rain-forest zone. Typical plants of white and yellow yams are easily distinguished on the basis of several gross morphological characters, but there are many intermediate forms. Several taxonomists have treated the two types as two discrete species: *Dioscorea rotundata* Poir. (white yam) and *D. cayenensis* Lamk. (yellow yam) (Hutchinson and Dalziel 1931; Burkill 1960; Ayensu 1972; Akoroda and Chheda 1983; Onyilagha and Lowe 1985), but others have considered them as two subspecies or varieties within a single species *D. cayenensis* (Chevalier 1936; Miège 1968, 1979; Martin and Rhodes 1978). Recently, Hamon and Toure (1990) proposed to treat this species complex as *D. cayenensis-rotundata*. No consensus has been established concerning the origin of these two types and the phylogenetic relationship between them. On morphological grounds, Miège (1982) proposed *D. abyssinica* Hochest ex. Knuth, *D. lecardii* De Wild., *D. liebrechtsiana* De Wild., *D. praehensilis* Benth. and *D. sagittifolia* Pax. as the possible wild progenitors of Guinea yams. Of these, the three species *D. abyssinica*, *D. lecardii* and *D. sagittifolia* are morphologically so similar to each other that their status as discrete species is questionable (Dumont 1982; Terauchi unpublished results). Other wild species which are morphologically closely related to Guinea yams include *D. burkilliana* J. Miège, *D. mangenotiana* J. Miège, *D. minutiflora* Engl., *D. smilacifolia* De Wild. and *D. togoensis* Knuth (Chevalier 1936; Miège 1982). The habitats of these wild species are the rain-forest for *D. burkilliana*, *D. liebrechtsiana*, *D. mangenotiana*, *D. minutiflora*, *D. praehensilis* and *D. smilacifolia* and the savanna for *D. abyssinica*, *D. lecardii*, *D. sagittifolia* and *D. togoensis*. Within each ecological zone these wild species are distributed widely without showing any geographical isolation. Chevalier (1936) created a new taxon, subsection Cayenenses Chev., under the Section Enantiophyllum Uline to encompass Guinea yams and their wild relatives.

In this paper, we report variation in the chloroplast DNA (cpDNA) and nuclear ribosomal DNA (rDNA) of Guinea yams and their wild relatives for the first time, with the aim of elucidating the origin and phylogeny of Guinea yams in a convincing framework.

Materials and methods

Plant materials

Variation in cpDNA and nuclear rDNA was studied in 26 accessions of Guinea yams and their wild relatives (Table 1). Among 14 accessions of the cultivars, seven were classified as typical white yam, five as typical yellow yam, and two (nos. 8 and 9) as intermediate. They were collected from the five major yam producing countries in the Yam zone. We tentatively adopt the nomenclature of Hutchinson and Dalziel (1931) by applying the name *D. rotundata* Poir. and *D. cayenensis* Lamk. to white yam and yellow yam, respectively. The intermediate type is designated here as *D. rotundata-cayenensis*. Seven wild relatives were identified according to the keys of Miège (1968). Among the ten species native to West Africa that are morphologically similar to Guinea yams, three species, *D. mangenotiana*, *D. lecardii* and *D. sagittifolia*, were not available for the present study because they are rare in Nigeria where the collection of the wild species was carried out. For all the accessions of the wild species studied, voucher specimens are preserved at IITA for future reference.

DNA extraction

DNA was extracted from a single plant of each accession. About 30 g of leaves was homogenized with 500 ml of a modified buffer

of Ogihara and Tsunewaki (1982) (0.44 *M* mannitol; 50 m*M* TRIS-HCl, pH 8.0; 3 m*M* EDTA; 5 m*M* 2-mercaptoethanol; 0.6% (w/v) PVP, MW 40,000) in a prechilled Waring blender. Homogenates were filtered through a single layer of cheesecloth and centrifuged at 150 *g* for 5 min. The supernatant was then centrifuged at 1,900 *g* for 10 min. The resulting pellet was used for DNA extraction. This procedure allowed us to wash out interfering polysaccharides and to enrich the chloroplast fraction in the pellet. Because this pellet still contained a significant nuclear fraction, both cpDNA and nuclear rDNA could be analysed using DNA extracted from it. After the lysis of chloroplasts and nuclei in a 2% sarkosyl and 200 µg/ml proteinase K solution (Kolodner and Tewari 1975), DNA was extracted once each by phenol and phenol/chloroform, and purified with CTAB/chloroform (Murray and Thompson 1980). About 100 µg DNA was obtained from 30 g fresh leaves.

Restriction digestion and Southern blotting

About 1 µg of DNA per accession was digested with each of five six-base cutters, *Bam*HI, *Dra*I, *Eco*RI, *Hin*dIII and *Pst*I, and three four-base cutters, *Hae*III, *Mbo*I and *Msp*I. The digested DNA was electrophoresed in 0.8% and 1.5% agarose gels when DNA was treated with six-base cutters and four-base cutters, respectively. Separated DNA fragments were blotted to Hybond N membranes (Amersham) after Southern (1975). The transferred DNA was cross-linked to the membrane by UV light (Spectroline TC-312A) irradiation for 2 min.

Characteristics of the DNA probes

CpDNA clone banks of two yam species, *D. bulbifera* L. and *D. opposita* Thunb., were previously constructed by Terauchi et al. (1989, 1991). From these banks, we used *Bam*HI fragments B5 (9.4 kb), B6 (9.0 kb) and B8 (6.55 kb) from *D. bulbifera* and *Sal*I fragments S2 (26.0 kb, DO#2), S5–6 (26.4 kb, DO#1) and S4–7 (30.0 kb, DO#3) from *D. opposita* as heterologous probes to detect cpDNA variation among Guinea yams and their wild relatives. The location of these fragments in physical maps of their respective cpDNAs is given in Fig. 1. The probe DO#1 spans the small single-copy region entirely, the probe DO#2 spans most of the inverted repeat and the probes DO#3, B5, B6 and B8 together span about 70% of the large single-copy region. Altogether, the probes cover 80% of the total chloroplast genome. To detect rDNA variation in Guinea yams and their wild relatives, we used heterologous rDNA clones: pRR217 obtained from rice (*Oryza sativa*) (Oono and Sugiura 1978) and pCE34.2 from taro (*Colocasia esculenta*) (Matthews 1990). The structure of these two fragments are given in Fig. 2. pRR217 (7.8 kb) contains the entire repeat unit of rDNA genes (18S, 5.8S and 26S rRNA genes) and non-transcribed spacer region; pCE34.2 (4.0 kb) is a fragment containing only the 3′ part of 18S, 5.8S and the 5′ part of 26S rRNA genes.

Hybridization

The DNA probes were chemically labelled with digoxigenin-dUTP and detected enzymatically according to the suppliers instructions (Boehringer, Mannheim). Labelled DNA was used repeatedly to probe different membranes. Hybridization was carried out at the standard conditions (68 °C, 12 h) as described in the kit. Hybridized membranes were washed under high stringency conditions (0.1% SDS, 0.1 × SSC, 68 °C) for all probes. Membranes were reprobed several times after removing previously hybridized probe DNAs.

Results

Chloroplast DNA variation

Six heterologous cpDNA probes were hybridized one by one to the DNAs of Guinea yams and their wild relatives that had been digested with the four-base cutters. All of the probes were mixed and hybridized together to the DNAs digested with the six-base cutters. The hybridization involving *Mbo*I failed because all of the fragments resulting from digestion with this enzyme were too small (less than 1.5 kb) to resolve satisfactorily. The number of fragments visualized by each enzyme for single or combined probes is given in Table 2. In total, 214 bands were scored, which corresponds to 227 sites and 1,120 base pairs, representing 0.7% of the total chloroplast genome. RFLPs were detected in ten probe/restriction enzyme

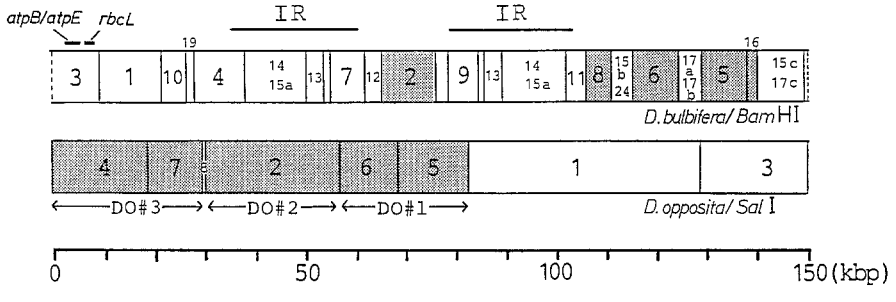

Fig. 1. *Bam*HI restriction map of *D. bulbifera* cpDNA and *Sal*I restriction map of *D. opposita* cpDNA. Hatched fragments were used as the probes for the Southern hybridization (Terauchi et al. 1991)

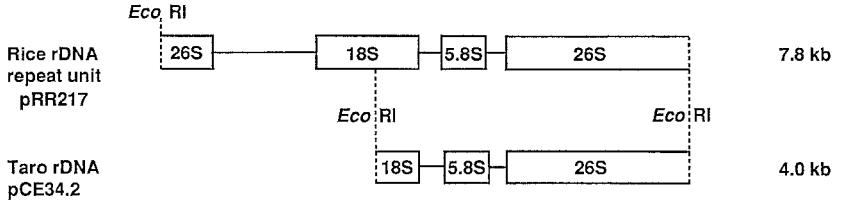

Fig. 2. Structure of ribosomal DNA fragments used for the probe

combinations, B5/*Hae*III, B5/*Msp*I, B6/*Hae*III, B6/*Msp*I, B8/*Hae*III, B8/*Msp*I, DO#1/*Hae*III, DO#1/*Msp*I, B5+B6+B8+DO#1+DO#2+DO#3/*Dra*I and B5+B6+B8+DO#1+DO#2+DO#3/*Hin*dIII. These RFLPs appear to be the results of nine restriction site changes and one length mutation (Table 3). Among the nine restriction sites changes, only in two cases (mutations 1 and 9), were the expected additive fragment sizes confirmed (Fig. 3a, b). For the other eight changes, this additivity was not observed (Figs. 3c–5), probably because one of the split fragments was too small to be detected or because the mutated site was located outside the extent of the probe used. Nevertheless, they were judged to be the result of base substitutions because other enzyme-digests hybridized to the same respective probe did not reveal any simultaneous size changes in the same DNA samples. A length mutation (mutation 10) was detected in the region corresponding to B5. A 0.1–0.15 kb longer fragment was always observed for DNA samples, 18–22, 25 and 26 compared with other DNAs, when the B5 fragment was hybridized to DNAs digested with *Dra*I (Fig. 3a), *Msp*I (Fig. 5), *Bam*HI and *Hin*dIII (data not shown).

Table 2. Number of bands scored [and the number of variable sites observed, if any (in brackets)] with each probe or combination of probes for each restriction enzyme. A length mutation is not included

Enzyme	Probe					
	B5	B6	B8	DO#1	DO#2	DO#3
*Hae*III	9 (2)	5 (1)	3 (1)	10 (1)	20	11
*Msp*I	4	6 (1)	5 (1)	9 (1)	15	13
	B5+B6+B8+D#1+DO#2+DO#3					
*Bam*HI	18					
*Dra*I	22 (1)					
*Eco*RI	21					
*Hin*dIII	23					
*Pst*I	18					

Ribosomal DNA variation

Fragments of 0.91 kb and 0.78 kb were variably detected after digestion with *Mbo*I and analysis with both pRR217 and pCE34.2 rDNA probes (Fig. 6). All of the samples of *D. rotundata* (samples 1–7), *D. praehensilis* (samples 15, 16), *D. liebrechtsiana* (sample 17), *D. abyssinica* (samples 23, 24) and one sample of *D. rotundata-cayenensis* (sample 9) displayed only the 0.78-kb fragment. *D. burkilliana* (sample 18), *D. minutiflora* (samples 19, 20), *D. smilacifolia* (samples 21, 22) and *D. togoensis* (samples 25, 26) had only the 0.91-kb fragment. One sample of *D. rotundata-cayenensis* (sample 8) and all of the samples of *D. cayenensis* (samples 10–14) possessed both of them. Only pRR217 was used as the probe for the rDNA analysis with other enzymes. These enzymes gave either monomorphic restriction patterns (*Bam*HI, *Eco*RI), smear patterns (*Hae*III and *Msp*I) or did not appear to have a restriction site within the rDNA repeat unit of Guinea yams and their wild relatives (*Dra*I, *Hin*dIII and *Pst*I).

Discussion

Classification of chloroplast genome and the maternal progenitor of Guinea yams

The ten mutational changes detected among 26 chloroplast DNAs of Guinea yams and their relatives (Table 3) allowed us to classify the DNAs into five chloroplast genome types: types A–E (Table 4). Chloroplast genome type A was shared by five species, *D. rotundata*,

Table 3. Mutations in chloroplast DNA of Guinea yams and their wild relatives

Mutation	Enzyme	Probe	Region[a]	Lost fragment (kb)[b]	Gained fragment (kb)[b]	Mutated samples
1	*Hae*III	B5	LSC	1.25+0.65	1.9	19, 20
2	*Hae*III	B5	LSC	2.2 [+0.1]	2.3	19, 20
3	*Hae*III	B6	LSC	4.4 [+2.1]	6.5	18
4	*Msp*I	B6	LSC	1.4 +[0.4]	1.8	25, 26
5	*Hae*III	B8	LSC	1.3 [+0.1]	1.4	18–22, 25, 26
6	*Msp*I	B8	LSC	3.45	3.2 [+0.25]	19, 20, 25, 26
7	*Hae*III	DO1	SSC	1.7	1.0 [+0.7]	18, 21, 22, 25, 26
8	*Msp*I	DO1	SSC	4.0 [+0.3]	4.3	18–22, 25, 26
9	*Dra*I	DO1/2/3	–	6.4	3.4+3.0	19, 20
10	–	B5	LSC	–	0.1 insertion	18–22, 25, 26

Brackets indicate the fragment that was not seen because it was too small or was located outside the extent of the probe, but it was invoked since length mutations were not seen with other enzymes

[a] LSC, Large single copy region; SSC, small single copy region

[b] Lost and gained fragments here are all with reference to *D. rotundata* cpDNA (DNAs 1–7)

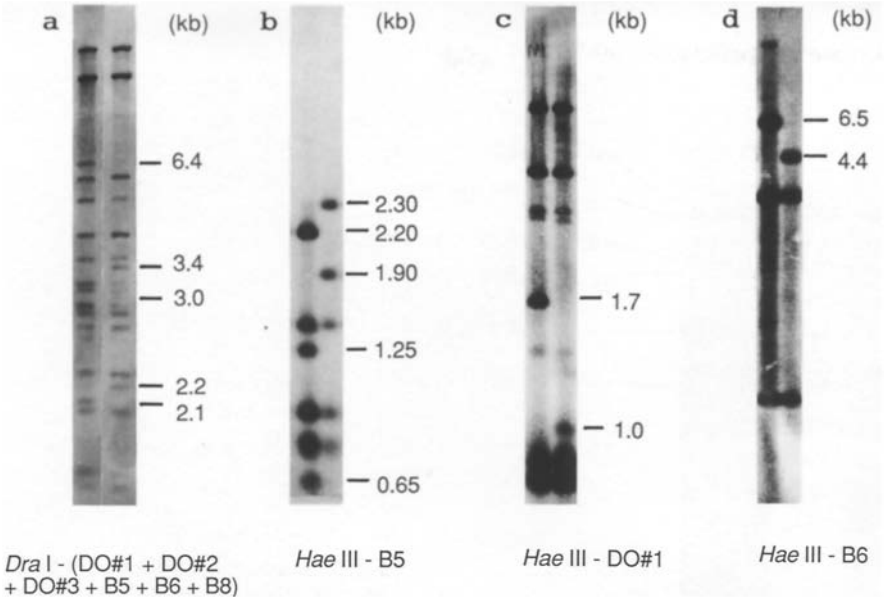

Fig. 3a–d. Southern blots showing restriction site mutations in cpDNAs of Guinea yams and their wild relatives. **a** *Dra*I digests were hybridized with the mixed probes of DO#1, DO#2, DO#3, B5, B6 and B8. A restriction site change (6.4 kb = 3.4 kb + 3.0 kb; mutation 9 in Table 3) and a length mutation (2.1 kb → 2.2 kb; mutation 10) are shown. **b** *Hae*III digests were hybridized with the B5 probe. Two independent restriction site changes are shown, namely, 1.25 kb + 0.65 kb = 1.90 kb (mutation 1) and 2.20 kb [+0.10 kb] = 2.30 kb (mutation 2; brackets indicate that the fragment was not seen because it was too small or was located outside the extent of the probe, but was invoked since length mutations were not seen with other enzymes). **c** *Hae*III digests were hybridized with the probe DO#1 (1.7 kb = 1.0 kb [+0.7 kb]; mutation 7). **d** *Hae*III digests were hybridized with the probe B6 (6.5 kb = 4.4 kb [+2.1 kb]; mutation 3)

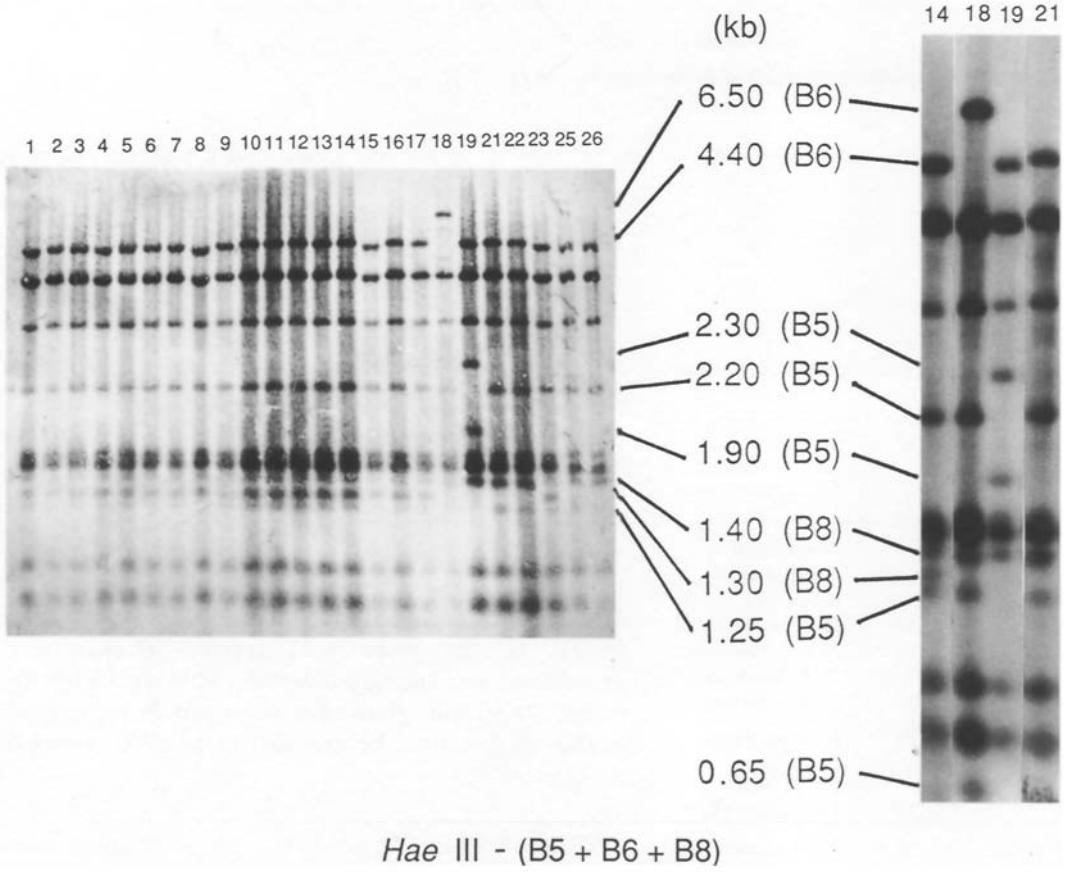

Fig. 4. Southern hybridization of B5 + B6 + B8 mixed probes with cpDNAs of Guinea yams and their wild relatives that were digested with *Hae*III

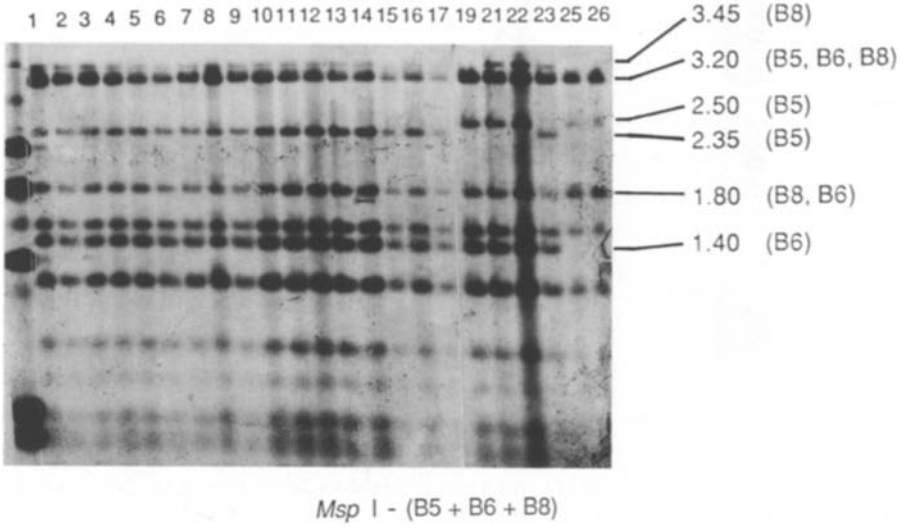

Fig. 5. Southern hybridization of B5 + B6 + B8 mixed probes to cpDNAs of Guinea yams and their wild relatives that were digested with *Msp*I

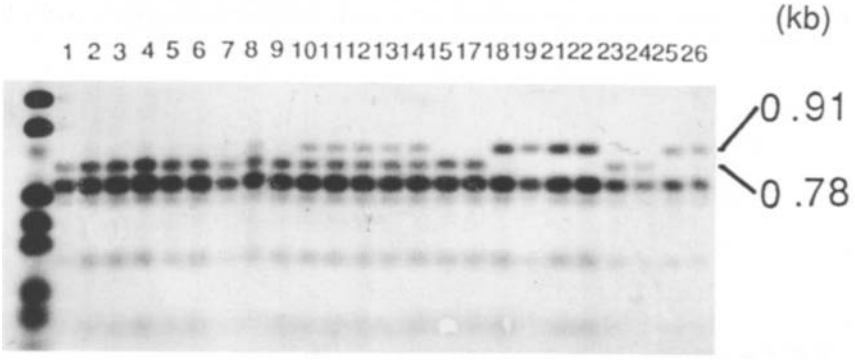

Fig. 6. Southern hybridization of taro rDNA, pCE34.2, to *Mbo*I digests of DNAs of Guinea yams and their wild relatives

Table 4. Classification of chloroplast genomes of Guinea yams and their wild relatives

Chloroplast genome	Mutation[a]	DNA no	Species
A	–	1–7	*D. rotundata*
		8, 9	*D. rotundata-cayenensis*
		10–14	*D. cayenensis*
		15, 16	*D. praehensilis*
		17	*D. liebrechtsiana*
		23, 24	*D. abyssinica*
B	1, 2, 5, 6, 8, 9, 10	19, 20	*D. minutiflora*
C	3, 5, 7, 8, 10	18	*D. burkilliana*
D	5, 7, 8, 10	21, 22	*D. smilacifolia*
E	4, 5, 6, 7, 8, 10	25, 26	*D. togoensis*

[a] Mutations lying between respective chloroplast genome and chloroplast genome A

D. cayenensis, *D. abyssinica*, *D. liebrechtsiana*, *D. praehensilis*, and the intermediate type, *D. rotundata-cayenensis*. Genome types B, C, D, E were possessed by *D. minutiflora*, *D. burkilliana*, *D. smilacifolia* and *D. togoensis*, respectively. No intraspecific variation was observed. Since maternal inheritance of cpDNA is predominant among angiosperms (Palmer et al. 1988), these results suggest that two cultivated species, *D. rotundata* and *D. cayenensis*, could have their maternal origin in one or several of the three wild species, *D. abyssinica*, *D. liebrechtsiana* and *D. praehensilis*. Wild species not included (*D. lecardii*, *D. mangenotiana* and *D. sagittifolia*) in this study cannot be excluded as possible maternal progenitors.

Sequence divergence

An estimate of the number of nucleotide substitutions per site (*d*) between the chloroplast genome types was

Table 5. Estimates of the number of nucleotide substitutions per site, *d* (given as 100 *d*; above diagonal), and number of mutations (inclusive of a length mutation; below diagonal) among the five chloroplast genomes of Guinea yams and their wild relatives

Cp genome	A	B	C	D	E
A		0.272	0.183	0.136	0.227
B	7		0.272	0.227	0.227
C	5	6		0.045	0.136
D	4	5	1		0.091
E	6	5	3	2	

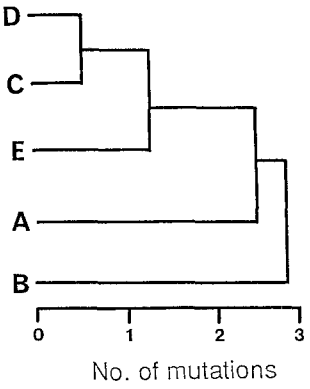

Fig. 7. A UPGMA tree showing a phylogenetic relationship among five chloroplast genomes of Guinea yams. Clustering has been made using mutation numbers between all pairs of the five chloroplast genomes (Table 5)

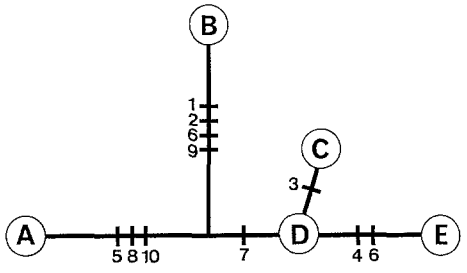

Fig. 8. Unrooted most parsimonious network for five cpDNA types of Guinea yams and their wild relatives based on restriction site changes and a length mutation. The number indicates the mutation number (see Table 3)

calculated by the maximum likelihood method of Nei and Tajima (1983) (Table 5) using the restriction site change data presented in Table 3. Values for *d* among five chloroplast genome types ranged from 0.0005 to 0.0027 with an average of 0.0018. These values are similar to those reported among *Lycopersicon* (Palmer and Zamir 1982), *Pennisetum* (Clegg et al. 1984) and *Zea* (Doebley et al. 1987), but are smaller than in *Brassica* (Palmer et al. 1983), *Pisum* (Palmer et al. 1985) and *Triticum-Aegilops* (Ogihara and Tsunewaki 1988). For another species, *Dioscorea bulbifera* L., we obtained the value $d = 0.0023 \pm 0.0007$ (data calculated from Terauchi et al. 1991) for an intraspecific sequence divergence between Asian and African chloroplast genome types that are assumed to have diverged from each other in the beginning of the Tertiary Miocene, approximately 10 million years ago (Burkill 1960). The diversification of the Guinea yam group (subsection Cayenenses Chev.) seems to have started around this time as indicated by the similar values of *d* between *D. bulbifera* and subsection Cayenenses.

Phylogenetic relationships among the five chloroplast genome types of Guinea yams and their wild relatives

On the basis of the distance matrix of mutation numbers (inclusive of a length mutation; Table 5), a phenetic tree (Fig. 7) was constructed by the UPGMA (unweighted pair group method with arithmetic mean) method of Sneath and Sokal (1973). Three primary clusters are recognizable: the first one consists of three chloroplast genome types, C, D and E, which represent *D. burkilliana*, *D. smilacifolia* and *D. togoensis*, respectively; the second one contains genome type A with the five species *D. rotundata*, *D. cayenensis*, *D. abyssinica*, *D. liebrechtsiana*, *D. praehensilis* and the intermediate type *D. rotundata-cayenensis*; the third one contains genome type B from *D. minutiflora*. An unrooted, most parsimonious network (Fig. 8) was constructed by cladistic assessment of the data in Table 3. The network requires 11 steps to account for ten mutations. Only mutation number six displays an apparent conversion or parallel gain of a *Msp*I restricition site in genome type B (*D. minutiflora*) and type E (*D. togoensis*). In this network three lineages are recognized. Within the third lineage, consisting of chloroplast genome types C, D and E, genome type D (*D. smilacifolia*) seems to be ancestral to genome types C (*D. burkilliana*) and E (*D. togoensis*). The location of *D. togoensis* in this network is somewhat surprising, because phenotypically *D. togoensis* is much closer to species with chloroplast genome type A than to those of genome types C and D. *D. togoensis* and all species of genome type A share annual stems and annually replaced tubers rather than the perennial stems and perennial large corms found among *D. burkilliana*, *D. minutiflora* and *D. smilacifolia*. This network indicates that the evolutionary change between annuality and perenniality occurred at least twice, independently, once during the speciation between chloroplast genome types A and D and again between genome types D and E.

Ribosomal DNA polymorphism and origin of Guinea yams

Ribosomal DNA polymorphism was detected not only with pRR217 but also with the probe pCE34.2, which does not contain the non-transcribed spacer region. The

most variable part within pCE34.2 is the intergenic spacer regions (IGS, spacers between 18S and 5.8S genes, and between 5.8S and 26S genes) (Rogers and Bendich 1987), therefore the two variable fragments detected in Fig. 6 most probably are found around these IGS regions. Because no simultaneous size difference was observed when the DNA was digested with other enzymes, the RFLP appears to be a result of *Mbo*I restriction site change. Although the IGS is not constant in general, it shows very little variability within and between species of Guinea yams and their wild relatives. Therefore, the two fragments observed are not likely to have arisen by convergent occurrences. Because *D. cayenensis* is a true cultigen without a wild type, the 0.78-kb and 0.91-kb fragments co-existing in *D. cayenensis* were most likely derived through hybridization between the species with only the 0.78-kb fragment and that with only the 0.91-kb fragment. This result, together with that of cpDNA, indicates that *D. rotundata* must have originated through the direct domestication of either one or several of wild species, *D. abyssinica*, *D. liebrechtsiana* and *D. praehensilis*, or through hybridization among them, and that *D. cayenensis* must have been derived from the hybridization between a female plant of either one of species with chloroplast genome type A (*D. rotundata*, *D. abyssinica*, *D. liebrechtsiana* and *D. praehensilis*) and a male plant of either one of species with the *Mbo*I-0.91-kb rDNA fragment (*D. burkilliana*, *D. minutiflora*, *D. smilacifolia* and *D. togoensis*) (Fig. 9). Morphological and ecological data support the hypothesis of the hybrid nature of *D. cayenensis*. The growth period of *D. cayenensis* is 8–12 months and some cultivars are even perennial, the corm of *D. cayenensis* is large, the leaves are thick and flat, and its habitat is mainly in the rain-forest zone. All of these are common characters of *D. burkilliana*, *D. minutiflora* and *D. smilacifolia*. The fact that some *D. cayenensis* cultivars have small folded compartments termed acarodomatia (De Wildeman 1904 cited by Burkill 1960) on the lower side of the leaf blade indicates their close relationship with *D. burkilliana* and *D. smilacifolia*. This hypothesis is also in good agreement with that proposed by Hamon (1988) based on an isozyme electrophoretic study. Since the hybridization event, the resulting from of *D. cayenensis* must have been propagated entirely by vegetative means since only male cultivars are known. The intermediate *D. rotundata-cayenensis* types (DNA sample 8) had more copies of the 0.78-kb fragment than of the 0.91-kb fragment judging from the intensity of the hybridization signal (Fig. 6). This is consistent with the opinion of yam breeders that our sample could be a hybrid between *D. rotundata* and *D. cayenensis*. The copy number difference may reflect the existence of more than two *Nor* loci in *D. cayenensis*. Another accession of *D. rotundata-cayenensis* (DNA 9) had only the 0.78-kb fragment, indicating that it is not of hybrid origin as such.

A new nomenclature

Based on the aforementioned hypothesis for the origin of Guinea yams, we propose a new nomenclature of Guinea yams as follows: the species name *D. rotundata* Poir. should be used to encompass all cultivars in subsection Cayenenses; *D. rotundata* Poir. *nomen nudum* could be applied to designate cultivars of white yam as before; the former *D. cayenensis* Lamk. (yellow yam) should be reclassified as a variety or subspecies of *D. rotundata*, such as *D. rotundata* var. × 'cayenensis'.

Effective Guinea yam breeding has been hampered by the shy-flowering of most cultvars, presumably as a result of continuous vegetative propagation and hybrid sterility. The possible wild progenitors of the Guinea

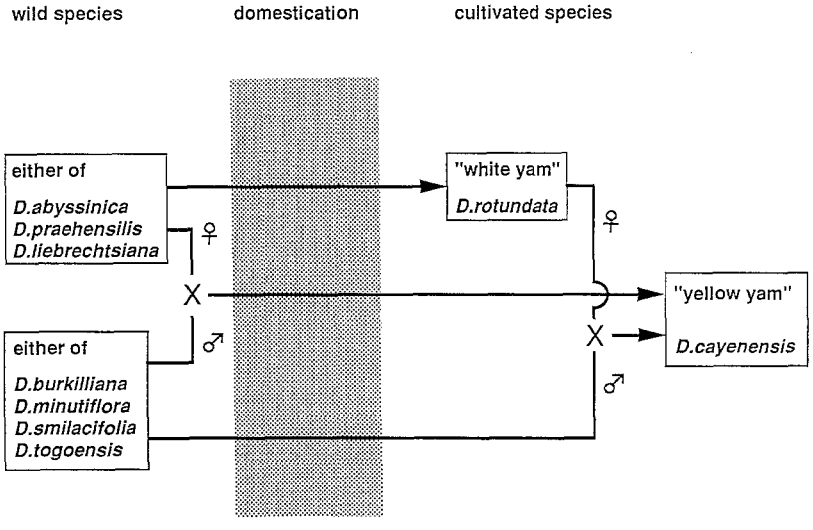

Fig. 9. A hypothetical scheme of the origin of white and yellow yams

yams reported in this paper usually undergo normal sexual reproduction. These wild species could be incorporated into breeding populations for the effective improvement of Guinea yam by overcoming the shy-flowering problem and for introducing new genetic traits. For that purpose, the exploration and preservation of Guinea yam wild relatives are important. As rapid deforestration is taking place in the region these tasks are urgent since the habitats of most wild yams are within or at the edge of forests.

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