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ORIGINAL PAPER

Microsatellite segregation analysis and cytogenetic evidence for tetrasomic inheritance in the American yam *Dioscorea trifida* and a new basic chromosome number in the *Dioscoreae*

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Abstract Despite the economic and cultural importance of the indigenous ''Amerindian'' yam *Dioscorea trifida*, very little is known about their origin, phylogeny, diversity and genetics. Consequently, conventional breeding efforts for the selection of *D. trifida* genotypes resistant to potyviruses which are directly involved in the regression of this species have been seriously limited. Our objective of this paper is to contribute to the clarification of the cytogenetic status, i.e., inheritance and chromosome number. Our results provide genetic evidence supporting tetrasomic behaviour of the genome of *D. trifida* based on chromosomal segregation pattern analysis using eight SSRs markers in three different crosses. This is the first reliable evidence of an autopolyploid species in the genus *Dioscorea*. The second major result in this study is the revealing of a new base chromosome number in the botanical section *Macrogynodium* to which *D. trifida* belongs. To date, our assumptions about the ploidy level of yams are based on the observations that the basic chromosome number is 10 or 9, and *D. trifida* was described as octoploid. The chromosome number of *D. trifida* accessions was also assessed using somatic chromosomic count techniques. Flow cytometry did not show significant variation of 2C DNA content among 80 accessions indicating homogeneity of the ploidy level of the cultivated *D. trifida*. This suggests that autotetraploidy is well established as well as the rule for the cultivated pool of *D. trifida*, even if the direct diploid ancestor remains to be identified. The data presented in this paper are significant and important for the effective breeding and conservation of the species and for elucidating the phylogeny and the origins of the yam and the evolution of the genus *Dioscorea*.

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Introduction

Yams, genus *Dioscorea*, family *Dioscoreaceae*, are important crop plants for sustainable food production in tropical and subtropical regions (Burkill 1960; Coursey 1967; Ayensu and Coursey 1972). Food yams are believed to have originated in tropical areas on three separate continents: Africa (*Dioscorea cayenensis–D. rotundata*), Southeast Asia and the South Pacific (*D. alata*), and South America (*D. trifida*). *D. trifida* is the only cultivated edible yam out of the dozen species to which Knuth (1924) referred to in the *Macrogynodium* section, and is also the most important edible American *Dioscorea*.

Potyviruses (genus *Potyvirus*, family *Potiviridae*) are the main limiting factor for the production of *D. trifida*. Potyviruses cause significant economic damage and seriously impede the development of the yam as

a viable crop, and are directly involved in the regression of the indigenous ''Amerindian'' yam *D. trifida* in the Caribbean and French Guyana (South America) (Degras 1993; Bousalem et al. 2000, 2003). Despite the economic and geo-cultural importance of *D. trifida*, very little is known about their origin, phylogeny, diversity and genetics. Consequently, conventional breeding efforts for resistance to potyviruses have been seriously limited. Our objective in this paper is to contribute to the clarification of the cytogenetic status, i.e., inheritance and chromosome number.

Although the polyploidy status of cultivated yams has been widely recognised, the yam genetics is one of the least understood among the major staple food crops (Zoundjihekpon et al. 1994). The existence of various ploidy levels and the lack of a diploid relative to the cultivated polyploid yams lead to complicated yam studies.

Observations have been restricted in most cases to the determination of chromosome numbers and chromosome pairing from mitotic (Sharma and De 1956; Raghavan 1958, 1959; Ramachandran 1968; Essad 1984) and meiotic cells (Abraham and Nair 1990; Abraham 1998).

To date, the conclusion about the ploidy of yams is based on the basic chromosome number of 10 or 9. From a summary table established by Essad (1984) based on published studies on *Dioscorea* (on 72 species), the basic chromosome number of yam species is acknowledged as being x=10 or x=9, with a high frequency of polyploid species. Tetraploid species are the most frequent, followed by 2x, 6x and 8x forms in similar proportions. The base chromosome number x=10 is reported in all the Asian species, but is found in only 52% of the African species and 13% of the American species examined so far. The remaining African and American species are considered to have a basic number of x=9. Since *D. trifida* has 80 chromosomes (Essad 1984), it should be an octoploid species in the case of a basic number of x=10.

However, determining ploidy levels in yam by counting chromosomes is tedious and difficult since yam chromosomes are small, generally dot-like and most often clumped together, complicating the counting (Baquar 1980; Zoundjihekpon et al. 1990).

Recent data has revealed two new basic chromosome numbers: x=6 (Segarra-Moragues and Catalán 2003; Segarra-Moragues et al. 2004) and x=20 (Scarcelli et al. 2005). If confirmed on a larger number of yam species, these results should lead us to reconsider the basic number of yams on a more general level and, as a consequence, to decrease the level of ploidy of at least some species.

More studies are necessary to identify the inheritance patterns of the polyploid yams. If multivalent formation is indeed more common in autotetraploid than in allotetraploid species, however, counting multivalents is not a completely reliable method since autotetraploid species do not always exhibit multivalent formation (Soltis and Riesberg 1986) and allotetraploid species have been shown to form multivalents at times (Watson et al. 1991). Therefore, analyses of segregating populations have been extensively used to assess inheritance patterns (Demarly 1958; Jackson and Casey 1982). Recently, segregation analysis using isozyme and microsatellites markers led to the conclusion that *D. rotundata* is a diploid species (Scarcelli et al. 2005). In segregating populations of water yam (*Dioscorea alata*) and Guinea yam (*Dioscorea rotundata* Poir.) ($2n = 4x = 40$), the observed segregation of AFLP markers reflected a disomic inheritance (Mignouna et al. 2002a, b). These results revealed an allotetraploid structure for *D. rotundata* and *D. alata*. More recently, Segarra-Moragues et al. (2004) concluded on the basis of microsatellites patterns that the two *D. pyrenaica* and *D. chouardii* endemic to the Pyrenees (Spain and France) are also allotetraploid.

Since they are highly polymorphic, simple-sequence repeat (SSR) markers, also referred to as microsatellites, are considered as valuable tools for determining meiotic behaviour (Qu and Hancock 1995). We recently developed SSR markers in *D. trifida* and assessed their polymorphism in selected *D. trifida* cultivars (Hochu et al. 2006). The portability of other microsatellite markers developed in distant yam species (Terauchi and Konuma 1994; Scarcelli et al. 2005) on *D. trifida* was low but some markers proved to be valuable. Individual patterns generally showed a maximum of four alleles, strongly suggesting that *D. trifida* is a tetraploid species (Hochu et al. 2006).

The aim of the present paper was to use SSR molecular markers to evaluate the inheritance patterns in *D. trifida* by examining parental and progeny genotypes from controlled crosses.

To ascertain the discussion of segregation data on the basic number of chromosome we assessed the chromosome number of *D. trifida* using somatic chromosomic count techniques. We also verified the uniformity of the ploidy level of the cultivated *D. trifida* using flow cytometry. The implications of our results for *D. trifida* and for the phylogeny and evolution of *Dioscorea* are discussed.

Materials and methods

Plant materials

Segregation analysis

Three progenies derived from crossing the male parent INRA 5-20 with three distinct female clones (Mh2, Mh22, Ah6) were analysed. All of the four genotypes used were breeding lines originating from a *D. trifida* selection programme at INRA Guadeloupe. Female plants were grown in isolated plots in a separate field and surrounded by INRA 5-20. Seed production varied from one population/plot to the next. Seeds were germinated in a growth chamber and transplanted in vitro. The three populations finally totalled 14 (INRA 5-20 × Ah6), 28 (INRA 5-20 × mh2) and 53 individuals (INRA 5-20 × Mh22), respectively.

DNA ploidy screening and chromosome counting

Eighty accessions of cultivated *D. trifida* were selected from in vitro germplasm collections (material now available at INRA Guadeloupe). Accessions (Table 1) were collected in the traditional areas of yam cropping and agro-ecosystems of French Guyana: in the traditional cropping areas along the Pacific Coast and the Maroni River, as well as in the indigenous ancestral cropping areas in the rain forest.

Mitotic chromosome counts were carried out on root tips of eight representative accessions selected from among the 80 samples tested using flow cytometry (Table 1).

Methods

DNA extraction

DNA was extracted from 100 mg of leaves taken from in vitro plants using the DNeasy Plant Mini Kit (Qiagen) with the following modification: 1% of polyvinylpyrrolidone (PVP 40 000) was added to the buffer AP1.

Microsatellite amplification

We used eight SSRs markers (Table 2). Six were specifically developed from the cultivated *D. trifida* (Hochu et al. 2006) and the two additional markers were selected from a set of 30 SSRs markers developed from the cultivated yams *D. alata*, *D. cayenensis–D. rotundata*, and the wild African yam *D. abyssinica* (Tostain et al. 2006). Amplification and visualisation conditions were as described by Hochu et al. (2006).

Microsatellite segregation analysis

Two types of tetrasomic segregation are often defined, referred to as chromosomal and chromatid segregation (Bever and Felber 1992). Chromosomal segregation refers to the case when the marker is sufficiently close to the centromere and crossovers between the centromere and the marker are rare, whereas chromatid segregation refers to the condition where the marker is far from the centromere and crossovers between the centromere and the marker are common. Under the chromosome model, segregation ratios are calculated as the possibility of drawing any pair from a set of four chromosomes, whereas under the chromatid model with free recombination between the marker and the centromere, segregation ratios can be calculated as a random drawing from the eight possible chromatids. Under the chromatid model, it is possible for two markers from sister chromatids to migrate into the same gamete [referred to as a double reduction, see Bever and Felber (1992) for a complete explanation]. In this study, we were primarily interested in whether segregation is tetrasomic or disomic; therefore, only chromosome segregation was considered. Since sample sizes were too small in most of the crosses in this study to reliably detect double reduction events, we will only report the genotypes we suspected to be the products of double reduction (see Table 3). Moreover, the chromosomal segregation method is considered to provide robust results in most cases (Olson 1997).

According to the chromatograms, parents of the segregating populations could be of different genotypes when they had less than four alleles. For example, we considered that genitors showing only two bands (ab) could be aabb (duplex) or aaab or abbb (one simplex + one triplex). We also considered that null alleles could be present in some cases. Therefore, a phenotype (ab) could be aab0, abb0 or ab00. We nonetheless used the ratio of the different allelic peaks on the chromatograms to remove unlikely genotypes for a given phenotype, e.g., if a parent showed three alleles (abc) on the chromatogram and if the relative area of one allele was close to 15% while the two others were higher than 30% each of the total peak area, we assumed that this allele had very little chance of being duplex.

All Population × Locus combinations in which one allele was simplex in one parent (for example, when this parent carried four different alleles) were analysed

Table 1 Accessions, origin, chromosome number, fluorescence ratio and ploidy level of the cultivated *Dioscorea trifida* from French Guyana

Accessions/ Source[a]	Origin		Chromosome number	2C DNA content (pg)[c]
	Location	Ethnic group[b]		
Along the Oyapock River (frontier with Brazil)				
St-G 1	Saint-George	Palikur		2.15
St-G 4	Saint-George	Palikur	80	**2.72**
St-G 6	Saint-George	Palikur	80	**2.80**
St-G 55	Saint-George	Palikur		**2.82**
Atlantic Ocean coast				
Cay 1	Cayenne	Mixed		**2.61**
Cay 2	Cayenne	Mixed		2.55
Cay 5	Cayenne	Mixed		2.64
Cay 6	Cayenne	Mixed		2.65
Cay 7	Cayenne	Mixed		2.62
Cay 9	Cayenne	Mixed		2.58
Cay 11	Cayenne	Mixed		2.68
Cay 12	Cayenne	Mixed		**2.80**
Cay 13	Cayenne	Mixed		2.58
Cay 16	Cayenne	Mixed		**2.63**
Cay 19	Cayenne	Mixed		2.65
Cay 22	Cayenne	Mixed		**2.66**
Cay 23	Cayenne	Mixed		2.47
Cay 26	Cayenne	Mixed		2.74
Régina 2	Régina	Mixed		**2.53**
Régina 3	Régina	Mixed		2.69
Ms 27	Montsinery	Mixed		2.76
Ms 28	Montsinery	Mixed		**2.62**
Ms 29	Montsinery	Mixed	80	2.80
Macouria 9	Macouria	Palikur	80	**2.69**
Sina 6	Sinnamary	Creole		**2.90**
Sinu 6	Sinnamary	Creole		**2.95**
Irakoubou 5	Irakoubou	Creole		**2.77**
Charvein 2	Charvein	Creole		2.64
Charvein 3	Charvein	Creole		2.60
Charvein 7	Charvein	Creole		2.55
Charvein 8	Charvein	Creole	80	2.71
Charvein 10	Charvein	Creole		2.69
Charvein 11	Charvein	Creole		**2.69**
Charvein 16	Charvein	Creole		**2.64**
Charvein 21	Charvein	Creole		**2.68**
Cacao 2	Cacao	H'Mong		2.85
Cacao 11	Cacao	H'Mong		2.54
Along the Maronie river (frontier with Surinam)				
Msl 1	Saint-Laurent	Kali'na, Arawak		**2.75**
Ms1 2	Saint-Laurent	Kali'na, Arawak		2.60
Apatou 3	Apatou	Paramaka		**2.90**
Papa 3	Papaïchton	Aluku		**2.74**
Papa 5	Papaïchton	Aluku	80	**2.86**
Papa 7	Papaïchton	Aluku	80	2.69
Papa 8	Papaïchton	Aluku	80	2.86
Papa 9	Papaïchton	Aluku		2.70
Papa 15	Papaïchton	Aluku		2.75
MPA 1	Maripassoula	Aluku		2.93
MPA 2	Maripassoula	Aluku		2.66
MPA 7	Maripassoula	Aluku		2.52
MPA 8	Maripassoula	Aluku		2.93
MPA 10	Maripassoula	Aluku		**2.84**
MPA 13	Maripassoula	Aluku		2.94
MPA 17	Maripassoula	Aluku		**2.77**
MPA 21	Maripassoula	Aluku		2.72

Table 1 continued

Accessions/ Source[a]	Origin		Chromosome number	2C DNA content (pg)[c]
	Location	Ethnic group[b]		
MPA 22	Maripassoula	Aluku		2.64
MPA 23	Maripassoula	Aluku		2.80
Elahé 1	Elahé	Wayana		2.71
Elahé 2	Elahé	Wayana		2.85
Elahé 5	Elahé	Wayana		2.97
Geographic centre of French Guyana, lowland moist forest				
Saul 1	Saül	Creole		2.73
Saul 2	Saül	Creole		2.71
Saul 4	Saül	Creole		2.68
Saul 11	Saül	Creole		2.84
Saul 21	Saül	Creole	80	**2.78**
Saul 23	Saül	Creole	80	2.67
Saul 24	Saül	Creole		2.65
Saul 25	Saül	Creole		2.83
Saul 26	Saül	Creole		2.87
Saul 27	Saül	Creole	80	2.80
Saul 28	Saül	Creole	80	2.58
Saul 30	Saül	Creole		**2.68**
Saul 31	Saül	Creole		2.71
Saul 36	Saül	Creole		2.80
SaulMong 7	Saül	H'Mong		2.61
SaulMong 17	Saül	H'Mong		2.80
SaulMong 20	Saül	H'Mong		2.83
SaulMong 21	Saül	H'Mong		**2.72**
SaulMong 22	Saül	H'Mong		2.64
SaulMong 23	Saül	H'Mong		2.73
Hybrid creates by INRA, used as reference in genetic study				
INRA 5 20	INRA	–		**2.79**

[a]Collection of the samples was performed by Mustapha Bousalem and Richard Arnolin (Institut National de la recherche Agrnomique (INRA-CRAG-URPV, Guadeloupe) and Gina Boulardin (Chambre d'Agriculture de Guyane)

[b]Amerindian populations: Kali'na, arawak, palikur; Noirs Marrons (Businenge) population: Aluku, Paramaka; Mixed population: Creole, Emigrants and Europeans

[c]When at least two replicated flow cytometry measurements were performed, DNA 2C (pg) reported (in bold) refer to the average of the obtained value

for evidence of double reduction by searching progeny inheriting only this simplex allele from this parent. In this case, it is reasonable to assume that the offspring carried two doses of the allele. For instance, in an abcd × efgh cross, double reduction is detectable by the presence of the afg phenotype in the progeny corresponding to the aafg genotype.

Statistical analysis

We adopted a Bayesian procedure to discriminate among the different inheritance hypotheses. It actually better fits the requirement of testing complex segregation patterns than the χ^2 statistics (Olson 1997). Moreover, in some cases, several possible parental heterozygous genotypes led to building several expected genotypic ratios in the progeny. The likelihood of the observed data L under different parental genotypic combinations is thus the probability of observing the real distribution of the offspring in the different genotypic classes under the three inheritance patterns we wanted to test–disomy (alloteraploidy), tetrasomy (autotetraploidy) or octosomy (autooctoploidy)–and this according to all the combinations of possible parental genotypes. A scenario $S(f,m,\mathrm{X})$ is built on a combination of the possible parental genotypes (f: father and m: mother) and an inheritance pattern (X). Under the scenario $S(f,m,\mathrm{X})$, we designated $p_{i,f,m,\mathrm{X}}$ as the expected frequency of the genotypic class i. The probability of observing the data consequently follows a multinomial distribution (Olson 1997) and the likelihood of the data under $S(f,m,\mathrm{X})$, $L(S(f,m,h))$ was computed as follows:

$$L(\mathrm{f},\mathrm{m},\mathrm{X}) = \frac{n!}{n_1!n_i!\ldots n_k!}\prod_{i=1}^{k} p_{i,f,m,\mathrm{X}}{}^{n_i}$$

Table 2 Characteristic of the microsatellites loci used in this study

Locus	Repeat motif	Species[a]	Primer sequences (5′–3′)[b]	TA (°C)[c]
MTI 2	$[CAA]_6$	*D. trifida*	TCATCAAGAGCATCAAAAAAC (F) GCCTCGTCTTTGAAGTTGGT (R)	50
MTI 3	$[TAA]_7$	*D. trifida*	ATAACAAACAAAAAATGAAAC (F) TAACAGTGATTGAGCTAGGA (R)	55
MTI 4	$[GTT]_8$	*D. trifida*	ACTTGGTGTTGTTGGATTGC (F) TATCACTCCCCAGACCAGA (R)	50
MTI 10	$[TC]_{17}$	*D. trifida*	TCGTGTCCATCTTGCTGCGT (F) GAAAAGCGGAGATGAAGAGCA (R)	55
MTI 11	$[CAA]_7$	*D. trifida*	CTCTTTTGCTTCTCATTTCA (F) ATGTAGCCAATCCAAAATAG (R)	55
MTI 12	$[CTT]_8$	*D. trifida*	CTGCCAGCGTTCCGATTC (F) CGTAGGACCTCTCGCATCAG (R)	55
Dab2C05	$[GA]_{19}$	*D. abyssinica*	CCCATGCTTGTAGTTGT (F) TGCTCACCTCTTTACTTG (R)	50
Dab2D07	$[TA]_{10}$	*D. abyssinica*	AACATATCTGGGTCAAGG (F) CAAGTTTTGGAAGCAAG (R)	50

[a]Microsatellites were isolated from *D. trifida* (Hochu et al. 2006) and *D. abyssinica* (Tostain et al. 2006)

[b]F, forward primer; R, reverse primer

[c]Optimum annealing temperature

where n is the total number of progeny and n_i is the observed number of progeny in the genotypic class i.

To test autotetraploidy (tetrasomy 4X) versus allotetraploidy (disomy 2X), we derived a Bayes factor that was slightly modified from Olson (1997) as the ratio of the respective sum of all the probabilities for the two inheritance patterns:

$$\begin{aligned} BF &= \Pr(\text{data}|\text{tetrasomy})/\Pr(\text{data}|\text{disomy}) \\ &= \frac{\sum \Pr(\text{data}|\text{Scenario}\, j, 4\text{X})}{\sum \Pr(\text{data}|S(\text{Scenario}\, i, 2\text{X})} \\ &= \frac{\sum_i \sum_j L(fi, mj, 4\text{X})}{\sum_i \sum_j L(fi, mj, 2\text{X})} \end{aligned}$$

where i and j are, respectively, the i^{th} (resp. the j^{th}) possible genotype for the female (resp. the male).

In the case of the disomic inheritance pattern (2X), we first considered that the two independently-segregating loci could present alleles of the same sizes (homoplasy). For example, a parent can be a/b at the locus in one genome and a/c in the second genome. This assumption is very conservative for testing inheritance patterns but leads to a lower discrimination between 2X and 4X patterns. We then considered that alleles of the same size could not be shared between the two loci under the 2X scenario. We computed the Bayes factor accordingly.

To take the observed but unexpected genotypes under a given scenario into account, we considered that these individuals were erroneously observed and placed them in a unique genotypic class that was assigned a class probability designated as P_{error}. We arbitrarily tested three values of P_{error}, i.e., 10^{-2}, 5×10^{-3} and 10^{-3}. This made it possible to weigh the importance of the observed genotypes that were not expected under a given scenario. The lower the P_{error} value, the less likely the scenario that does not produce theoretically some observed genotypes. The Bayesian factor was computed for the three error values to test the robustness of the model fit.

Chromosome preparations

Only mitotic chromosome counts were made. Roots were harvested from plants cultivated in pots. They were treated with 0.04% 8-hydroxyquinoline for 8 h, fixed for 48 h in 3:1 ethanol:acetic acid and stored in 70% ethanol at 4°C. The fixed roots were rinsed twice in H_2O for 10 min each, treated in 0.25 N HCl for 15 min, rinsed for 10 min in H_2O and placed in a digestion buffer (0.01 M citrate buffer pH 4.5, 0.075 M KCl) for 5 min. The root tips were cut and placed in an enzyme solution (5% Onozuka R-10 cellulase, 1% Y-23 pectolyase in a digestion buffer) in a microtube at 37°C for 30–180 min (the time varying with the size of the roots). Root tips were then rinsed in H_2O and spread on a slide with a drop of 3:1 ethanol:acetic acid. Slides were stored at –70°C. The chromosomes were counterstained with DAPI (4′,6-diamidino-2-phenylindole). The slides were mounted in vectashield antifade solution (Vector Lab) observed under a OLYMPUS

Table 3 Distribution of expected and observed genotypes in the progeny INRA5–20 × Mh22 at the MTI4 locus under tetrasomic (4X) or disomic inheritance (2X). Two different assumptions are tested for INRA 5-20 genotype assuming that Mh22 is known without error: (1) cdde; (2) ccde. Logarithms of likelihood for all the possible segregation patterns are given and the Bayes Factor is computed using two hypothesis as indicated in the text. Three error probabilities have been considered for the unexpected genotypes 0.01, 0.005 and 0.001. For convenience, log likelihood are given

Parental genotype	Progeny genotype																													Log (L) probability		
	df	def	cdf	cdef	bd	bdf	bdef	bde	bcf	bcef	bce	bcdf	bcde	bcd	adef	ade	ad	adf	acf	acef	acdf	acde	acd	abe	abde	abd	abc	abce	abcd	0,01	0,005	0,001
observed genotypes																																
		2		3			1	5		1	1	3	1	5	6	5	1			2	1	1	2	1	7	1		2	2			
Distribution of expected progeny for the parental genotypes: cdde x abdf under tetrasomic inheritance (4x, 1/36)																																
cdde X abdf	1	2	2	1	1	1	2	2		1		2	1	2	2	2	1	1		1	2	1	2		2	1		1	2	-19	-19	-21
Distribution of expected progenies for the parental genotypes: cdde x abdf under disomic inheritance (2x, 1/16)																																
cd/de x ab/df					1	1	1	1		1		1	1	1	1	1	1	1		1	1	1	1							-37	-43	-56
cd/de x ad/bf	1	1	1	1	1			1					1	1	1			1		1	1				1	1		1	1	-33	-38	-49
cd/de x af/bd	1	1	1	1		1	1			1		1				1	1					1	1		1	1		1	1	-42	-48	-64
cd/de x bd/af	1	1	1	1		1	1			1		1				1	1					1	1		1	1		1	1	-42	-48	-64
cd/de x bf/ad	1	1	1	1	1								1	1	1			1		1	1				1	1		1	1	-33	-38	-49
cd/de x df/ab					1	1	1	1		1		1	1	1	1	1	1	1		1	1	1	1							-37	-43	-56
ce/dd x ab/df *							2	2				2		2	2	2					2		2							-42	-49	-67
ce/dd x ad/bf *		2	2					2						2	2						2				2				2	-43	-50	-68
ce/dd x af/bd *		2	2				2					2				2							2		2				2	-54	-63	-85
ce/dd x bd/af *		2	2				2					2				2							2		2				2	-43	-63	-85
ce/dd x bf/ad *		2	2					2						2	2						2				2				2	-42	-50	-68
ce/dd x df/ab *							2	2				2		2	2	2					2		2							-42	-49	-67
dd/ce x ab/df *							2	2				2		2	2	2					2		2							-42	-49	-67
dd/ce x ad/bf *		2	2					2						2	2						2				2				2	-43	-50	-68
dd/ce x af/bd *		2	2				2					2				2							2		2				2	-54	-63	-85
dd/ce x bd/af *		2	2				2					2				2							2		2				2	-54	-63	-85
dd/ce x bf/ad *		2	2					2						2	2						2				2				2	-43	-50	-68
dd/ce x df/ab *							2	2				2		2	2	2					2		2							-42	-49	-67
de/cd x ab/df					1	1	1	1		1		1	1	1	1	1	1	1		1	1	1	1							-37	-43	-56
de/cd x ad/bf	1	1	1	1	1			1					1	1	1			1		1	1				1	1		1	1	-33	-38	-49
de/cd x af/bd	1	1	1	1		1	1			1		1				1	1					1	1		1	1		1	1	-42	-48	-64
de/cd x bd/af	1	1	1	1		1	1			1		1				1	1					1	1		1	1		1	1	-42	-48	-64
de/cd x bf/ad	1	1	1	1	1			1					1	1	1			1		1	1				1	1		1	1	-33	-38	-49
de/cd x df/ab					1	1	1	1		1		1	1	1	1	1	1	1		1	1	1	1							-37	-43	-56
Distribution of expected progeny for the parental genotypes: ccde x abdf under tetrasomic inheritance (4x, 1/36)																																
ccde x abdf		1	3	2			1	1	1	2		2	2	3	1	1			1	2	2	2	3		1		1	2	2	-24	-25	-28
Distribution of expected progeny for the parental genotypes: ccde x abdf under disomic inheritance (2x, 1/16)																																
cc/de x ab/df *										2		2	2	2						2	2	2	2							-66	-77	-103
cc/de x ad/bf *			2	2									2	2						2	2							2	2	-66	-77	-103
cc/de x af/bd *			2	2						2		2										2	2					2	2	-70	-82	-109
cc/de x bd/af *			2	2						2		2										2	2					2	2	-70	-82	-109
cc/de x bf/ad *			2	2									2	2						2	2							2	2	-66	-77	-103
cc/de x df/ab *										2		2	2	2						2	2	2	2							-66	-77	-103
cd/ce x ab/df							1	1	1	1		1	1	2	1	1			1	1	1	1	2							-37	-43	-57
cd/ce x ad/bf		1	2	1				1					1	2	1				1	1	1				1		1	1	1	-34	-39	-51
cd/ce x af/bd		1	2	1			1		1	1		1				1						1	2		1		1	1	1	-46	-53	-70
cd/ce x bd/af		1	2	1			1		1	1		1				1						1	2		1		1	1	1	-46	-53	-70
cd/ce x bf/ad		1	2	1				1					1	2	1				1	1	1				1		1	1	1	-34	-39	-51
cd/ce x df/ab							1	1	1	1		1	1	2	1	1			1	1	1	1	2							-37	-43	-57
ce/cd x ab/df							1	1	1	1		1	1	2	1	1			1	1	1	1	2							-37	-43	-57
ce/cd x ad/bf		1	2	1				1					1	2	1				1	1	1				1		1	1	1	-34	-39	-51
ce/cd x af/bd		1	2	1			1		1	1		1				1						1	2		1		1	1	1	-46	-53	-70
ce/cd x bd/af		1	2	1			1		1	1		1				1						1	2		1		1	1	1	-46	-53	-70
ce/cd x bf/ad		1	2	1				1					1	2	1				1	1	1				1		1	1	1	-34	-39	-51
ce/cd x df/ab							1	1	1	1		1	1	2	1	1			1	1	1	1	2							-37	-43	-57
de/cc x ab/df *										2		2	2	2						2	2	2	2							-66	-77	-103
de/cc x ad/bf *			2	2									2	2						2	2							2	2	-66	-77	-103
de/cc x af/bd *			2	2					2	2												2	2					2	2	-70	-82	-109
de/cc x bd/af *			2	2						2		2										2	2					2	2	-70	-82	-109
de/ccx bf/ad *			2	2									2	2						2	2							2	2	-66	-77	-103
de/cc x df/ab *										2		2	2	2						2	2	2	2							-66	-77	-103
Bayes factor hypothesis A : analysis integrating all the possibilities																														6 10^{13}	9 10^{17}	6 10^{27}
Bayes factor hypothesis B : analysis excluding the possibilities to have the same allele in two differents locus. The considered alleles are marqued by an *																														10 ^{22}	6 10^{28}	7 10^{44}

DP50 microscope and photographed with analySIS (R) de Soft-Imaging-System software.

DNA ploidy screening by flow cytometry

Plant preparation

Approximately 0.5 cm^2 of individual vitroplant leaves were chopped up with the same amount of young leaves of a *Citrus madurensis* tree (used as an internal standard) in 500 µl of ice-cold LB01 buffer (Dolezel et al. 1989) with a double-edged razor blade. The obtained nuclei suspension was filtered through 50µm pore sized nylon Scrynel filter just prior to being stained with 80 µl of propidium iodide aqueous solution (1 mg/1 ml) and being measured.

Flow cytometric analysis

Relative fluorescence intensity of stained nuclei was analysed using a flow cytometer FACScan with Cellquest Software, Becton Dickinson (Mountain View, CA, USA) located at INSERM U291, Montpellier, France. Fluorescence intensity histograms obtained from the analysis showed two main peaks whose positions on the fluorescent intensity channels are proportional to their 2C DNA amount (DNA ploidy). Since our internal standard DNA ploidy is known and equal to 0.76 pg (Patrick Ollitrault, personal communication), we were able to convert the intensity peak ratio into sample 2C DNA content: Sample 2C DNA = (sample intensity/standard intensity) × 0.76 pg.

Results

Segregation analysis

Population screening for selfing and contaminating genotypes

We first checked to see if some individuals could have a different male parent than INRA 5-20 or could result from selfing. Five individuals from the INRA 5-20 X Mh2 cross were identified as contaminating genotypes, with alleles not detected in the two parents. Finally, eight polymorphic loci could be reliably scored and the three segregating populations totalled 53, 23 and 14 individuals, respectively. To exemplify the computation of the Bayes factor, we first illustrated (Table 3) the results for the Mti4 on the INRA 5-20 × Mh22 population. INRA5-20 has three alleles, 107, 113 and 115 bp (phenotype cde), and the female Mh22 has four alleles, 101, 104, 113 and 118 pb (phenotype abdf).

Octosomic inheritance likelihood

If we observe only 3–4 alleles in the two parents, this would mean that all or part of the alleles have more than one copy in the case of octoploidy. Therefore, some frequent gametes would carry all the alleles observed in the two parents and, by mating, would produce genotypes with all of the six observed alleles, i.e., 101/104/107/113/115/118 (abcdef). However, no genotype carried more than four alleles in the progeny and this makes octosomy very unlikely at this locus. Since this pattern has never been observed in any of the segregating populations for any locus under study, we will not further consider octosomic inheritance as a possible pattern.

Tetrasomic versus disomic inheritance

Since INRA5-20 has only three alleles, it could be either duplex for one of its alleles. From the peaks observed on the chromatogram, the most likely possibility is that INRA5-20 could be duplex for the 113 pb allele (42% of the cumulated peak area) or for the 107 bp allele (35%), while there is surely only one copy of the 115 bp allele. This leads to the following genotypes for INRA 5-20: (1) cdde or (2) ccde. This yields two scenarios for the 4X inheritance and 24 scenarios for each of the INRA 5-20 possible genotypes, i.e., 48 scenarios under the 2X inheritance pattern. Results are given in Table 3. Bayes factor testing 4X versus 2X inheritance clearly indicates that the tetrasomic inheritance is much more likely than the disomic pattern. With the more conservative of the three values for P_{error} (P_{error}=0.01), the tetrasomic inheritance is 10^{13} times more likely than the 2X inheritance for the Mti4 locus. Comparing the likelihoods of the two possible INRA5-20 genotypes for the 4X inheritance, the 'cdde' genotype appeared more likely than the 'ccde' one.

The Bayes factors under the different assumptions and the three segregating populations are given in Table 4. In the large population (INRA 5-20 × Mh22) one locus is unequivocally under tetrasomic inheritance. For the other loci, no statistical difference clearly appeared between the likelihood of 4X and that of 2X. In some cases, the 2X inheritance pattern appeared slightly more likely than the 4X scenario, e.g., Mti2 Bayes factor = 0.02. The value of P_{error} was of little impact compared to the effect of considering only the scenario of 2X inheritance for which the two loci did not share common allelic sizes. In these cases, the

Table 4 Bayes factors testing the likelihood of a 4X versus a 2X inheritance pattern in three segregating populations of *Dioscorea trifida* on eight microsatellites locus (see text)

Population	Locus	Total number of different allele	Probability of observing unexpected genotypes (P_{error})					
			0.01		0.005		0.001	
			a	b	a	b	a	b
INRA 5-20 × MH22 (N = 53)	**Mti4**	6	$\mathbf{6 \times 10^{13}}$	$\mathbf{1 \times 10^{22}}$	$\mathbf{9 \times 10^{17}}$	$\mathbf{6 \times 10^{28}}$	$\mathbf{6 \times 10^{27}}$	$\mathbf{7 \times 10^{44}}$
	Mti3	5	$\mathbf{1 \times 10^{2}}$	$\mathbf{2 \times 10^{24}}$	$\mathbf{1 \times 10^{2}}$	$\mathbf{2 \times 10^{24}}$	$\mathbf{1 \times 10^{2}}$	$\mathbf{2 \times 10^{24}}$
	Mti12	4	$\mathbf{2 \times 10^{0}}$	$\mathbf{4 \times 10^{28}}$	$\mathbf{5 \times 10^{0}}$	$\mathbf{1 \times 10^{36}}$	$\mathbf{5 \times 10^{0}}$	$\mathbf{3 \times 10^{53}}$
	Dab2C05	5	2×10^{-2}	$\mathbf{5 \times 10^{8}}$	1×10^{-1}	$\mathbf{4 \times 10^{12}}$	$\mathbf{2 \times 10^{1}}$	$\mathbf{5 \times 10^{21}}$
	Mti10	3	$\mathbf{3 \times 10^{0}}$	$\mathbf{1 \times 10^{8}}$	$\mathbf{3 \times 10^{0}}$	$\mathbf{2 \times 10^{23}}$	$\mathbf{3 \times 10^{0}}$	$\mathbf{1 \times 10^{33}}$
	Mti11	3	1×10^{-1}	$\mathbf{4 \times 10^{46}}$	1×10^{-1}	$\mathbf{5 \times 10^{57}}$	1×10^{-1}	$\mathbf{4 \times 10^{83}}$
	Dab2D07	3	1×10^{-1}	$\mathbf{3 \times 10^{7}}$	1×10^{-1}	$\mathbf{3 \times 10^{7}}$	1×10^{-1}	$\mathbf{3 \times 10^{7}}$
	Mti2	3	2×10^{-2}	$\mathbf{4 \times 10^{2}}$	2×10^{-2}	$\mathbf{1 \times 10^{4}}$	2×10^{-2}	$\mathbf{4 \times 10^{7}}$
INRA 5-20 × MH2 (N = 23)	Mti4	4	3×10^{-2}	$\mathbf{8 \times 10^{14}}$	1×10^{-1}	$\mathbf{7 \times 10^{18}}$	1.5×10^{-1}	$\mathbf{8 \times 10^{27}}$
	Mti3	6	$\mathbf{1 \times 10^{0}}$	$\mathbf{5 \times 10^{8}}$	$\mathbf{2 \times 10^{1}}$	$\mathbf{1 \times 10^{12}}$	$\mathbf{1.3 \times 10^{4}}$	$\mathbf{5 \times 10^{19}}$
	Mti12	4	5×10^{-2}	$\mathbf{6 \times 10^{13}}$	5×10^{-2}	$\mathbf{2 \times 10^{18}}$	5.0×10^{-2}	$\mathbf{6 \times 10^{28}}$
	Dab2C05	4	5×10^{-3}	$\mathbf{6 \times 10^{11}}$	5×10^{-3}	$\mathbf{1 \times 10^{14}}$	5.0×10^{-3}	$\mathbf{6 \times 10^{22}}$
	Mti10	4	1×10^{-1}	$\mathbf{8 \times 10^{7}}$	1×10^{-1}	$\mathbf{2 \times 10^{10}}$	1.4×10^{-1}	$\mathbf{8 \times 10^{15}}$
	Mti11	2	1×10^{-1}	$\mathbf{1 \times 10^{16}}$	1×10^{-1}	$\mathbf{5 \times 10^{19}}$	1.0×10^{-1}	$\mathbf{1 \times 10^{28}}$
	Dab2D07	3	9×10^{-2}	$\mathbf{2 \times 10^{0}}$	9×10^{-2}	$\mathbf{8 \times 10^{0}}$	9.0×10^{-2}	$\mathbf{2 \times 10^{2}}$
	Mti2	3	3×10^{-2}	3×10^{-2}	3×10^{-2}	3×10^{-2}	3.0×10^{-2}	3×10^{-2}
INRA 5-20 × AH6 (N = 14)	Mti4	5	1×10^{-2}	1×10^{-2}	1×10^{-2}	1×10^{-2}	1×10^{-2}	1×10^{-2}
	Mti3	6	$\mathbf{8 \times 10^{0}}$	$\mathbf{1 \times 10^{4}}$	$\mathbf{3 \times 10^{0}}$	$\mathbf{8 \times 10^{5}}$	$\mathbf{3 \times 10^{0}}$	$\mathbf{1 \times 10^{10}}$
	Mti12	4	3×10^{-3}	3×10^{-3}	6×10^{-2}	6×10^{-2}	3×10^{-2}	3×10^{-2}
	Dab2C05	5	$\mathbf{1 \times 10^{0}}$	$\mathbf{1 \times 10^{0}}$	$\mathbf{1 \times 10^{1}}$	$\mathbf{1 \times 10^{1}}$	$\mathbf{1 \times 10^{3}}$	$\mathbf{1 \times 10^{3}}$
	Mti10	4	3×10^{-2}	$\mathbf{2 \times 10^{1}}$	6×10^{-2}	$\mathbf{1 \times 10^{2}}$	3×10^{-1}	$\mathbf{2 \times 10^{4}}$
	Mti11	2	1×10^{-1}	$\mathbf{6 \times 10^{4}}$	1×10^{-1}	$\mathbf{2 \times 10^{6}}$	1×10^{-1}	$\mathbf{6 \times 10^{9}}$
	Dab2D07	3	2×10^{-2}	$\mathbf{5 \times 10^{2}}$	2×10^{-2}	$\mathbf{8 \times 10^{3}}$	2×10^{-2}	$\mathbf{5 \times 10^{6}}$
	Mti2	3	2×10^{-3}	$\mathbf{2 \times 10^{2}}$	2×10^{-3}	$\mathbf{3 \times 10^{4}}$	2×10^{-3}	$\mathbf{2 \times 10^{9}}$

Bold values indicate that 4X is more likely than 2X, in light otherwise

a = Similar alleles can be shared by the two locus under the 2X scenario

b = Alleles have to be different at the different locus under the 2X scenario

4X inheritance appeared more likely in all the cases for this INRA 5-20 × Mh22 population. The same effect was observed in the INRA 5-20 × Mh2 population (N=23), with a significant effect of giving more weight to unexpected genotypes by decreasing P_{error}. Under the most restricting conditions (non-shared allelic size and P_{error}=10^{-3}), the likelihood of the 4X inheritance was much higher than that of the 2X inheritance for all loci but one. In the small population (INRA 5-20 × AH6), the same trend was confirmed except for two loci, even if the Bayes factors were lower (10^{4}–10^{9}) compared to the other populations.

It should be observed that in the case for which 2X appeared more likely than 4X (Bayes factor <1), the Bayes factor was not lower than 5×10^{-3}, which appeared as a slight difference compared to the maximum for the reciprocal situation (3.9×10^{83}). Furthermore, the number of individuals that were unexpected under the scenario was much higher for the 2X scenario than for the 4X one. This firstly suggests that the 4X inheritance is on average much more likely than the 2X inheritance and secondly that 2X inheritance could be confused with 4X inheritance when the number of alleles segregating in the population is too low.

However, some of the genotypes observed under the 4X pattern were not expected. For example, bce and abe individuals were observed at the Mti4 locus in the INRA5-20 × Mh22 progeny (Table 3). These individuals could have arisen through double reduction during the female meiosis (genotype bce) and in the male (genotype abe), assuming that the 'e' allele is indeed in one copy in INRA5-20. Double reduction was also suspected in two other loci: Mti3 in the 5-20 × Mh22 population and Mti10 in the 5-20 × Mh2 population. The occurrence of null alleles was suspected at the Mti2, Mti4, Mti10 and Dab2DO7 loci.

Ploidy levels among *D. trifida* cultivars as inferred by flow cytometry and chromosome counts

All of the 80 genotypes analysed in this study had 2C DNA amounts that varied from 2.47 to 2.93 (Table 1). Eighty chromosomes were counted in chromosome

preparations of the eight representative accessions selected among the 80 samples tested by flow cytometry (Table 1, Fig. 1). Based on these two results, we could assume that all cultivars had the same ploidy level. Difficulties in yam chromosome counts and observed chromosome counts were in agreement with those previously reported for *D. trifida* (Essad 1984). This lack of precision is due to the difficulty in precisely assessing such a large number of small chromosomes.

Discussion

The Bayesian approach: experimental aspect, statistical and genetic significance

The Bayesian approach made it possible for us to simultaneously consider all the possible parental genotypes and to compute a likelihood ratio that also took the frequencies of unexpected genotypes in the sample into account, even when the sample was small (as low as n=14). This type of an approach is not easy using Chi-square (Olson 1997). The possibility of differentiating between the 4X and 2X inheritances clearly depended on the number of alleles segregating in the populations and the size of the segregation populations. When the two loci were allowed to share common allelic size under the 2X scenarios, more genotypic classes were possible and the observed genotypes had more of a chance to fit in one of the expected classes. Nevertheless, even in this favorable case for the 2X inheritance, the highest values of the Bayes factor in favour of 2X were not more than 5×10^{-3}, i.e., much lower than the probabilities observed in favour of 4X inheritance. When accepting homoplasy for the 2X patterns, the size of our populations was too small to differentiate the 4X versus 2X distribution, except when the number of segregating alleles (six) made it possible to arrive at that conclusion. When excluding homoplasy between the two loci in the 2X pattern, 4X was by far the likely pattern for the three populations (even in the smallest one), except for very few non-significant exceptions. *Dioscorea trifida* therefore appears to have a clear 4X inheritance pattern. As for any locus, the 2X inheritance appeared sufficiently non-significant, we have no footprint of segmental allotetraploids (Soltis and Soltis 1999; Soltis et al. 2003, 2004) in our data. Recent cytological and molecular data suggest that many traditionally recognised autopolyploids can be treated as segmental allopolyploids (Sybenga 1969; Allendorf and Danzmann 1997; Fjellstrom et al. 2001, Jannoo et al. 2004). In our study, the number of loci was too low to draw any conclusion on this point.

We also suspected double reductions in some cases when one allele was in a single dose in the parents. The determination of the overall ratio rate of double reduction in *D. trifida* will require the analysis of larger populations, but the existence of double reduction is coherent with the tetrasomic inheritance and may be indirect evidence of tetravalent formation.

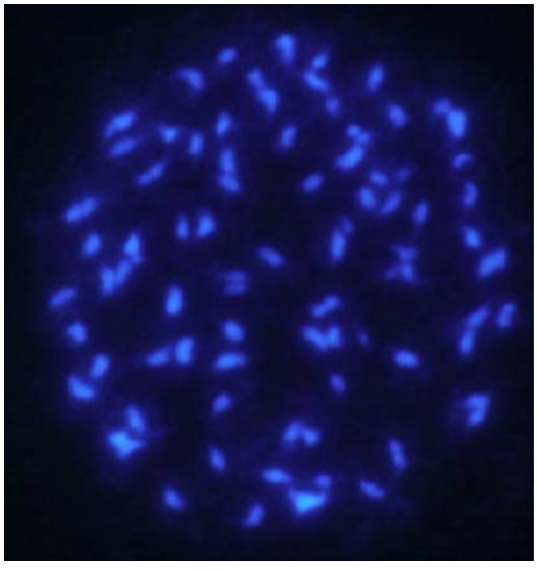

Fig. 1 Mitotic chromosome counts from root-tip preparations of *D. trifida* cultivar charvein ($2n$ = 80). The chromosomes are counterstained with DAPI (blue fluorescence)

First reliable evidence of the autotetraploid species in the genus *Dioscorea*

Our results provide genetic evidence supporting tetrasomic behaviour of the genome of *D. trifida* based on chromosomal segregation pattern analysis using eight SSRs markers in three different crosses. This is the first reliable evidence of an autopolyploid species in the genus *Dioscorea*.

In general, four types of information are used to distinguish among different origins of tetraploids: segregation patterns of genetic markers, the presence or absence of multivalent formation, tracing genetic markers from putative parental diploids to tetraploid derivatives and regeneration of tetraploids from the parental diploid(s) (Stebbins 1950). Several types of information are often combined before a firm conclusion is drawn because no single type of information is conclusive. For *Dioscorea*, observations have been restricted in most cases to the determination of chromosome numbers and chromosome pairing from mitotic (Sharma and De 1956; Raghavan 1958, 1959;

Ramachandran 1968; Essad 1984) and miotic cells (Abraham and Nair 1990; Abraham 1998).

However, the observation of meiotic cells is not a suitable method for distinguishing between auto- and allopolyploidy. Multivalents may be observed only at the early stages of meiotic division (e.g., if chiasma are rare) and remain undetected because they have already dissociated at the stages generally observed (Jackson and Casey 1982). The characterisation of allele segregation in tetraploids as either disomic or tetrasomic should therefore be considered as valuable evidence for determining whether species have either autotetraploid or allotetraploid origins (Krebs and Hancock 1989).

Polyploid species of *Dioscorea* are reported mainly among the Asian and African species and most of them are allopolyploids except for the Asian species *D. hispida* (Jos et al. 1976) and *D. esculenta* (Bai et al. 1978), which were assumed to be autopolyploid species. Despite the fact that American *Dioscorea* represent more than one third of the species in the genus *Dioscorea*, there is a complete absence of ploidy data in the New World species, except for an isolated report about the alloploidy of *D. floribunda* (Martin and Ortiz 1963).

Evidence for x=20 in *D. trifida* by combined analysis of SSRs markers and chromosome counts

D. trifida is commonly described as an octoploid species and our results clearly rejected autooctoploidy. Assuming this octoploid status, *D. trifida* should then be a complex allo-auto polyploid species like those already encountered in other highly polyploid species, which rarely appear to be complete autopolyploids (Stebbins 1950; Jannoo et al. 2004). Consequently, if we agree that *D. trifida* is octoploid, our observation of a 'simple' autotetraploidy would lead to the conclusion that the eight SSR markers amplified only one tetraploid genome and failed to amplify on the other present genome(s) in *D. trifida*. These companion genomes could be either another autotetraploid genome or two genomes that form an allotetraploid state. For example, Shiotani and Kawase (1989) proposed that sweet potato has a 'tetradisomic' genetic constitution with two different genomes (B1B1B2B2B2B2) and the recent inheritance of SSRs supported this (allo-2X auto-4X) hexaploidy. We propose here that the ploidy level of *D. trifida* may be simply 4X and not 8X, as usually considered. This comes from converging evidence from our different results. First, we used two SSR primers derived from two African wild species belonging to the botanical section *Enantiophyllum* from which *D. trifida* (*Macrogynodium* section) is very distant. Their capacity to amplify on these distant species makes it unlikely that they were not able to amplify the putative and yet undiscovered homologous genomes involved in *D. trifida* if it really is an 8X species.

There have been conflicting chromosome counts on *D. trifida* reported in both older and more recent reports. Three levels of ploidy ($2n$ = 54, 72, 81), which have a basic number of 9, were described by Henry (1967). Martin and Degras (1978) indicated the existence of clones with $2n$ = 18, 36 and 54 chromosomes. The most reliable study is that of Essad (1984) who observed a number of chromosomes = 80. Essad (1984) concluded that there is a base number x=10 in American species, which was previously in doubt. Our chromosome counts are in agreement with those of Essad (1984), but since the octoploidy pattern is very unlikely and autotetraploidy strongly suggested, we propose on the basis of our results that the basic number is x=20.

No significant variation of 2C DNA content was observed among a large collection of cultivated *D. trifida* cultivars collected in very different ethnic groups from all the main areas of yam cropping and agro-ecosystems of French Guyana. This suggests that cultivated *D. trifida* has a regular basis of 80 chromosomes and a unique ploidy level. It does not therefore form a complex of different polyploid plants as already reported in species with high chromosome number and clonal propagation. We thus consider that autotetraploidy is well established as well as the rule for the cultivated pool of *D. trifida*, even if the direct diploid ancestor remains to be identified.

Our proposal for a basic chromosomic number of x=20 in *D. trifida* is in accordance with recent studies (Scarcelli et al. 2005) on the African yam *Dioscorea cayenensis–D. rotundata* classified in the botanical section *Enantiophyllum* that is phylogenetically distant from the *Macroyinodium* section to which *D. trifida* belongs. These congruent results contradict the common assumption that there are only two base chromosome numbers in the genus *Dioscorea* (Essad 1984).

Genetic and evolutionary significance

Considering the potential for multiple origins of polyploidy (Thompson and Lumaret 1992; Soltis and Soltis 1993), one must be very careful when assuming multisomic inheritance. In such cases, one can at least conclude that tetraploidy occurred either via autopolyploidisation or via allopolyploidisation involving very close species. The paucity of information about molecular *Dioscorea* evolution makes the interpreta-

tion of our data even more complicated. Unlike the situation in more thoroughly studied polyploidy crops, there are no prior hypotheses of the origin of *D. trifida* derived from morphological, cytological or molecular data, and some of the reports are conflicting.

The disclosure of a basic chromosome number of 20 raises the question of the validity of the current ploidy data in the genus *Dioscorea*. A similar result was recently found in the African complex *D. cayenensis–D. rotundata* belonging to the botanical section *Enantophylum* (Scarcelli et al. 2005). Even more surprisingly, Segarra-Moragues et al. (2004) concluded that the two species of the Bordera section, *D. pyrenaica* and *D. chouardii* (Caddick et al. 2002) endemic to the Pyrenees, are allotetraploid with the chromosome base number of x = 6, which was not previously reported within the *Dioscoreaceae*. These results should lead us to reconsider the basic number of yams on a more general level and should be integrated into the global reconstruction of chromosomal evolution hypothesis.

Conclusion and future prospects: towards a new model of polyploidy?

In conclusion, the data presented in this paper are significant in that SSR markers, chromosome counts and flow cytometry have been used with success to determine the mode of inheritance and the level of ploidy and provide new evidence for a base chromosome number of 20. This knowledge is important for the effective breeding and conservation of the species, and for elucidating the phylogeny and the origins of the yam and the evolution of the genus *Dioscorea*.

The genus *Dioscorea* is an attractive model for the investigation of ploidy events and chromosome evolution in a wild and cultivated species in relation to the vegetative propagation and the process of domestication. With the discovery of the diploid progenitor, phylogenetic reconstruction and the study of the level of genetic variation are the next steps that must be taken to build an appropriate strategy for breeding *D. trifida*.

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