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Genetic diversity of the greater yam (*Dioscorea alata* L.) and relatedness to *D. nummularia* Lam. and *D. transversa* Br. as revealed with AFLP markers

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Abstract

Amplified fragment length polymorphism markers were used to assess the genetic relatedness between *Dioscorea alata* and nine other edible *Dioscorea*. These species include *D. abyssinica* Hoch., *D. bulbifera* L., *D. cayenensis-rotundata* Lamk. et Poir., *D. esculenta* Burk., *D. nummularia* Lam., *D. pentaphylla* L., *D. persimilis* Prain. et Burk., *D. transversa* Br. and *D. trifida* L. Four successive studies were conducted with emphasis on the genetic relationship within *D. alata* and among species of the Enantiophyllum section from Vanuatu. Study 1 was carried out to select a set of polymorphic primer pairs using 11 combinations and eight species belonging to five distinct sections. The four most polymorphic primer pairs were used in study 2 among six species of the Enantiophyllum section. Study 3 focussed mainly on the genetic relationship among 83 accessions of *D. alata*, mostly from Vanuatu (78 acc.) but also from Benin, Guadeloupe, New Caledonia and Vietnam. The ploidy level of 53 accessions was determined and results indicated the presence of tetraploid, hexaploid and octoploid cultivars. Study 4, included 35 accessions of *D. alata*, *D. nummularia* and *D. transversa* and was conducted using two primer pairs to verify the taxonomical identity of the cultivars 'langlang', 'maro' and 'netsar' from Vanuatu. The overall results indicated that each accession can be fingerprinted uniquely with AFLP. *D. alata* is an heterogeneous species which shares a common genetic background with *D. nummularia* and 'langlang', 'maro' and 'netsar'. UPGMA cluster analysis revealed the existence of three major groups of genotypes within *D. alata*, each assembling accessions from distant geographical origins and different ploidy levels. The analysis also revealed that 'langlang', 'maro' and 'netsar' clustered together with the cultivar 'wael' (*D. transversa*) from New Caledonia. Results are discussed in the paper.

Introduction

Dioscorea alata or the greater yam is the most widely cultivated yam species throughout the tropics. The origin of the species is still a matter of debate but it is generally stated that *D. alata* belongs to the Enantiophyllum section of Southeast Asia. De Candolle (1886) first suggested an Indo-Malayan origin of the species. Later on, Prain and Burkill (1939) placed it further northward in the Indochinese peninsula where the wild presumed relatives *D. hamiltonii* Hook. and *D. persimilis* Prain. et Burk. are found. Although there is no further evidence to support this second hypothesis, it has been widely accepted (Alexander and Coursey 1969; Martin 1974; Degras 1986; Mignouna et al. 2002a).

Analyses of the morphological variation present in a worldwide collection of *D. alata* revealed a highly plastic species for which the greatest

Table 1. Number of accessions analysed.

Species	Sections	Geographical origins[a]	Studies			
			1	2	3	4
D. abyssinica	Enantiophyllum	BE = 1	–	1	–	–
D. alata	Enantiophyllum	BE = 1, GU = 1, NC = 5, VN = 4, VU = 78	4	26	83	20
D. bulbifera	Opsophyton	VU = 1	1	–	–	1
D. cayenensis	Enantiophyllum	VU = 2	2	2	–	–
D. esculenta	Combilium	VU = 3	3	–	–	1
D. nummularia	Enantiophyllum	VU = 7	–	3	–	7
D. pentaphylla	Botryosicyos	VU = 2	2	–	–	1
D. persimilis	Enantiophyllum	VN = 1	1	1	–	–
D. transversa	Enantiophyllum	NC = 1, VU = 7	2	8	–	8
D. trifida	Macrogynodium	VU = 1, GU = 1	2	–	–	–
Total			17	41	83	38

[a]BE – Benin; GU – Guadeloupe; NC – New Caledonia; VN – Vietnam; VU – Vanuatu.

variability is found in Papua New Guinea and the Philippines islands (Martin and Rhodes 1977).

Various ploidy levels analyses have indicated the existence of tetraploid, hexaploid and octoploid cultivars with $x = 10$ as basic chromosome number (Abraham and Nair 1991; Gamiette et al. 1999). No diploid forms are found.

Isozyme variation was assessed among 269 accessions of *D. alata* originating from the South Pacific, Asia, Africa and the Caribbean (Lebot et al. 1998) and identified 66 distinct zymotypes using four polymorphic enzyme systems. However, the rather low polymorphism of these markers could not reveal any correlation between zymotypes groups, geographical origins, ploidy level and/or phenotypic traits.

Random amplified polymorphism DNAs (RAPD) have been used to assess the genetic relationships among *D. alata* cultivars (Asemota et al. 1996) and amplified fragment length polymorphism (AFLP) have been used to study the genetic relationships of Guinea yams (Mignouna et al. 1998) and to construct genetic maps of three *Dioscorea* species (Terauchi and Kahl 1999; Mignouna et al. 2002a, b). However, AFLP markers have not been used to study the extent of genetic variation existing within *D. alata* and between species of the Enantiophyllum section confined to southeast Asia-Oceania.

The aim of this paper is to assess the usefulness of AFLP markers to study genetic relationships among cultivars of *D. alata*, *D. nummularia* and *D. transversa* using accessions maintained in the germplasm collection of Vanuatu.

Materials and methods

Plant material

The number of accessions studied per species is presented in Table 1. Most of the accessions of *D. alata* originated from the Vanuatu germplasm collection. This collection was established within the scope of the *South Pacific Yam Network* project which had the main objectives of studying the genetic diversity of *D. alata* using national core collections established in Melanesian countries, that are, Fiji, New Caledonia, Papua New Guinea, the Solomon Islands and Vanuatu. The Vanuatu germplasm collection was also described using international standardised morphological descriptors (IPGRI/IITA 1997). A core collection was then established based on a UPGMA clustering method analysis of the morphological data (Malapa 2000). Accessions were extracted within each cluster to represent up to 10% of the variability according to the core collection's strategy established by Brown (1989). Fewer accessions of *Dioscorea* spp. (Table 1) were also included in this study for comparative analyses and were obtained from various institutions: Centre International de Recherches Agronomiques pour le Développement (CIRAD, Montpellier, France), Institut Agronomique Calédonien (IAC, Nouméa, New Caledonia), Institut de Recherches pour le Développement (IRD, Montpellier, France), Vanuatu Agriculture and Technical Center (VARTC) and Vietnam Agriculture Science Institute (VASI, Hanoi).

AFLP studies

DNA extracts were done in Vanuatu. Leaf samples of each accession were collected on well-developed plants, put separately into plastic tube (15 mL) and kept in portable container of liquid nitrogen. Total genomic DNA was extracted from 5 g of young green leaves. A MATAB procedure (Risterucci et al. 2000) was used with minor modifications as follows: each sample was ground in liquid nitrogen with a mortar and pessel. The fine powder was quickly transferred to 50 mL Falcon tubes containing 15 mL of the extraction buffer: 100 mM Tris–HCl (pH 8), 1.4 M NaCl, 20 mM EDTA (pH 8), 2% MATAB, 2% PEG (6000) and 0.5% Na sulfite. Extraction was done at 74 °C for 1 h. Proteins were precipitated with 15 mL of a 24 : 1 solution of chloroform : isoamyl alcohol. The mixture was centrifuged at 5000 rpm for 30 min using a 'Universal 32' centrifuge (Hettich Zentrifugen).

DNA was precipitated with cold ethanol (2.5 v) and hooked with a sterile Pasteur pipette. It was dissolved in 1 mL buffer (50 mM Tris–HCl (pH 8), 0.7 mM NaCl, 10 mM EDTA). Purification of DNA was done using the QIAGEN-tip protocol, with miniprep-volumes. Purified DNA was checked on agarose gel (0.8%) and the concentration determined by Hoechst 33258 staining using a fluorometer. Extracts were then diluted in Merck water to obtain DNA concentrations of 50 ng/μL. Commercial Invitrogen kits (Life Technologies^{TM}) with the given pair of restriction enzymes (*Eco*RI and *Mse*I), were used for AFLP procedure. Radioactive labelling was done using γ^{33}P and the amplified fragments were separated on a 5% Urea-PAGE.

Primers with three selective nucleotids at the 3′-end were used for PCR amplification. They proved in earlier genetic studies to produce a sufficiently and rationally scorable interspecific level of polymorphism within the genus *Dioscorea* (Mignouna et al. 1998; Malapa 2000). Our four studies presented here used a total of 12 combinations and different samples (Tables 1 and 2). For each analysis, two *D. alata* accessions were used as internal controls in addition to the tomato DNA control of fragment sizes. The purpose was not only to check the repeatability of the data, but also to calibrate each gel on already known band levels, in order to avoid any shift or confusion from one gel to another for a given primer pair:

Study 1 aimed at testing 11 combinations and to assess the most polymorphic ones on a set of 17 accessions belonging to eight different species.

Study 2 was conducted with the four most polymorphic primer pairs on 41 accessions belonging to six different species of the Enantiophyllum section.

Study 3 was conducted using the two most polymorphic primer pairs on 83 accessions of *D. alata* to study the infraspecific variation.

Study 4 was conducted using another set of two primer pairs to confirm the relationships between *D. alata*, *D. nummularia* and *D. transversa*.

Table 2. Number of polymorphic bands scored per primer pairs.

	Study 1	Study 2	Study 3	Study 4
E-AAC/M-CTA	39	–	–	–
E-AAC/M-CAG	53	40	–	–
E-ACA/M-CAT	52	–	38	–
E-ACC/M-CTA	55	–	–	–
E-ACC/M-CAT	46	54	–	38
E-ACA/M-CAA	40	–	–	–
E-ACG/M-CTA	43	–	–	–
E-AAC/M-CAT	32	31	–	–
E-ACT/M-CTA	40	–	–	–
E-ACT/M-CTC	53	–	–	–
E-ACA/M-CAC	40	–	–	–
E-ACA/M-CAG	–	31	37	30
Total	493	156	75	68
Mean	44.8	39	37.5	34

Data analysis

Amplified fragment length polymorphism revealing multilocus and dominant markers could bias genetic distances in case of redundant band levels. The redundancy between loci could be minimized if independent loci are exploited and determined according to a genetic map. Such maps exist for species of the genus *Dioscorea* (Terauchi and Kahl 1999; Mignouna et al. 2002a, b) and were constructed using combinations with two selective nucleotides at the 3′-end. For our studies, no preliminary screening of redundant loci was done. We therefore include bootstrap analysis to evaluate the solidity of the genetic relationships revealed within and among the species analysed in studies 1 and 2.

Due to the dominant nature of AFLP markers, binary data matrices were coded for each band level (1 = present, 0 = absent) and for each accession. Very weak bands were excluded from the analyses and band intensity was not taken into account. Studies 1 and 2 were conducted by two different persons and this permitted to assess the consistency and reliability of scoring AFLPs. Only unambiguous signal intensities and banding patterns were retained after doing two independent readings of AFLP profiles. Pairwise distance matrices were computed using the Dice and Simple Matching coefficients. The resulting matrices were subjected to Unweighted Pair Group Method Analysis (UPGMA) to construct dendrograms with the software package NTSYS-PC (Rohlf 1995). The distance matrices were also analysed using the software Darwin 4.2 (Perrier et al. 2003) for calculating the bootstrap values using 1000 replicates.

Ploidy level analysis

Fifty-three accessions of *D. alata* were planted in a greenhouse in Montpellier, France, for ploidy level analysis. Chromosomes were counted on slide preparations of root protoplasts following D'Hont et al. (1996). Young root tips (5 mm in length; 0.5 mm thick) were removed from pot plants with fine forceps and immersed directly into a 0.04% solution of 8-hydroxyquinoline. They were kept 2 h each at room temperature and 4 °C. Then, they were fixed in a freshly prepared 3 : 1 solution (ethanol : acetic acid glacial) for 48 h at room temperature.

Protoplasts were then prepared as follows: root tips were rinsed for 10 min in distilled water and immersed in 0.25 N HCl solution for 10 min. They were then rinsed for 10 min in distilled water and immersed in citrate digestion buffer (1.47 g trisodium citrate-dihydrate, 1.05 g citric acid-monohydrate, 500 mL distilled water) for 10 min at room temperature. Root tips were cut to 1 mm length, then immersed in enzyme solution of 5% cellulase (Onozuka R-10, Yakult Honsha Co. Ltd., Japan) and 1% pectolyase (Y-23, Seishin Pharmaceutical Ltd., Japan) and hydrolysed for 1 h at 37 °C in microtubes. Tips were removed with a sterile Pasteur pipette and rinsed in distilled water for 1 min. Smears were then prepared using 3 : 1 solution (ethanol : acetic acid glacial) and stained with 12 μL of DAPI (4,6-diamidino-2-phenylindole). Slides were observed for taking chromosome counts under UV fluorescent light (340–380 nm) using a Leica DMRXA microscope equipped with a wavelength filter passing under 430 nm.

For flow cytometry, the protocol used was adapted from Gamiette et al. (1999). About 0.5 cm^2 of an adult green leaf was used for nuclei isolation. Each sample was chopped with a new razor blade in a plastic Petri dish in 2 mL of icecold extraction PBS buffer. The suspension of released nuclei was filtered through a 30 μM mesh nylon filter into a microtube to which icecold propidium iodide was added to a final concentration of 50 ppm. The sample was incubated for 5 min at room temperature. The fluorescence of the nuclei was measured using a Faxscan flow cytometer equipped with an argon ion laser tuned to 488 nm. A tetraploid cultivar (cv. 'tamate') and an octoploid cultivar (cv. 'ifit') of *D. alata* were used successively as internal controls to adjust the G1 peaks at channels 150 and 300, respectively. Ploidy levels were then determined by comparing the relative fluorescent peak intensities of the sample and the internal control for 3000 nuclei examined as done in Gamiette et al. (1999).

Results

AFLP studies

All AFLP primer pairs produced highly polymorphic patterns within and among the *Dioscorea* species analysed. Patterns were complex in the intensity and size of bands, and several species-specific band levels were observed. The sizes of the AFLP fragments ranged from 50 to 350 bp. The average numbers of polymorphic band levels scored per primers pairs in the four studies were 44.8, 39, 37.5 and 34 bands (Table 2). Results are presented below.

Study 1

The comparisons of pairwise genetic similarities among the eight *Dioscorea* spp. are presented in Table 3. Mean values ranged from 53.7% between *D. alata* and *D. transversa* to 0.3% between *D. cayenensis-rotundata* and *D. trifida*. The results are consistent with the systematics of these species

Table 3. Average genetic similarities (%) within and among eight *Dioscorea* sp. (Study 1).

	ala[a]	tra	per	cay	pen	bul	esc	tri
D. alata	87.7							
D. transversa	53.7	94.1						
D. persimilis	25.7	31.4	100					
D. cayenensis	14.7	14.7	13.0	78.3				
D. pentaphylla	08.8	10.9	07.0	02.6	84.7			
D. bulbifera	08.4	06.8	10.1	05.8	02.2	100		
D. esculenta	05.7	05.2	05.0	03.9	06.2	03.0	94.2	
D. trifida	05.5	05.1	04.9	00.3	01.1	03.3	01.3	74.5

[a]ala – *D. alata*; bul – *D. bulbifera*; Cay – *D. cayenensis*; esc – *D. esculenta*; pen – *D. pentaphylla*; per – *D. persimilis*; tra – *D. transversa*; tri – *D. trifida*.

regarding their botanical sections and geographical origins. *D. alata*, *D. persimilis*, *D. transversa* and *D. cayenensis-rotundata* belong to the Enantiophyllum section. The former three species occur in Asia-Oceania whereas *D. cayenensis-rotundata* in Africa. Moreover *D. bulbifera* (sect. Opsophyton), *D. esculenta* (sect. Combilium), *D. pentaphylla* (sect. Botryosicyos) and *D. trifida* (sect. Macrogynodium) belong to different sections with the former three species originating in Asia-Oceania whereas *D. trifida* in America (Table 1).

Comparison of pairwise genetic similarity between species indicates that *D. alata* is genetically closer to *D. transversa* (53.7%) rather than to *D. persimilis* (25.7%) (Table 3). This relationship is further supported by the cluster analysis (Figure 1) of the distance matrix and strong bootstrap values (100% for the *D. alata* vs. *D. transversa* relationship and 98% for the relationship between *D. persimilis* and the former two species).

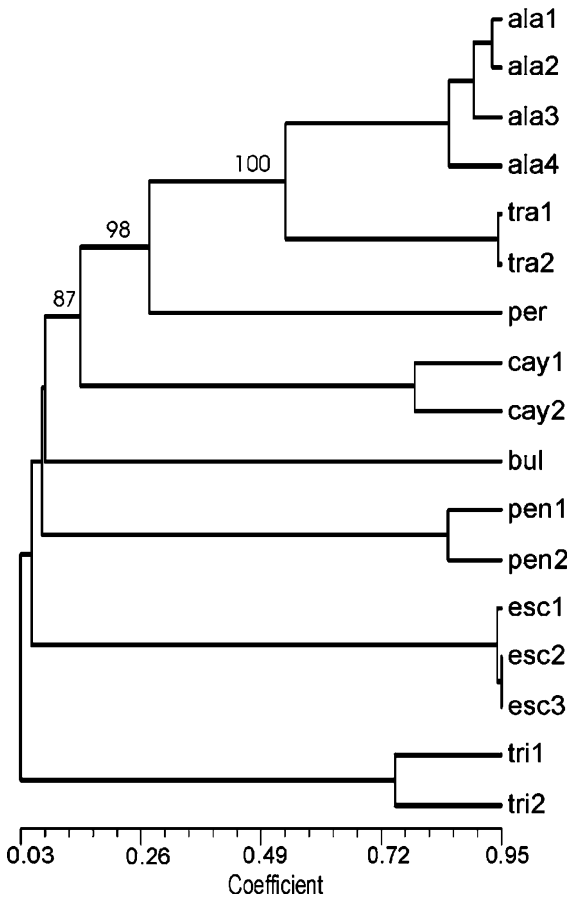

Figure 1. Strict consensus tree (UPGMA and Dice coefficient) from an AFLP data matrix using eleven primer pairs among eight edible *Dioscorea* species. Bootstrap values are indicated upon lines. *D. alata* (ala), *D. bulbifera* (bul), *D. cayenensis-rotundata* (cay), *D. esculenta* (esc), *D. pentaphylla* (pen), *D. persimilis* (per), *D. transversa* (tra), *D. trifida* (tri).

Study 2

The four most polymorphic primer pairs generated 156 polymorphic bands in 41 accessions belonging to six Enantiophyllum section species: *D. abyssinica*, *D. alata*, *D. cayenensis-rotundata*, *D. nummularia*, *D. persimilis* and *D. transversa* (Tables 1 and 2). Cluster analysis (Figure 2) of the distance matrix revealed a tree topology with five major groups as supported with high bootstrap values ranging from 67.3% to 99.7%. Clusters nos. 1, 2, 3 and 4 include, respectively, *D. alata* (ala1 to ala26), *D. transversa* (tra27 to tra34), *D. nummularia* (num35 to num37) and *D. persimilis* (per38). The African species, *D. abyssinica* (aby39) and *D. cayenensis-rotundata* (cay40 and cay41) are included in cluster no. 5 which is separated from clusters nos. 1, 2, 3 and 4 by a genetic distance of 31% similarity and supported with a bootstrap value of 98.3%.

The general topology of the tree revealed an interspecific relationship which is partly consistent with results of study 1. *D. alata* (cluster 1) is closely related to *D. transversa* (cluster 2) and *D. nummularia* (cluster 3), but genetically distant from *D. persimilis* (cluster 4). Cluster no. 2 groups cvs. 'netsar' (tra27), 'maro' (tra28, tra29) and 'langlang' (tra30 to tra34).

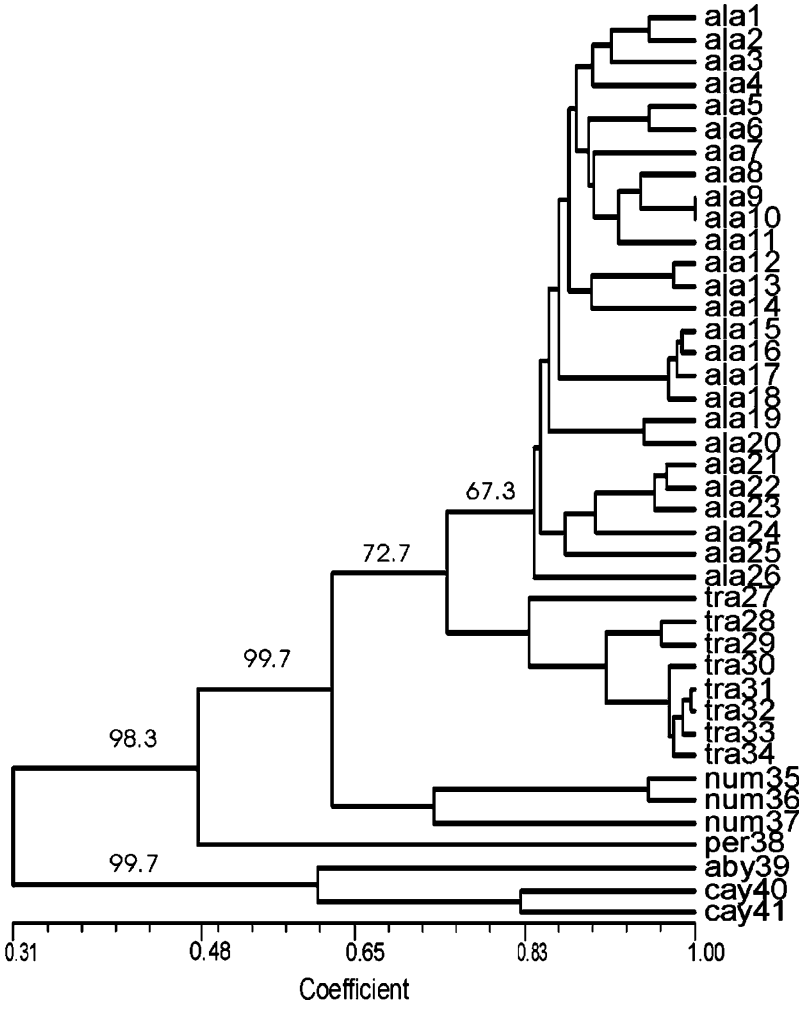

Figure 2. Strict consensus tree derived from an AFLP data matrix of six *Dioscorea* species of the Enantiophyllum section using four primer pairs (UPGMA and Dice coefficient): *D. abyssinica* (aby), *D. alata* (ala), *D. cayenensis-rotundata* (cay), *D. nummularia* (num), *D. persimilis* (per), *D. transversa* (tra).

'langlang' and 'maro' are morphologically similar and could be distinguished from 'netsar' by the absence of discrete wings along stems and the presence of anthocyaned pigments on stems and young leaves. Cluster no. 3 includes three wild accessions (num35 to num37) of *D. nummularia* collected in Vanuatu. The genetic relationship between these three species is also supported with a high bootstrap value of 99.7% between clusters nos. 1 to 3. The mean numbers of species-specific bands found for *D. alata*, *D. transversa* and *D. nummularia* are 5.25, 8.25 and 10 bands, respectively (Table 4). They indicated that 'langlang', 'maro' and 'netsar' are closely related to each other and belong to a taxa which is distinct from the species *D. alata* and *D. nummularia*, but share a common ancestor with them.

Study 3

Pairwise genetic distances between accessions using the Simple Matching coefficient raised similarities varying from 49.3% to 100% with the lowest similarity being observed between accessions from Vietnam (acc. 45) and Vanuatu (acc. 83). The UPGMA cluster analysis of the AFLP data (Figure 3) reveals three main clusters which are separated by small genetic distances. Overall,

Table 4. Specific AFLP bands for *D. alata*, *D. transversa* and *D. nummularia* (Study 2).

Primer pairs	Total bands	Polymorphic bands	Specific bands		
			D. alata 26 acc.	*D. transversa* 8 acc.	*D. nummularia* 3 acc.
E-AAC/M-CAG	47	40	4	10	15
E-AAC/M-CAT	43	31	8	9	6
E-ACA/M-CAG	43	31	6	7	4
E-ACC/M-CAT	58	54	3	7	15
Mean	47.75	39	5.25	8.25	10
SD	7.09	10.86	2.22	1.50	5.83

subclusters with more than 85% of genetic similarity generally assemble cultivars with a same morphotype, though some exceptions were observed.

Cluster no. 1 (acc. 01–44) includes accessions sharing from 68.5% to 100% of average genetic similarity. These accessions are phenotypically highly variable and field observations of plant morphology revealed a continuum of shape and size of leaves from ovoid and narrow to round and large (Figure 3). Accessions 19–24 belong to the same morphotype showing 90.8% genetic similarity. This morphotype develops very ruffled wings along stems and is highly appreciated for the firm consistence, white colour and good organoleptic properties of the tuber flesh. Also, tubers are shallow and thus easily harvested and widely distributed through out the Vanuatu archipelago. Accessions 30–44 have heart-shaped leaves although their morphotypes are highly variable. Male (acc. 31, 32 and 33) and female (acc. 35, 36 and 38) accessions are included within this subgroup. All produce regular cylindrical shaped tubers with white or purple flesh. All accessions which clustered with 85% genetic similarity are phenotypically homogenous. Three duplicates were identified (i.e., acc. 12 and 13; acc. 46 and 47; acc. 73 and 74), each being phenotypically identical to its respective duplicates but morphologically distinct from one another.

Cluster 2 includes 21 accessions (acc. 45–65) sharing from 63.6% to 100% average genetic similarity. Accessions 64 and 65 belong to the same morphotype and may be regarded as an outstanding group but we included them herein owing to their closest relatedness with clusters nos. 1 and 3. Accessions 45, 56, 57, 58 originated from Vietnam and accession 59 from Benin. They clustered together with accessions from Vanuatu which produced irregular tuber shape and white or anthocyaned tuber flesh. Their wings are developed and ruffle. Accessions 45, 46, 47, 48, 49, 50, 52 and 53 belong to the same morphotype and shared more than 89.6% genetic similarity. Their tuber flesh is characterized by an elastic consistency when cooked.

Cluster 3 includes 18 late maturing accessions sharing from 67.2% to 100% average genetic similarity. Leaves are ovoid or triangular but much narrower as compared to accessions from the two previous clusters. Two morphotypes are clearly distinguished separating the accessions 66–78 from accessions 79–83. The first morphotype, includes 13 accessions with 87.5% genetic similarity. They produce irregular, ovoid or cylindrical shapes of tubers, all having white to purple flesh. All cultivars included in this subcluster are male plants. The second morphotype includes 5 accessions (acc. 79–83) with a narrower genetic basis (93.2%). They are non-flowering, produce the most diverse tuber shape (round, ovoid, cylindrical, flattened, triangular and irregular) and are resistant to anthracnose. Tuber flesh colour is also variable from white to purple, their leaves are ovoid and narrow.

Study 4

The two most polymorphic primer pairs combination were used to assess the genetic relationship between *D. alata*, *D. nummularia* and *D. transversa*. Three non-related *Dioscorea* spp. were also included for comparison in the cluster analysis (Table 1). Overall, AFLP generated 68 markers (Table 2).

Clearly, only two primer pairs combinations permitted the separation of *D. alata* (ala),

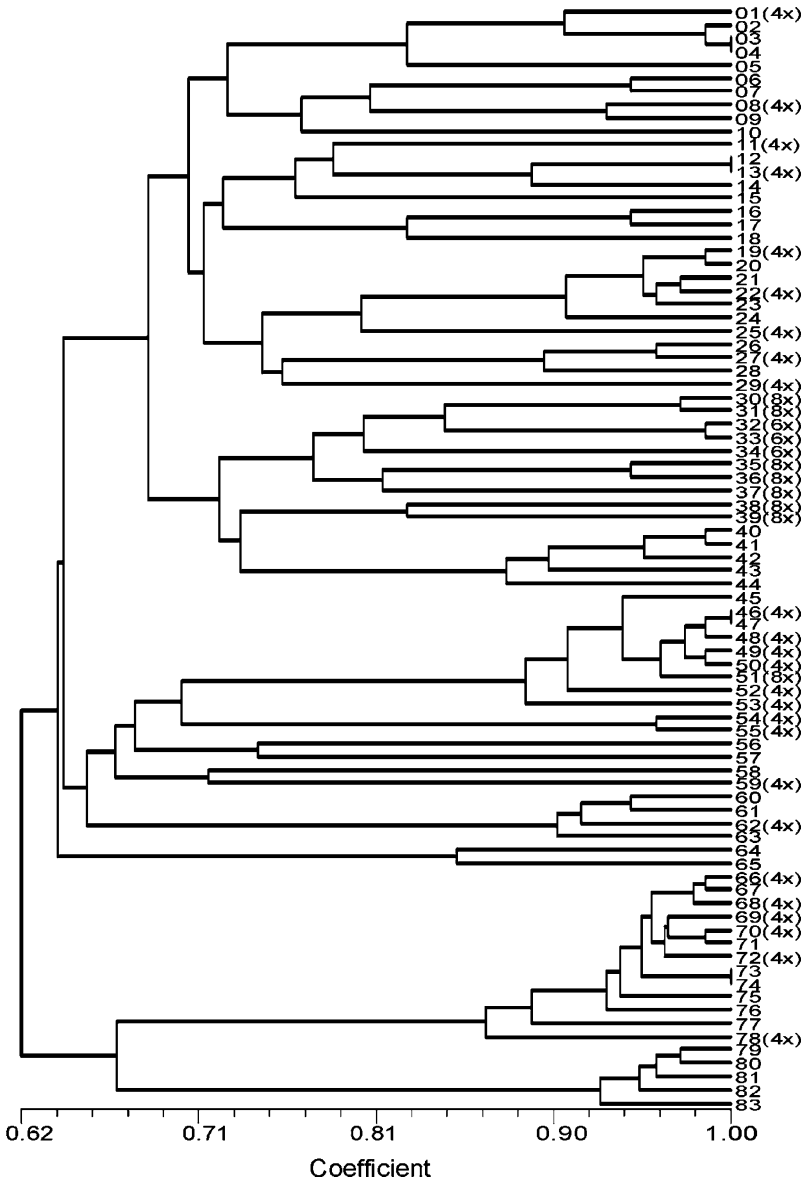

Figure 3. Strict consensus tree derived from an AFLP data matrix of 83 accessions of *D. alata* using two primer pairs (UPGMA and Simple Matching coefficient). Ploidy levels are indicated in brackets.

D. nummularia (num) and *D. transversa* (tra) from each other (Figure 4). AFLPs reveal that the Vanuatu cultivars 'netsar' (tra1), 'maro' (tra2, tra3, tra4, tra7) and 'langlang' (tra5 and tra6) cluster together with the cultivar 'wael' (tra8nc) which had been identified as *D. transversa* in New Caledonia (Bourret 1973). According to Lebot (1997), 'wael' has all the morphological characteristics of the cultivar 'maro' from Vanuatu. Their growth cycle, vegetative characteristics and the high dry matter content of their tubers differentiate them from *D. alata*. AFLPs also reveal that cultivars 'timbek' (num1), 'net' (num2) and the wild forms 'buts' (num3 to num7) belong to the species

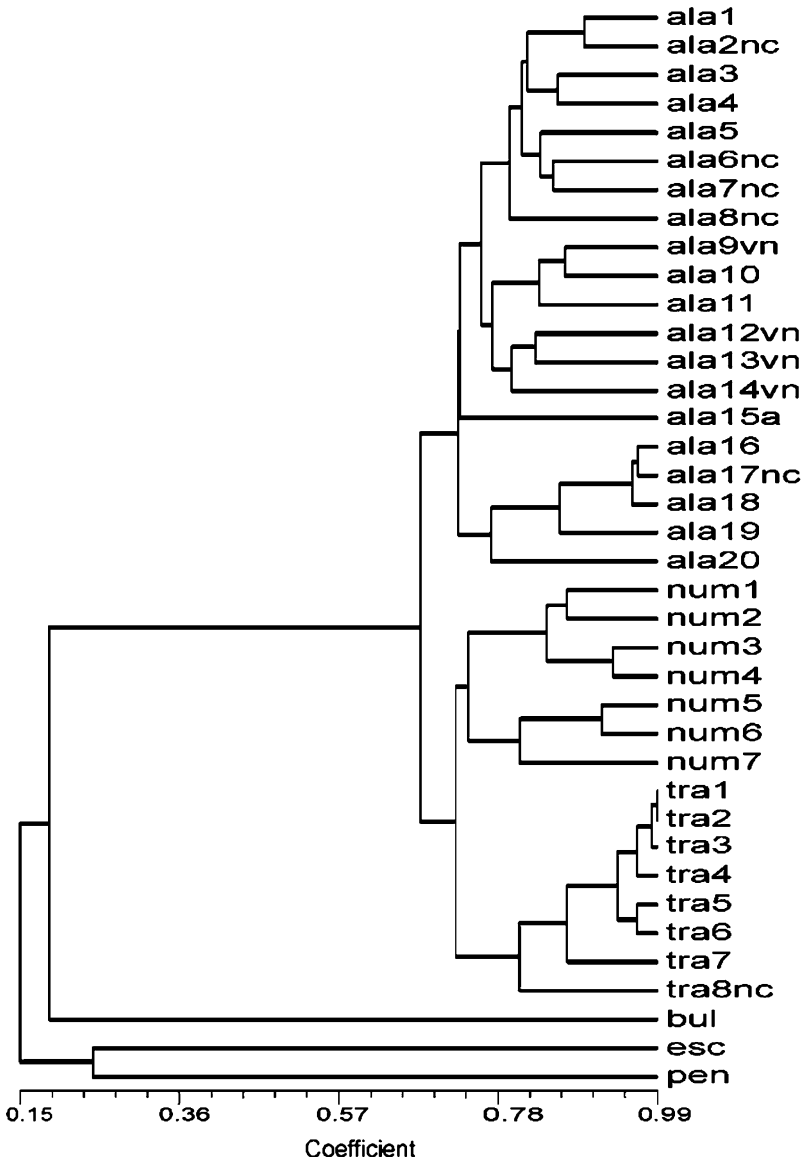

Figure 4. UPGMA and Dice coefficient of 38 accessions including *D. alata* (ala), *D. bulbifera* (bul), *D. esculenta* (esc), *D. nummularia* (num), *D. pentaphylla* (pen) and *D. transversa* (tra).

D. nummularia as suggested by their morphological and floraltraits similarities.

Ploidy levels

Overall, 53 accessions of *D. alata* were analysed and their ploidy levels confirmed with both chromosome counts and flow cytometry (Table 5). Twenty-nine tetraploids, five hexaploids and nineteen octoploids were identified. No diploids were found. Tetraploids and octoploids are widely distributed geographically throughout the Vanuatu archipelago. The five hexaploids originate all from the southern part.

Comparison between morphotypes and cytotypes reveal that tetraploid accessions regroup cultivars with narrow leaves and hexaploid and octoploid accessions assemble cultivars with thick, dark

Table 5. Fluorescent G1 peak intensity ranges of *D. alata* accessions analysed by flow cytometry.

Accession number	Means and ranges of relative fluorescent peak intensities (au)[a]	Ploidy level	Internal references
29	134.32 < 158.76 < 190.01	4×	cv. *Ifit* (8×)
5	237.14 < 258.55 < 284.98	6×	cv. *Ifit* (8×)
19	254.65 < 322.10 < 385.60	8×	cv. *Tamate* (4×)

[a]Arbitrary unit.

green waxy and cordate lamina. Male accessions include five tetraploids (acc. 19, 68, 69, 70 and 78), two hexaploids (acc. 32 and 33) and two octoploids (acc. 30 and 31) and female accessions include three octoploids (acc. 35, 36 and 38) (Figure 3).

Observation of clustering patterns did not reveal any concordance with ploidy levels as tetraploid, hexaploid and octoploid accessions cluster together with 72% of genetic similarity (Figure 3).

Discussions

In this paper we have made a number of points as discussed below.

D. alata exhibits an important morphological variation in Vanuatu as shown by the various cultivars presenting different colours and shapes for both their aerial and underground organs. Numerous morphotypes are recognized in folk taxonomy and have different uses. Previous studies based on phenotypic criteria (Martin and Rhodes 1977; Lebot et al. 1998; Malapa 2000) have revealed that a vast continuum of morphological variation exists, but without, the existence of well-differentiated groups.

Amplified fragment Length polymorphism indicate that the genus *Dioscorea* can be fingerprinted at species and variety levels because of the generation of high polymorphism and specific banding patterns. Interspecific studies revealed that Enantiophyllum section's species are clearly distinguished from each other and are genetically distant from species from other sections. AFLPs obtained from studies 1 and 2 also revealed that African species (*D. abyssinica*, *D. cayenensis-rotundata*) are distant from the southeast Asian-Oceanian species, *D. alata*, *D. nummularia*, *D. persimilis* and *D. transversa*.

D. alata, *D. nummularia* and *D. transversa* are closely related to each other as observed from AFLP-fingerprinting profiles. The genetic similarities among taxa reveal that *D. alata* is genetically close to 'langlang', 'maro', 'netsar' and 'wael' but distant from *D. persimilis* (Figure 2). These results does not support previous hypothesis that *D. persimilis* was the progenitor of *D. alata* (Prain and Burkill 1939) but comfort Telford's (1986) statement regarding the proximity between *D. alata* and *D. transversa*. To date, the origin of *D. alata* is still a matter of debate as *D. persimilis* is confined to the Indochinese peninsula (from Myanmar in the east to Vietnam in the west) while *D. nummularia* and *D. transversa* are restricted to southeast Asian islands and Oceania (Bourret 1973; Lebot 1997). Our findings suggest that *D. alata* may belong, together with *D. nummularia* and *D. transversa*, to a southeast Asian-Oceanian genepool which is rather confined to the former Sahulian and Wallacean regions (Yen 1995; Lebot 1999).

The non-distinction between Asian, African and Melanesian cultivars of *D. alata* strengthens the inferences based on previous isozyme studies (Lebot et al. 1998) which indicated that clones have been widely distributed. This inference must however be taken with caution as not enough material from diverse geographical origins was included in the present study. UPGMA analysis indicates that tetraploid, hexaploid and octoploid cultivars of *D. alata* clustered together with very little genetic distances between them (Figure 3). These results suggest that higher ploidy levels may rise from autopolyploidisation involving tetraploid cultivars. Hence, the substantial variation observed within *D. alata* could also reflect phenotypic and genomic variability which are further increased by the outcrossing mating system imposed by dioecism. Investigations using *in situ* hybridization techniques should confirm these findings.

The implications of these findings have bearing on developing long-term strategies for genetic resources and breeding programmes. Some authors already proposed that Melanesia is the area of diversification of *D. alata* (Bourret 1973; Martin and Rhodes 1977; Degras 1993; Lebot et al. 1998) and others also reported the vulnerability of the species because of genetic erosion (Lebot 1992) and anthracnose disease (Degras 1993). As our study reveals the heterogeneous composition of *D.*

alata in Vanuatu, a more comprehensive survey within Melanesian and southeast Asian islands needs to be carried out with AFLP to provide a better understanding of the genetic structure. Furthermore, such study will help in defining a proper strategy for conservation purposes in order to support future efforts in genetic improvement programmes. However, the use of powerful co-dominant markers such as microsatellites (single sequence repeats) is necessary in order to confirm our findings.

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