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Characterization of the genetic diversity of the Tall coconut (*Cocos nucifera* L.) in the Dominican Republic using microsatellite (SSR) markers

Reina Teresa Martinez · Luc Baudouin · Angélique Berger · Michel Dollet

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Abstract The predominant coconut variety cultivated in the Dominican Republic is a local Tall, known as *criollo*. It was never characterized genetically. The Malayan Dwarf and its hybrid with the local Tall are also present. Thirteen accessions, representing nine localities, are planted in a collection at the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF). We explored genetic diversity in 114 individuals from this collection. The main aim was to detect possible relationship with resistant varieties to coconut lethal yellowing (LY) disease. Contrarily to what happened in other Caribbean countries, LY never became an epidemic in the Dominican Republic. Thirteen simple sequence repeats markers from a kit dedicated to coconut diversity were used. In addition to diversity parameters, we used Bayesian assignment tests and cluster analysis to determine its population structure and its relationship with other coconut populations. The *criollo* coconut proved to be a typical Indo-Atlantic variety and is probably highly susceptible to the usual LY pathogens. Local conditions and the nature of the local phytoplasma strain probably explain the particular epidemiology of LY in the Dominican Republic. As a cross-pollinating variety, the *criollo* presents polymorphism within a population, but there is little if any variation among populations. The marker study confirmed the hybrid status of each member of two accessions and, thus, the reliability of the sampling.

Keywords Genetic diversity · *Cocos nucifera* · Lethal yellowing disease · Microsatellite · SSR

Abbreviations

Coconut population abbreviations: full name–country

Pacific group = A

MYD	Malayan Yellow Dwarf–Malaysia

Indo-Atlantic group (typical) = B1

BRTxx	various populations from the Brazilian Tall–Brazil
CALT	Calangute Tall–India
CKT	Cameroon Kribi Tall–Cameroon
DRT	Dominican Republic Tall–Dominican Republic
JMT	Jamaica Tall–Jamaica
LMT	Laccadive Micro Tall–India
MXAT	Mexican Atlantic Tall–Mexico
SCT	Seychelles Tall–Seychelles Islands
SKGT	Sakhi Ghopal Tall–India
SLT	Sri Lanka Tall–Sri Lanka

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R. T. Martinez
Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF), Proteccion Vegetal,
Calle Rafael Augusto Sanchez No. 89,
Ensanche Evaristo Morales,
Santo Domingo, Dominican Republic
e-mail: meliasai13@hotmail.com

L. Baudouin (✉) · A. Berger
Centre International de Recherches en Agronomie pour le Développement (CIRAD),
TA A-96/03, Avenue Agropolis,
34398 Montpellier Cedex 5, France
e-mail: luc.baudouin@cirad.fr

M. Dollet
Centre International de Recherches en Agronomie pour le Développement (CIRAD),
TA A-29/F, Campus International de Baillarguet,
34398 Montpellier Cedex 5, France

TTOT	Trinidad and Tobago Tall–Trinidad and Tobago
WAT4+, WAT6	West African Tall–Côte d'Ivoire and Benin, respectively
WCT	Indian West Coast Tall–India

Indo-Atlantic group (introgressed) = B2

ADOT	Andaman Ordinary Tall–India
CMT	Comoro Moheli Tall–Comoros
EAT, EAT12	Two populations of the East African Tall–Tanzania
EAT15	East African Tall–Kenya
ECT	Indian East Coast Tall–India
KPDT	Kappadam Tall–India
LCT	Laccadives Ordinary Tall–India
MZT, MZTa, MZTb, MZTc, MZTd	various populations of the Mozambique Tall–Mozambique
SLT12	"Margaret" population of the Sri Lanka Tall–Sri Lanka
SNRT01	"Clovis" population of the San Ramon Tall–Sri Lanka

Introduction

Coconut palm (*Cocos nucifera* L.) is a crop of economic, social, and ecological importance for the Dominican Republic. It is grown in commercial plantations for exportation as copra and as dehydrated products (CEID-RD 2008). It is also used locally as food and for manufacturing nonedible products for cosmetics and substrate for agricultural purposes.

Besides its agricultural importance, coconut palm is a part of the landscape and represents an asset for the tourism industry, which is very important for the country. Of the total area under coconut palms, 85–90% are cultivated in the northeast (Maria Trinidad Sanchez Province) and east (Samana and in the Hato Mayor, El Seibo, and La Altagracia Provinces; Benítez and Díaz 1997). Estimated extent of coconut is 35,000 ha and the average annual production is 107,000 T of coconuts (FAOSTAT; http://faostat.fao.org/site/291/default.aspx). Most farmers are smallholders.

The traditionally grown Tall coconut type, locally known as *criollo*, is thought to result from an introduction at the beginning of the sixteenth century from South Asia, probably via West Africa. It would thus belong to the Indo-Atlantic group or group B (Lebrun et al. 2005). It is cross-pollinating. Self-pollinating Dwarf coconuts were introduced from Malaysia during the twentieth century. They belong to the other group of coconut cultivars: the Pacific group or group A. Hybrids between the Malayan Yellow Dwarf (MYD) and the local Tall are planted in a few large plantations.

Farmers depend on very old plantations that give very poor yields. Lately, small farmers have tended to lose interest on the crop because they depend on the traders to sell their nuts and get insufficient benefit. In order to improve the coconut industry in the Dominican Republic, implementation of a new policy would probably be necessary and should take agronomic, biotic, and abiotic factors into account. In addition to the above problems, diseases as *Phytophthora palmivora* Butler and coconut lethal yellowing (LY) contribute to the reduction of the yield. LY existed in the north part of the Dominican Republic since the early 1960s (Carter 1962) and a new outbreak appeared in the southeast in 2006. The pathogen was classified as a member of the 16S rDNA restriction fragment length polymorphism (RFLP) group 16SrIV, subgroup E (16SrIV-E; Martínez et al. 2008). However, the disease has not become an epidemic and the reasons remain unclear.

The main purpose of this work is to identify possible relationships between genetic features of the Dominican *criollo* and the slow dissemination rate of LY, in comparison to countries like Jamaica, Honduras, and Mexico where LY has become an epidemic (Romney 1983; Roca et al. 2002). To study its genetic diversity, simple sequence repeats (SSR) microsatellite markers were used. This technique has been used successfully by many scientists to characterize the genetic diversity of the coconut population (Perera et al. 2000; Rivera et al. 1999) A 13-marker kit for characterizing coconut cultivar was set up and used in a worldwide set of 131 populations (Lebrun et al. 2005). This made it possible to improve the genetic classification of coconut varieties already outlined based on RFLPs in Lebrun et al. (2003). The kit makes it possible to compare the observed genotypes with a wide range of genotyped populations and is being used at the international level. It was used to assess trueness to type of extended planting material in Jamaica (Baudouin et al. 2008) and to characterize coconut diversity in the Andaman Islands (Rajesh et al. 2008).

Material and methods

Plant material

Coconuts were collected from nine different localities in order to represent overall genetic diversity of coconut in the Dominican Republic. The collection sites were plantations for commercial as well as for domestic use, located in various parts of the country (Table 1). All sites were planted with Tall coconut except La Totuma which was planted

Table 1 Details of the accessions used in the study

Region	plantation site (town)	Accession name[a]	Number of trees analyzed
North–East	La Gorda–La Totuma (Nagua)	GT-A04	7[b]
		GT-V04	8[b]
	Los Yayales (Nagua)	NY-V04	9
	La Senda (Nagua)	NS-R04	8
East	Yagrumas (Samana)	SY-R05	9[b]
	Las Terrenas (Samana)	ST-R05	9
		ST-V05	9
	Miches	M-R04	7
		M-V04	10
	Higuey	NH-R05	10
		NH-V05	8
South	Jaquimes (Barahona)	J-P04	10
	Cabral (Barahona)	CB-R05	7
Total			111 (out of 114)

[a] The last three characters of the name refer to the color of the mother tree (*A* yellow, *V* green, *R* reddish brown, and *P* greenish brown) and to the planting date (2004 or 2005)

[b] One sample did not produce usable results

with hybrids between the local Tall and imported MYDs. At each site, the nuts were collected under one or two trees (which were thus assumed to be their mother trees).

The seeds were germinated and planted in a variety garden at the Experimental Station of Palmarejo, belonging to the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF). This garden consists of 13 accessions corresponding to the 13 mother trees and the total number of palms planted was 130 (one 10-tree plot per accession). We collected leaf samples from the 114 surviving trees and transported them to the genotyping center at Centre Internationale de Recherches en *A*gronomie pour le Développement (CIRAD; Table 1).

DNA extraction

DNA was isolated from fresh adult leaves of coconut palm. Total DNA was extracted from 450 mg of each coconut leaf using the MATAB protocol according to Risterucci et al. (2000). The tissues frozen in liquid nitrogen were powdered with a mortar and mixed with 5 ml of extraction buffer (1.4 M NaCl, 100 mM Tris–HCl pH8, 20 mM ethylenediaminetetraacetic acid (EDTA), 0.5% sodium sulfite, 1% PEG 6000, and 2% MATAB). The plant extract was incubated 30 min at 74°C and mixed with an equal volume of chloroform–isoamyl alcohol (24:1 *v*/*v*). After a centrifugation at 9,000×*g* during 15 min, the supernatant was precipitated by adding 0.8 volume of isopropanol per volume of supernatant. The DNA pellets were removed with a glass hook and resuspended in 400 µl of sterile water. The DNA concentration was estimated by separating 3 µl aliquots in 1% agarose and staining with ethidium bromide. The remaining samples were diluted 1:9 and stored at −20°C until use.

Marker set and reference genotype database

In order to compare the results obtained in the Dominican Republic with the available data, we used 13 microsatellite or SSR markers from the "microsatellite kit for coconut cultivar identification" (Baudouin and Lebrun 2002). These markers have already been scored with 1,215 genotypes of known origin. The resulting database consists in 131 populations representing the global coconut diversity. The markers used are CnCirA3, CnCirA9, CnCirB12, CnCirB6, CnCir C12, CnCirC7, CnCirE10, CnCirE12, CnCirE2, CnCirF2, CnCirG11, CnCirH4′, and CnCirH7. Details are given in Lebrun et al. (2005) and available at http://tropgenedb.cirad.fr/en/coconut.html.

PCR amplification

For each SSR marker, one of the primers was designed with a 5′ end M13 extension (CACGACGTTGTAAAACGAC; Stiffens et al. 1993). polymerase chain reactions (PCR) were carried out in 384-well plates in a 10-µl final volume of reaction mixture containing: 25 ng of template DNA, 1X buffer (10 mM Tris–HCl pH8, 50 mM KCl, and 2 mM $MgCl_2$), 0.2 mM dNTP mix, 0.1 U/µl *Taq* polymerase, 0.08 µM of the M13 labeled primer, 0.1 µM of the other primer, and 0.1 µM of M13 primer-fluorescent dye IR700 or IR800 (Biolego, The Netherlands). The PCR program started with an initial step of denaturation at 94°C for 5 min, then 35 cycles of 94°C for 30 s, 51°C for 1 min, 72°C for 1 min, and stopped after the final elongation at 72°C for 5 min. Each mixture of PCR products contained one IR700 and IR800 labeled M13 reverse complement extensions, diluted to one fourth with formamid blue; 1.1 µl of the PCR products was separated on 6.5% of denaturing polyacrylamide gels in

a 1X Tris/borate/EDTA buffer and detected by the infrared fluorescence scanning system of sequencer (Li-Cor 4300).

Scoring

The genotypes of all individuals were determined by scoring DNA fragment lengths in the presence of a ladder (Ladder Small Size, CIRAD) and the control DNA samples from known genotypes (West African Tall [WAT] and Malayan Dwarf). The controls are required to determine the exact length of the fragments, since slight difference in apparent length may occur due to uncontrolled variations of experimental conditions.

Diversity parameters

The number of alleles and the percentage of heterozygote genotype were recorded for each population. The expected value of heterozygosity in Hardy–Weinberg equilibrium was estimated based on unbiased Nei's diversity index (Nei 1978). *F* statistics (Wright 1969) were calculated for the all "pure" Tall accessions to evaluate the overall degree of differentiation among populations (F_{ST}) as well as the heterozygote deficit within accession (F_{IS}) and in the whole population (F_{IT}). In addition, we calculated pairwise F_{ST} to evaluate the degree of differentiation among populations. Calculations were made using software *Genetix* (Belkhir et al. 1996–2004).

Family structure

According to the sampling protocol, all the nuts of each accession were supposedly collected under a single tree. As a result, the accessions should be half-sib families. We checked this assertion using the following rules. Rule 1: Homozygote progenies must have one of the maternal allele. There should not be more than two different homozygote genotypes at each locus. Rule 2: Each individual must have at least one maternal allele at each locus. If one of these rules does not apply to an accession, at least one individual did not come from the mother tree.

Bayesian assignment tests

Hybrids between populations

Two accessions (GT-A04 and GT-V05) were collected from a plantation of F_1 hybrids between the exotic cultivar MYD and the local Tall variety. In order to ascertain this origin, we used the Bayesian assignment procedure proposed by software *NewHybrid* (Anderson and Thompson 2001). In this procedure, it is assumed that each individual belongs to one of six genotypic classes, namely, two Mendelian parental populations, the F_1 and F_2 hybrids, and the backcrosses on each parent. Using a Markov chain Monte Carlo numeric simulation algorithm, it simultaneously estimates the allelic frequencies in the parental populations and calculates for all individuals their posterior probabilities of belonging to each of the six genotypic classes. Knowing the exotic parent, we were able to improve the accuracy of the results by including two MYD genotypes in the dataset. We also ran the same procedure without MYD genotypes as a test of the hypothesis that the MYD was the other parent of the hybrid.

Population assignment

F statistics provide us with an overall assessment of differentiation among accessions. We can examine it more precisely using a Bayesian assignment test. If there is no differentiation, individuals will be assigned randomly to one of the accessions. Deviation from randomness is thus evidence of some degree of differentiation in one or more accessions. We used *Geneclass* 2 (Piry et al. 2004) and the reference data were the 13 studied samples. All individuals were tested and assigned to the most probable accession. To avoid systematic bias due to rare alleles, the "leave one out" option of *Geneclass* 2 was used: the tested individual was excluded from the reference dataset.

Bayesian assignment can also be used to identify populations showing affinities with the local genotypes. In fact, the likelihood *L* of a population is the probability of obtaining the tested genotype in that population. Likewise, the probability of obtaining all *criollo* genotypes is the product of the likelihoods. We used the sum of the scores issued by *Geneclass* 2 ($-\log L$) as a dissimilarity measure.

Cluster analysis

We used cluster analysis to represent the relationship of the *criollo* coconuts with comparable populations. For the sake of clarity, we limited the analysis to the Indo-Atlantic cultivars. The MYD is added as outgroup. We preferred Cavalli-Sforza's distance to Nei's distance because there are evidence that the role of genetic drift, migration, and selection is more important than mutation in the evolution of this group. The weighted pair group method with arithmetic mean dendrogram was produced using the *DARwin* software (Perrier and Jacquemoud-Collet 2006).

Results and discussion

Overall diversity parameters

Out of 114 DNA samples analyzed, one (from accession GT-A04) did not amplify; two other samples amplified for

only a small number of markers and were not considered further. Among the 111 remaining individuals, 96 amplified for all 13 loci and the rest had no more than two missing data per individual, resulting in 1% missing data only. The number of alleles, allele length range, and observed and expected heterozygosities are listed in Table 2 for each locus and in Table 3 for each population.

The observed and expected heterozygosities are slightly higher than the average values in the typical Indo-Atlantic group (0. 408 and 0.428, respectively) but lower than in the introgressed Indo-Atlantic group (0.587 and 0.612, respectively). These parameters tend to be higher in the hybrids.

In microsatellite markers, the number of alleles depends highly on the sample size. Figure 1 compares the values found in the Dominican Republic (DRT) to those of group B1 and also shows the values observed in the "introgressed" Indo-Atlantic group (B2), found in East Africa, which is closely related to B1, but has received genes from Southeast Asia. The observed values in the *criollo* conform to what is found in the typical Indo-Atlantic populations (B1, which originated in South Asia) for similar sizes. This applies to each accession and to the whole set of *criollo* coconuts as well.

The overall genetic structure in the Dominican Republic can be explored using Wright's F statistics (Table 4). The F_{IT} expresses the deviation of the whole Tall population from the Hardy–Weinberg equilibrium. It is significantly different from 0, showing a slight deficit of heterozygotes. The F_{ST} represents the contribution of the divergence between populations to this deficit. It is also positive and significantly different from 0, showing that allelic frequencies differ among accessions. But we noted earlier that each accession is supposed to come from a single tree. In other words, they would be half-sib families. If the whole population is in Hardy–Weinberg equilibrium, the F_{ST} should be 0.125, which corresponds to the upper value of the confidence interval. This suggests no differentiation among plantations. But the hypothesis (half-sib families) is not strictly valid, as shown below.

The F_{IS} corresponds to the within-population allelic correlations, which is influenced by inbreeding. The overall value differs significantly from 0, suggesting a slight tendency toward inbreeding.

The values found for the diversity parameters of the local Talls studied here conform to what we can expect from a typical Indo-Atlantic coconut variety. Slight but significant deviations from the Hardy–Weinberg equilibrium indicate some degree of differentiation among accessions as well as variation in the inbreeding level. But this differentiation can result either from genetic difference among populations or from the fact that each accession is supposed to descend from a single tree. We thus examined how strictly this rule was followed.

Table 2 Numbers of alleles amplified, range of allele sizes, and observed (h_o) and expected (h_e) heterozygosities for each locus

Marker	Alleles	Range	h_o	h_e
CnCirA3	3	228–234	0.219	0.278
CnCirA9	4	89–103	0.579	0.541
CnCirB6	5	196–208	0.578	0.599
CnCirB12	8	137–173	0.506	0.618
CnCirC7	5	157–167	0.426	0.504
CnCirC12	5	167–183	0.470	0.497
CnCirE2	10	115–177	0.682	0.737
CnCirE10	4	232–246	0.454	0.457
CnCirE12	2	162–174	0.436	0.477
CnCirF2	2	193–205	0.104	0.108
CnCirG11	5	188–210	0.610	0.630
CnCirH4′	3	218–232	0.234	0.278
CnCirH7	4	123–149	0.449	0.463
Average	4.6		0.442	0.476

The values are calculated based on the Talls only. The hybrids had only one original allele in CnCir E2

Table 3 Average numbers of alleles amplified and observed (h_o) and expected (h_e) heterozygosities for each locus

	Average alleles number	h_o	h_e
Hybrids			
GT_A04	3.538	0.495	0.593
GT_V04	3.308	0.558	0.597
Average	3.423	0.5265	0.595
Talls			
NY_V04	2.692	0.439	0.468
NS_R04	2.769	0.429	0.427
SY_R05	2.846	0.466	0.489
ST_R05	2.923	0.462	0.456
ST_V05	3.385	0.410	0.524
M_R04	2.308	0.257	0.429
M_V04	2.846	0.419	0.427
NH_R05	3.077	0.562	0.508
NH_V05	3.077	0.453	0.525
J_P04	3.077	0.515	0.549
CB_R05	2.538	0.451	0.432
Average	2.867	0.442	0.476

Family structure

Seven of the 13 accessions did not follow the above rule 1. There were three or more different homozygous genotypes at a given locus. Two more accessions did not follow rule 2. At least one genotype lacked a maternal allele. This

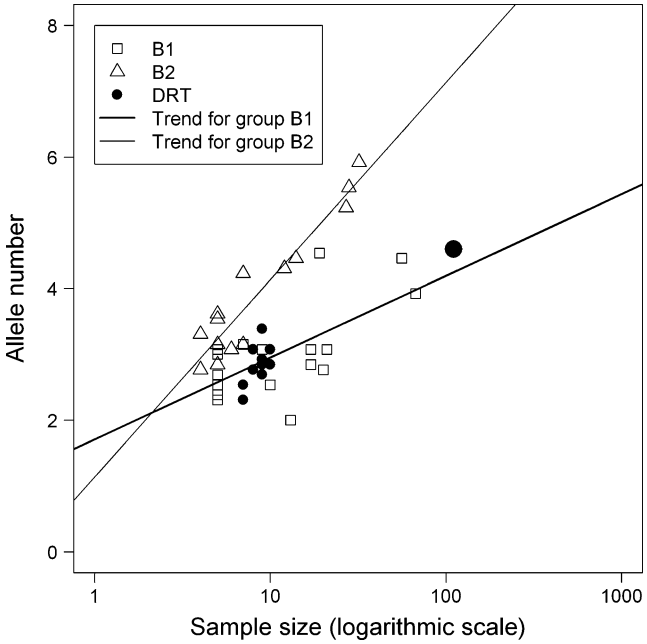

Fig. 1 Average allele number per locus in the Dominican accessions and in two groups of Indo-Atlantic coconut populations as a function of the sample size. The *small dots* correspond to the individual accessions and the *large dot* to the whole Tall population

suggests that the sampling method was not followed strictly. Either one or more nuts collected under the chosen tree had actually rolled from a neighboring tree, or the chosen tree did not have enough mature nuts at the collection time and some nuts had to be collected elsewhere in the plantation. Whichever is the case, this does not question the fact that each individual is representative of its collection site: the next two sections suggest they are.

Bayesian assignment tests among accessions

When applying the *Geneclass* 2 assignment method with the local accessions as reference dataset, the individuals of the two hybrid accessions from La Totuma were assigned indistinctly to one of them. Hybrids form a distinct and homogenous group. Among the 96 local Talls, 45 were assigned to their own accession, showing that there are differences between accessions. The expected value for the null hypothesis of no differences between accession is 6.9 (χ^2=225***).

Table 4 *F* statistics in the local Tall accessions

Parameter	Estimate	Confidence interval (α=0.95)
F_{IT}	0.153	0.098–0.207
F_{ST}	0.091	0.055–0.126
F_{IS}	0.068	0.026–0.114

Confidence intervals are estimated by bootstrapping

Do these differences result only from the family structure of the accessions? In spite of the deviations from sampling protocol noted above, members of the same accession are still more likely to be related than individuals of different accessions. Three plantations (Miches, Samana, and Higuey) are represented by two accessions with a total of 53 individuals, among which 21 were assigned to their own accession. Out of the 32 others, ten were assigned to the other accession of the same plantation. The expected value, if the plantations were equivalent, is 4.2 (χ^2=9.41**). This tends to mitigate the conclusion suggested by the overall F_{ST} value (see above): a small part of the differences between accessions is due to differences between the plantations.

Identification of hybrid genotypes

The assignment test indicated that accessions GT-A04 and GT-V04 are well differentiated from the others. In fact, they were collected in a F_1 hybrid plantation. This can be verified using another Bayesian test. Using genotypic information from the 111 local genotypes and two MYD individuals, software *NewHybrid* confirmed the genetic status of each individual. The 96 putative Talls were assigned probabilities 0.889 to 0.999 of being pure *criollos*. The 15 progenies from the F_1 hybrids were assigned probabilities 0.780 to 1.000 of being F_2 hybrids, as expected.

We could also confirm the identity of the MYD as the exotic parent. In the run performed without MYD individuals, this parent was not represented in the data. Our interest was mainly directed to the posterior expectation of its allele frequencies. At each locus, the most probable allele was the one found in the MYD (like all Dwarf coconuts, the MYD is self-pollinating; it is homozygous at all loci). Of course, omitting one of the parents of the hybrid in the dataset made the results somewhat more ambiguous, but the most probable origin remained correct.

Having confirmed the status of the hybrid and of the pure *criollo* coconuts, we can undertake to assess the relationships between the latter and other coconut germplasm.

Comparison with other germplasm

Assignment assay to known populations

A Bayesian assignment test can be used to explore similarities between populations. The populations that obtain the highest assignment score are those which have the highest probability of producing the same genotypes as in the tested individuals. According to the Bayesian assignment procedure, the most probable origin corresponded to a composite population corresponding to three populations of the Brazilian Tall from a region located between Aracaju and Salvador de Bahia (BRTsul in Fig. 2).

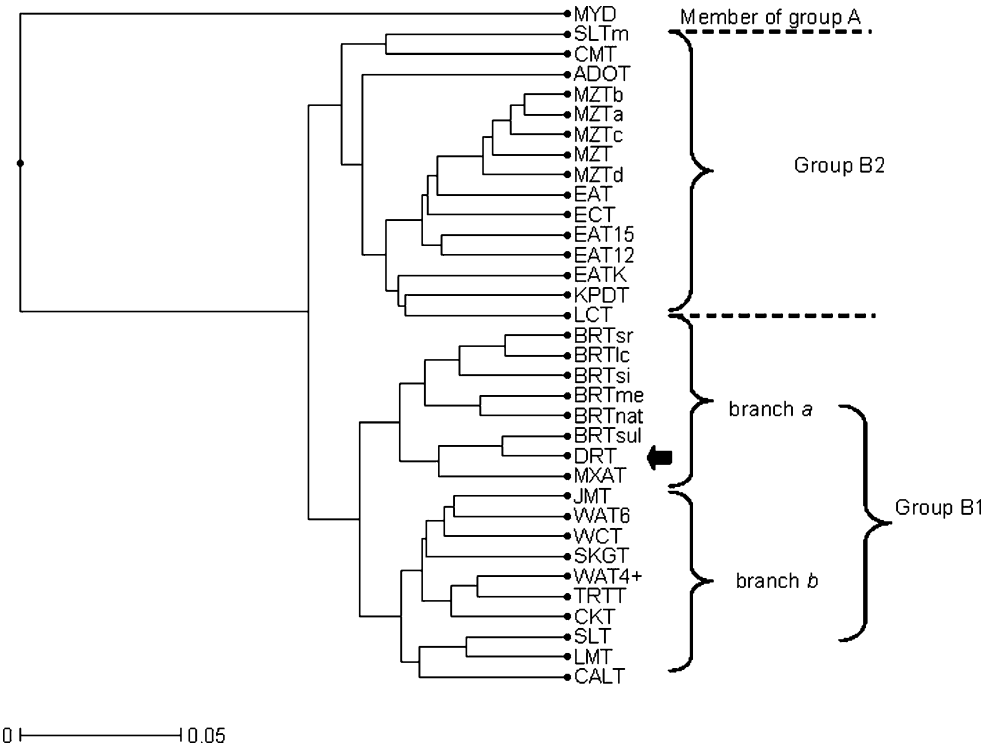

Fig. 2 Cluster analysis of 33 Indo-Atlantic coconut populations including the DRT. The MYD from the Pacific group is used as an "outgroup" in order to locate the root of the dendrogram. The Talls from the Dominican Republic are indicated by an *arrow*. The meaning of the codes is given in the abbreviation list

This does not mean that the *criollos* actually come from this region of Brazil, but simply that they have somewhat specific genetic features in common. Other populations that had fairly high likelihoods were the Jamaica Tall (JMT), the Indian West Coast Tall, the Mexican Atlantic Tall (MXAT), the Cameroon Kribi Tall, etc. All of them are Indo-Atlantic populations and this is general: the Indo-Atlantic populations are more likely than those from the Pacific group. The decimal logarithm of the likelihood ratio *in favor* of BRTsul ranged from 3.2 to 12.3 (average 5.8) in the case of the Indo-Atlantic and from 12.3 to 43 (average 21.5) for the Pacific group.

The fact that populations showing affinities with the coconuts from the Dominican Republic are located on three continents should not be a surprise: the coconut is a long-lived species and they were introduced to West Africa and America in the very beginning of the sixteenth century. A few generations back, all their ancestors were in South Asia. The test clearly confirms that the *criollo* coconut belongs to the Indo-Atlantic group, which was expected. Their affinities with some Brazilian populations were less expected at first sight. Cluster analysis can be used to illustrate the relationships between the studied coconuts and the other Indo-Atlantic cultivars.

Cluster analysis

The dendrogram presented in Fig. 2 represents the genetic relationships of 33 Indo-Atlantic populations, including the population from the Dominican Republic (DRT). The first branching isolates the MYD (the only member of the Pacific group used as outgroup) and thus represents the root of the Indo-Atlantic group. The second one divides the "introgressed" part of this group (B2, upper part of the dendrogram) from the typical Indo-Atlantic group (B1). The populations from the "introgressed" group are located in different regions of the Indian Ocean and have received part of their genes from the Pacific group. The only "typical" member in this branch is the Laccadive Ordinary Tall. The "typical" branch is further divided into two branches. The lower branch corresponds to India, West Africa, and two Caribbean Islands (Jamaica and Trinidad). The other one groups Brazil, the Atlantic Coast of Mexico, and the Dominican Republic. This suggests some similarities in the history of the introduction of coconut to these three countries.

The *criollo* is thus genetically close to the highly susceptible MXAT. Other notoriously susceptible cultivars, such as and the JMT and the WAT, also belong to the "typical" Indo-Atlantic group. No connections were found with Pacific cultivars and resistance factors are thus unlikely to exist in the Dominican *criollo*.

Conclusion

Contrarily to what is observed in Jamaica and Yucatan, the pace of extension of LY in the Dominican Republic is relatively slow. The primary aim of this study was to check whether this could be attributed to genetic differences between the local coconuts from these countries. Field

testing has shown that typical Indo-Atlantic cultivars (i.e., group B1) are generally highly susceptible to the disease while a few Pacific cultivars (group A) present some degree of resistance (even though none of them can be guaranteed as fully and permanently resistant). Most of them come from Southeast Asia (Been 1981; Dery et al. 2008; Batugal et al. 2009).

Based on the microsatellite kit, the Dominican *criollo* coconuts are typical of the Indo-Atlantic group and quite similar to the germplasm found in Brazil and in other Caribbean countries. This is in agreement with what is known of the pattern of introduction of coconut into the region. It also makes the existence of resistance factors in the Dominican *criollo* highly improbable. Moreover, the low degree of differentiation among populations indicates that the contribution of exotic germplasm is virtually inexistent. In fact, the introduction of the MYD and the production of F_1 hybrids are recent and their impact is limited to a few large plantations, such as La Totuma.

If coconut genotypes are not responsible for the low rate of transmission of the disease, it might be due to the local phytoplasma strain. Edaphic conditions, eradication policy, and natural barriers to contamination may also be involved (Martínez et al. 2008). In these conditions, early eradication of diseased trees is probably sufficient to keep the disease at an economically acceptable level. However, in prevision of a possible epidemic outbreak, it is recommended here to introduce a certain number of Tall and Dwarf cultivars from Southeast Asian origin. Besides their potential good behavior in the presence of LY, the Dwarf coconuts are early bearing and can be profitable in a tourism area: the water of its immature nuts is sweet and tasty.

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References

Anderson EC, Thompson EA (2001) A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229

Batugal P, Bourdeix R, Baudouin L (2009) Coconut breeding. In: Priyadarshan PM (ed) Breeding plantation tree crops: tropical species. Springer, Berlin, pp 327–376

Baudouin L, Lebrun P (2002) The development of a microsatellite kit and dedicated software for the use with coconuts. BUROTROP Bull 17:16–20

Baudouin L, Lebrun P, Berger A, Myrie W, Been BO, Dollet M (2008) The Panama Tall and the Maypan hybrid coconut in Jamaica: did genetic contamination cause a loss of resistance to lethal yellowing? Euphytica 161:353–360

Been BO (1981) Observations on field resistance to lethal yellowing in coconut varieties and hybrids in Jamaica. Oléagineux 36(1): 9–12

Belkhir K, Borsa P et al (1996–2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Montpellier (France). Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II

Benítez O, Díaz J (1997) Perfil de Inversión de Coco Seco. Revista No.6 Serie documentos técnicos. Junta Agroempresarial Dominicana, Inc. (JAD), Santo Domingo, República Dominicana, p 45

Carter W (1962) Report of visit to the Dominican Republic 5–15 December 1962, pp 12–14. In: Pujals Nolasco y Hichez Frías 1974, Informe Sobre la Situacion del Amarillo Letal en las Plantaciones de Cocoteros en Areas de la Republica Dominicana. Revista de Sanidad Vegetal de la Secretaria de Estado de Agricultura, Santo Domingo, Republica Domincana

CEID-RD (2008) Report from the Centro de Exportación e Inversiones de la República Dominicana, (CEI): Exportaciones de la República Dominicana comprendida de año 2000–2007. Departamento de Estadísticas

Dery SK, Philippe R, Baudouin L, Quaicoe RN, Nkansah Poku J, Owusu Nipah J, Arthur R, Dare D, Yankey N, Dollet M (2008) Genetic diversity among coconut varieties for susceptibility to Cape St. Paul Wilt disease. Euphytica 164(1):1–11

Lebrun P, N'cho Y-P, Bourdeix R, Baudouin L (2003) Coconut. In: Hamon P, Seguin M, Perrier X, Glaszmann J-C (eds) Diversity of cultivated tropical plant. Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, pp 219–238

Lebrun P, Berger A, Hodgkin T, Baudouin L (2005) Biochemical and molecular methods for characterizing coconut diversity. Coconut genetic resources. In: Batugal P, Ramanatha Rao V, Oliver J (eds) Coconut genetic resources. International Plant Genetic Resources Institute—Regional Office for Asia, the Pacific and Oceania (IPGRI-APO), Serdang, pp 225–251

Martínez RT, Fabre S, Harrison NA, Oropeza C, Dollet M, Hichez E (2008) Coconut lethal yellowing on the southern coast of the Dominican Republic is associated with a new 16Sr IV group phytoplasm. Plant Pathol 57:336

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89(3):583–590

Perera L, Russel JR, Pronan J, Powel W (2000) Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.). Genome 43:15–21

Perrier X, Jacquemoud-Collet JP (2006) DARwin software. Available at http://darwin.cirad.fr/darwin

Piry S, Alapetite A, Cornuet JM, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Heredity 95(6):536–539

Rajesh MK, Nagarajan P, Jerard BA, Arunachalam V, Dhanapal R (2008) Microsatellite variability of coconut (*Cocos nucifera* L.) from Andamans and Nicobar Islands. Curr Sci 94(12):1627–1631

Risterucci AM, Grivet L, N'Goran JAK, Pieretti I, Flament MH, Lanaud C (2000) A high density linkage map of *Theobroma cacao* L. Theor Appl Genet 101:948–955

Rivera R, Edwards KJ, Barker JHA, Arnold GM, Ayad G, Hodgkin T, Karp A (1999). Isolation and characterization of polymorphic microsatellites in *Cocos nucifera* L. Genome 42: 668–675

Roca M, Bustamante M, Aguilar E, Castillo M, Harrison N, Oropeza C (2002) La Epidemia de Amarillamiento Letal del

Cocotero en la Cuenca del Caribe. Escuela Agrícola Panamericana, El Zamorano, Honduras. Universidad de Florida, EEUU, Centro de Investigación Científica de Yucatán, México, p 44

Romney DH (1983) Brief review of coconut lethal yellowing. Meetings Abstracts of the 48th Annual of the InterAmerican Society for Tropical Horticulture, p20

Stiffens DL, Sutter SL, Roemer SC (1993) An alternate universal forward primer for improved automated sequencing of M13. Biotechniques 15:63–68

Wright S (1969) Evolution and the genetics of populations, vol. 2. The theory of gene frequencies. University of Chicago Press, Chicago, p 511