

# Coconut

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The coconut is an emblematic plant of tropical coastal countries, but it is also a vital resource for many populations of these regions. It is cultivated over around 11 million ha, 94% of which is located in Asia and the Pacific (Bourdeix et al., 1997). The major producer countries are the Philippines and Indonesia. It is essentially a smallholder crop, and the large plantations represent less than 10% of the total production.

Almost all parts of the coconut tree are exploited in numerous ways (Persley, 1992). The wood, although difficult to work with, is of excellent quality. The leaves are used to make roofs. The leaf midribs are used to make brooms. The sap tapped from inflorescences yields sugar and fermented beverages. The roots are used as dyes and in traditional medicines.

But coconut is best known for its fruit: the epidermis of this drupe covers a thick husk, the fibres of which are widely used as coir. Inside this is a voluminous seed—the coconut—comprising a brown, lignified shell and an albumen, the peripheral part of which is solidified at maturity. The remaining cavity encloses the liquid part of the albumen: the coconut water. The immature tender coconut provides a sweet and refreshing drink. When it is mature, it is mostly valued for its solid albumen, which can be consumed directly or after various transforming processes. The dried albumen, called copra, is an important item of international trade. It is the source of one of the principal oils of the lauric type, particularly useful in soap-making. This oil is also used in foods and in cosmetics.

## TAXONOMY AND GENETIC RESOURCES

### Botany and Taxonomy

The coconut (*Cocos nucifera* L.) is a diploid arborescent monocotyledon ( $2n = 2x = 32$ ) of the family Arecaceae. It is a monospecific genus, without any closely related wild species. It is a palm tree, the unbranched trunk of which bears a crown of fronds produced at the rate of about one a month. At the

base of each frond, a ramified inflorescence emerges. Each branch of the inflorescence has some female flowers at the base and a large number of male flowers at the summit.

The flowering is protandrous and the development of its cycles explains the reproductive behaviour of the two main types of coconut, between which are ranged the various cultivars (Rognon, 1976). The 'Tall' coconuts are mainly allogamous: the female flowering begins after the end of the male flowering. A certain rate of autogamy is, however, possible when the male flowering overlaps the female flowering of the preceding inflorescence. The Tall coconuts are also characterized by rapid growth, the presence of a voluminous bole at the base of the trunk, and widely spaced leaf scars. The Dwarf coconuts are most often autogamous: the female flowering occurs entirely (or mostly, as for the Brazilian Green Dwarf) before the male flowering ends. They are a small part of the world population and are generally located close to habitations. Apart from their autogamy, the Dwarfs are distinguished by slower growth, closely spaced leaf scars, greater precocity, and nuts that are smaller and often have a vividly coloured epidermis. There is also a variety of coconut similar to the Dwarfs in size, but allogamous: the Dwarf *Niu Leka*.

## Genetic Resources

### ORIGIN AND AREA OF DISTRIBUTION

Even though an American origin has been suggested, most authors now agree that the coconut is related to the Indo-Malayan centre of origin, as Vavilov has defined it (Child, 1964; Zohary, 1970). It is at present distributed in all the tropical coastal zones. The seeds were disseminated by flotation to the South Pacific, as well as by human intervention, which has long been the major means of dissemination. There are occasional spontaneous populations, but the overwhelming majority of coconut trees have been planted.

### THE DIVERSITY OF POPULATIONS

In the collections conserved around the world, more than 300 local varieties ('cultivars' or 'ecotypes') of coconut have been numbered, according to their geographic provenance and the plant and fruit morphology. Apart from the distinction between Dwarfs and Talls, Harries (1978) proposed that coconuts be grouped into two subtypes: The *Niu Kafa* type, with a slender trunk and elongated fruits that are triangular in section and rich in fibre, represent the wild type, adapted to dissemination by ocean currents. The *Niu Vai* type, with a thicker trunk, more rigid shape, and rounded fruits rich in water, have been domesticated since ancient times. A certain number of populations that have intermediate characters come from an introgression into 'wild' populations by domesticated cultivars. Both types are good for the production of copra and were widely planted from the end of the 19<sup>th</sup> century.

Various methods have been used to characterize the cultivars of coconut and study the relations between populations. The evaluation of morphological and agronomic characters, for which N'cho et al. (1993) contribute valuable information on the diversity as well as the potential use of cultivars, has a limited efficacy because of the influence of the environment. The enzymatic markers studied by Benoit and Ghesquiere (1984) reveal low polymorphism and give little information. They were taken up later in Indonesia (Hartana et al., 1993) and in Sri Lanka (Fernando and Gamini, 1997) with greater success, but the number of usable systems remains small. The polyphenols have provided promising results (Jay et al., 1989), but they proved to be not reproducible from one environment to another. Molecular markers have been the subject of active research using various methods: ISTR (Rohde et al., 1995), RAPD (Ashburner et al., 1997), AFLP (Perera et al., 1998), RFLP (Lebrun et al., 1998a, b), and microsatellites.

#### USE IN VARIETAL IMPROVEMENT

Hybridization between Dwarf coconuts began in Fiji (Marechal, 1928), and Patel (1938) created the first Dwarf  $\times$  Tall hybrids. In the 1940s to 1960s, comparative tests of Dwarf  $\times$  Tall and Tall  $\times$  Tall hybrids enabled the demonstration of the superiority of hybrids in terms of potential production (de Nuce de Lamothe and Bernard, 1985). Thammes (1955) proposed interplanting of two varieties in isolation, one used as male and the other as female, which made possible the mass production of hybrid varieties. Ultimately, this method was replaced by assisted pollination, which consists of applying a massive input of exogenous pollen on emasculated inflorescences (de Nuce de Lamothe and Rognon, 1972).

A strategy for coconut improvement was proposed by Bourdeix et al. (1990, 1991a, b). It was inspired from a recurrent reciprocal selection scheme that was designed by Comstock et al. (1949) for maize. Its originality lies in the simultaneous operation of two selection axes, one oriented towards the Tall  $\times$  Tall hybrids and the other towards the Dwarf  $\times$  Tall hybrids (Fig. 1).

The characterization of many available cultivars is essential to their use in improvement and to the search for heterosis, as well as to their conservation as a source of variability. This chapter summarizes the results of the study of Lebrun et al. (1998b) and attempts to find out what the tools of molecular markers can contribute to the knowledge and improvement of coconut in addition to the methodologies used earlier.

## ORGANIZATION OF DIVERSITY

### Nuclear and Mitochondrial Diversity Revealed by RFLP

For the nuclear RFLP, 25 probe-enzyme combinations used reveal 60 polymorphic bands among the 289 trees analysed. The Cox 1 probe reveals two mitochondrial profiles.

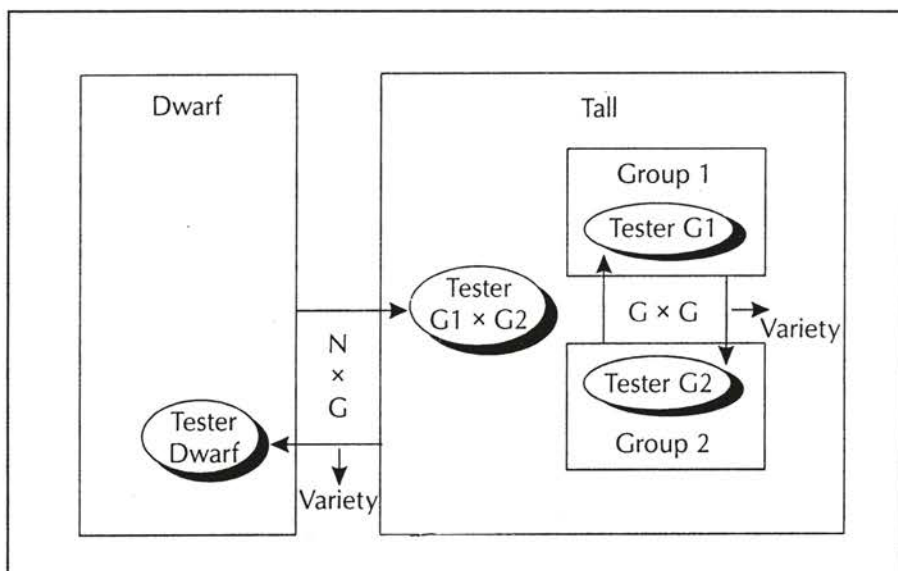


Fig. 1. Relationship between the two axes of recurrent reciprocal selection in coconut (Bourdeix et al., 1991a).

Correspondence analysis (CA) and geographic information can be used to constitute groups of relatively homogeneous cultivars and thus to identify the distinctive markers of each group.

#### THE TWO MAJOR ZONES OF DIVERSITY OF TALL COCONUTS

On the right of the CA (Fig. 2) can be distinguished a primary group that corresponds to the set of Tall cultivars from the ecogeographic zone of the Pacific, comprising Southeast Asia and the South Pacific, to which are added all the Dwarf cultivars and the Panama coconuts: this primary group is called the 'Pacific group'. Another group, located at left, corresponds to the coconuts of India, Sri Lanka, and West Africa: this is the 'Indo-Atlantic group'. These two groups are separated by a third group of three cultivars along the Indian Ocean, called the 'Indian Ocean group'.

In terms of nuclear markers, the Indo-Atlantic group is distinguished by the predominant presence of five markers, which are nearly absent otherwise, and of three others, which they share out with the single populations of Southeast Asia. Twenty-one markers are absent or have a low frequency in this group, while they are well represented in most of the other Talls. Finally, the 'rapid' allele of the cytoplasmic marker Cox 1 seems characteristic of this group: out of 45 individuals that it is made up of, 35 have this allele, while all the individuals of the Pacific group, except the individual Tonga Tall, have the 'slow' allele. The Kappadam Tall is a particular case since the five

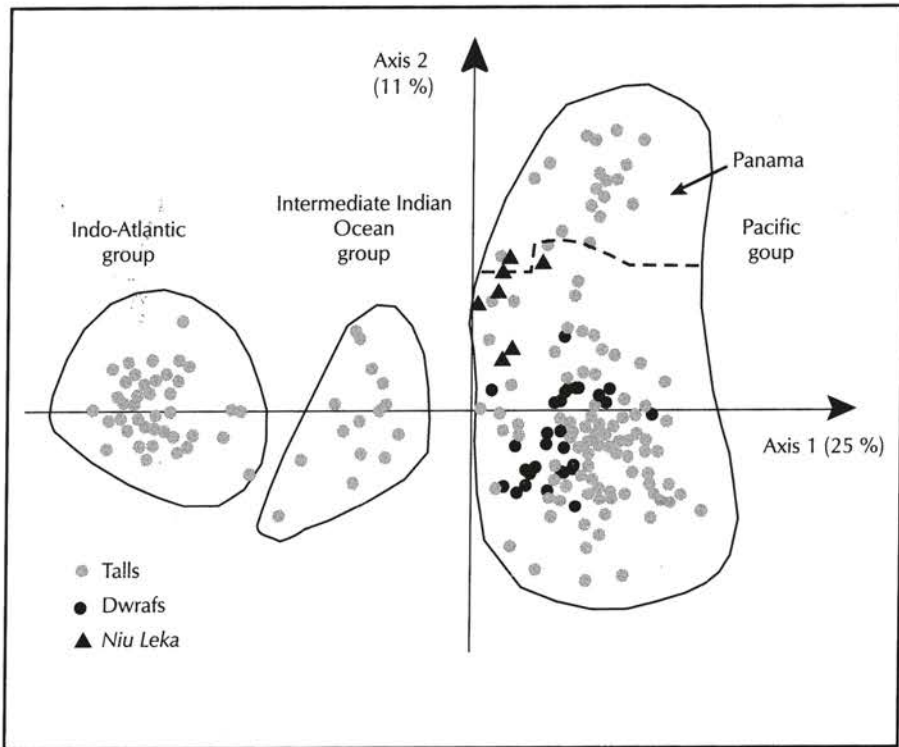


Fig. 2. Synthesis of RFLP data: the major groups of cultivars.

individuals that represent it have the 'slow' allele. In addition, even though its nuclear markers are essentially characteristic of the Indo-Atlantic group, a higher frequency is observed than that expected for some nuclear markers generally found in the Pacific group. It will be seen later that these two apparent anomalies could constitute the key of the origin of the Kappadam Tall, the fruit characters of which are very peculiar for this region.

These two groups are also distinguished by their degree of polymorphism: the Pacific group has more markers common to different cultivars—42 against 33 for the Indo-Atlantic group—and more markers of intermediate frequency, between 10% and 90%, 20 to 38 bands depending on the cultivar, as against 11 to 20 for the Indo-Atlantic group.

The intermediate Indian Ocean group has almost all the nuclear markers of the two primary groups and thus contains the widest molecular diversity. The cytoplasmic allele is the 'slow' allele, except for two individuals of the Mozambique Tall.

#### DIVERSITY IN THE PACIFIC GROUP

In order to characterize further the diversity of the Pacific group, a second CA was done (Fig. 3). In the first place, a geographic gradation can be seen



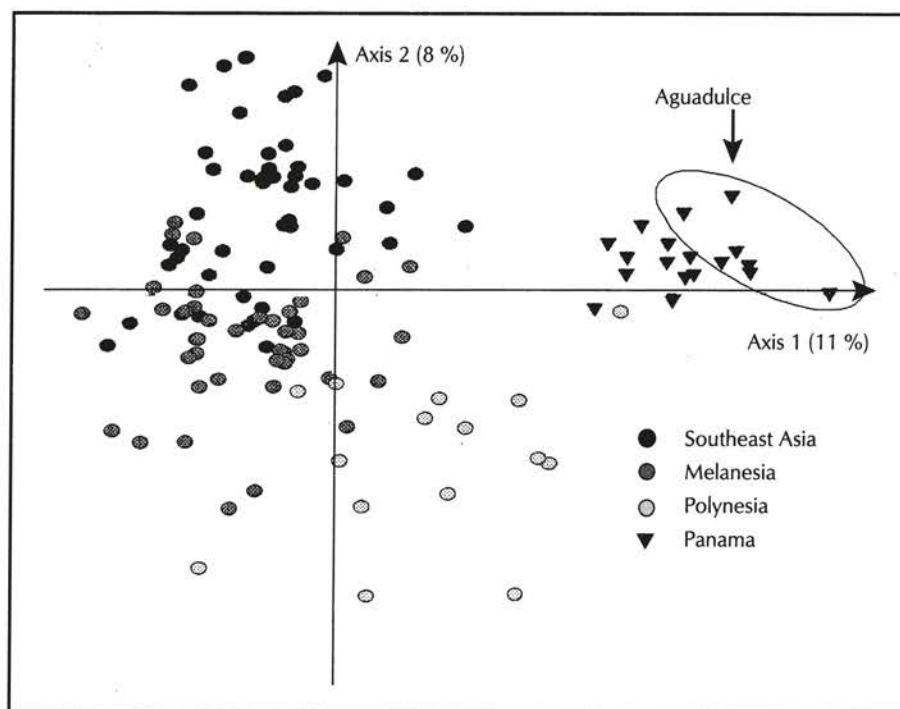


Fig. 3. Synthesis of RFLP data: diversity within the Pacific group.

between Southeast Asia and the South Pacific according to the second axis: the coconut trees of Polynesia appear at the lower part of the figure, those of Southeast Asia at the upper part, and those of Melanesia in the intermediate position. Contrary to the divergences revealed between the groups, the differences here appear to lie less in the existence of specific alleles than in frequency variations. There is also some overlapping between neighbouring subregions.

In the second place, it can be seen very clearly that the three populations of Panama are individualized on the first axis. They are related to the other cultivars of the Pacific group as to their RFLP profile, to the extent that all the markers frequent in Panama are present in this group. However, 19 bands of this group are absent or rare in Panama. Certain very frequent markers in the populations of Panama are more frequent in Polynesia than in the rest of the Pacific group. For the others, it is the reverse. The markers of intermediate frequency are few (12 to 17). The Aguadulce population is slightly distinct from the two other Panamanian populations, especially because of the presence of some bands specific to the Indo-Atlantic, with a low frequency. On the other hand, the two other populations, Monagre and Bowden (Jamaica), seem indistinguishable.

## DWARF COCONUT

The Dwarf coconuts are essentially autogamous. This characteristic is expressed in the near absence of bands of intermediate frequency and by a very low rate of heterozygosity. The two exceptions are *Niu Leka*, the only clearly allogamous Dwarf cultivar, and Malayan Green Dwarf, known to be partly allogamous. All the bands common to Dwarfs are found in the Pacific group, but 13 bands present in this group are absent from most of the Dwarfs. Among these latter, several correspond to the fixation of alleles of intermediate frequency in the Talls, which strongly suggests a common origin.

Four Dwarf cultivars of distinct origin and colour have a nearly identical profile: the Green Dwarfs from Sri Lanka and Kiribati, and the Brown Dwarfs from Ternate and Madang. On the other hand, the Malayan Dwarfs form a homogeneous group distinguished by the fixation of five alleles different from those found in the other Dwarfs. The seven Ghana Yellow Dwarfs and the 15 Malayan Yellow Dwarfs have exactly the same profile, which confirms the identity of these two cultivars. The three Dwarf cultivars of the Philippines, as well as the Brazilian Green Dwarf, also have common traits, while the profile of two Red Dwarfs of the South Pacific and that of the Cameroon Red Dwarf (a cultivar taken from Cameroon but probably exotic) appear clearly divergent even though certain traits are close to the Dwarf group.

Finally, the *Niu Leka* Dwarf has a profile that recalls that of the Talls of its region of origin (Tonga Tall and Rotuma Tall) and has the 'rapid' allele of Cox 1, rare in the region but present in a Tonga Tall individual. The presence of an allele typical of the Indo-Atlantic group in this region cannot be explained through historic data or through the morphoagronomic characters. It may be that the two regions have two different but indistinguishable alleles. Whatever the case, the *Niu Leka* is distinct from the other Dwarfs, which seem to be closer to the Southeast Asian or Melanesian coconut.

## The Polyphenols

Figure 4 summarizes the principal results of the study on polyphenols conducted by Jay et al. (1989). It represents a discriminant analysis, done on 32 cultivars (or ecotypes), each of them being represented on average by five individuals. The cultivars studied are included in the RFLP analysis, with the exception of Thailand Green Dwarf and the Tahiti Tall. Contrary to the CA done on the molecular markers, the origin of the individuals figures explicitly in the data provided for the statistical analysis. Even when this difference is taken into account, the major conclusions of this study converge with those of the molecular data. For example, we note that the Tall cultivars are distributed around three major zones: Africa, the South Pacific, and the Far East, these latter two being partly overlapped. The Indian coconuts are distributed between several groups. Those of Lakshadweep, an archipelago situated southwest of India, are close to the African coconuts, while those of

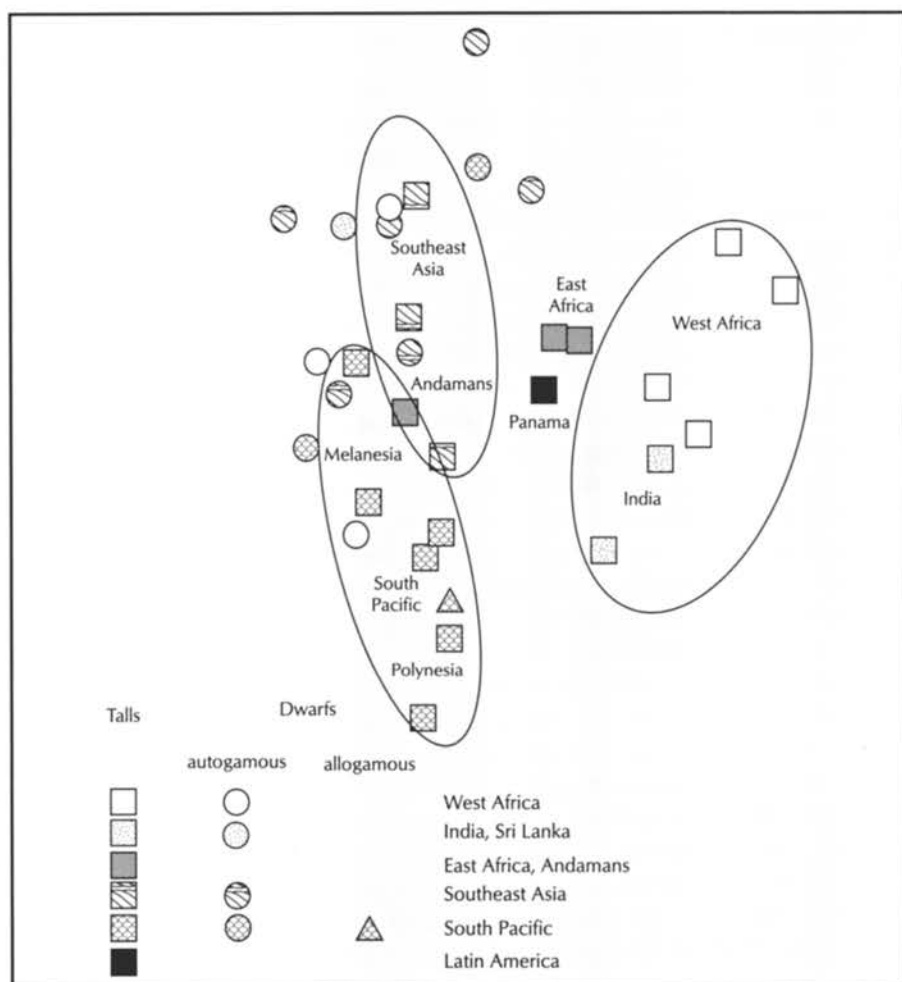


Fig. 4. Polyphenolic data: discriminant analysis of individuals according to the cultivar criterion (1-2 plane) (Jay et al., 1989).

the Andamans fall between the populations of the Far East and those of the Pacific. The East African cultivars are also found towards the centre of the figure in relation to the other African cultivars. Moreover, all of the Dwarfs seem close to the Far East and Melanesian Talls. However, there is no apparent link between the geographic origin of a Dwarf and its polyphenolic profile. This is particularly the case with those collected in Sri Lanka and in Africa, the exotic origin of which is clearly confirmed.

Thus, despite the differences in the method of statistical analysis and in the mode of presentation, the results of the two approaches are overall convergent. The most notable difference lies in the situation of the Panama Tall, which here appears intermediate and relatively close to the African cultivars.



## Agromorphological Data

Figure 5 shows the two primary axes of a discriminant analysis made on morphological and production data for 17 Tall cultivars, each represented by 30 individuals (N'cho et al., 1993). The first axis represents 34% of the total variability and comprises variables linked to the general vigour of the plant and, more particularly, that of the trunk. The second (21% of the variability) is positively linked to the size of the fruit and of the cavity left by the albumen (corresponding to the volume of water in the nearly mature fruit). It is also associated negatively with the number of nuts produced.

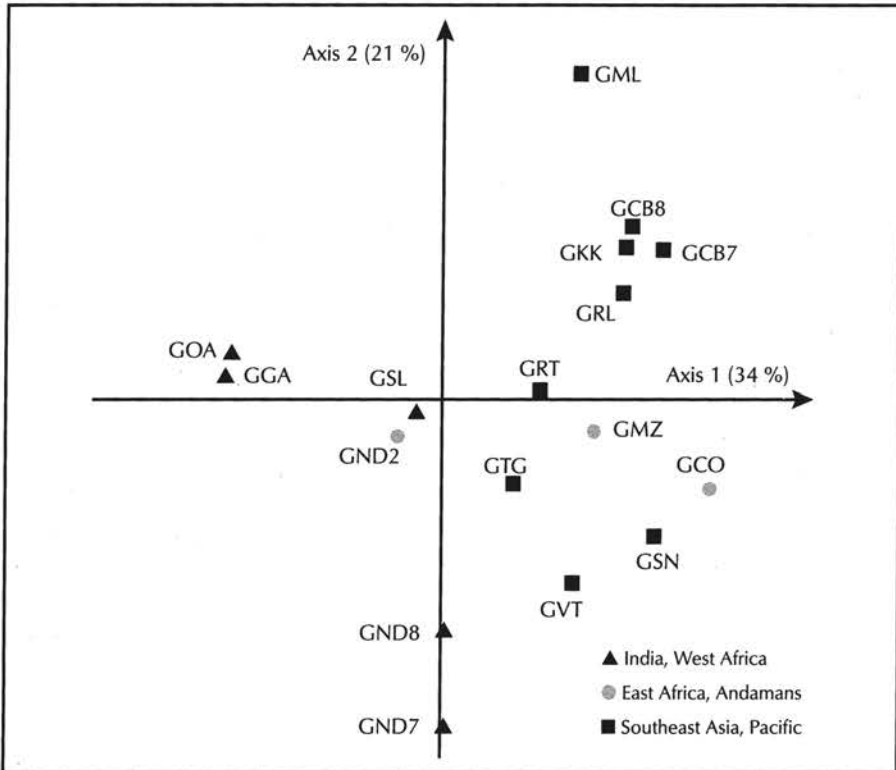


Fig. 5. Synthesis of morphological data (N'cho et al., 1993).

Following this representation, the cultivars of India and West Africa are characterized by a relatively slender trunk and by several small fruits, enclosing a small volume of water. Those of East Africa and the Melanesian islands are distinguished by a more robust trunk, with small fruits (except the Rennell Tall), while those of Southeast Asia and Papua New Guinea are robust and have large nuts. The cultivars of Polynesia occupy a median position. A third axis, which is not represented (13% of the variability) and is associated with the length of the different parts of the inflorescence, contains the Rennell Tall alone.

As with polyphenolic markers and RFLP, we find an east-west zonation, however less obvious. The intermediate position of the East African cultivars is not observed here.

## DISCUSSION AND CONCLUSION

### Contribution of Molecular Markers to the History of Coconut Diversification

The molecular analyses presented here are overall in agreement with the results of earlier studies. The major differences pertain to the better reproducibility of results and the independence of RFLP with respect to the environment, which results in greater precision. The RFLP markers thus contribute to a better understanding of the major events that marked the diversification of coconut (Lebrun et al., 1998b). In order to highlight the specific contributions of molecular markers, we place them in the context of a brief history of the coconut (Fig. 6).

The most probable region of origin is between Southeast Asia and Papua New Guinea (Zohary, 1970; Child, 1964). The coconut is adapted to dispersal by flotation, and the distribution of the islands in this region allows the nut to easily reach a coast favourable to its germination. Presumably, there was a noticeable variation in the composition of the fruit from the beginning. Gradually, the coconut tree migrated spontaneously by flotation towards the Pacific and, eventually, closer towards India. In these long-distance

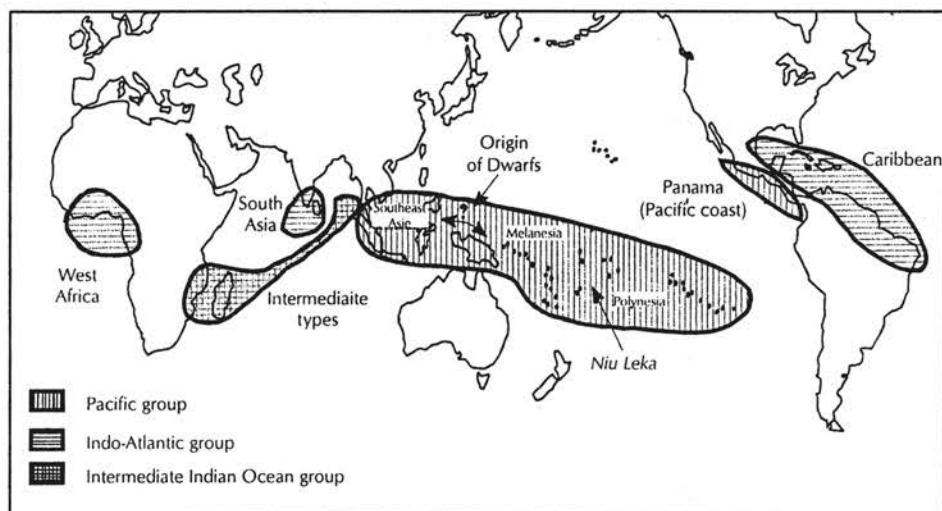


Fig. 6. Geographic location of the major coconut groups.

migrations, it was undoubtedly the form best adapted to marine transport that was favoured, the *Niu Kafa* type of Harries (1978).

At a very distant and uncertain date, the populations typical of the Indian subcontinent were isolated from the Pacific group. Even though it is not considered an indigenous plant, the coconut has grown in India for at least 3000 years (Child, 1964). The fact that the *Niu Kafa* type is predominant in India could suggest transportation by flotation, although it does not prove it. The later imports from Southeast Asia were limited and could not greatly modify the genetic structure.

- The distribution of cultivars into two major groups is not new. On the other hand, several RFLP markers have a great disequilibrium of frequency, which could go as far as specificity, between the Pacific and Indo-Atlantic groups. This indicates an ancient divergence probably due to prolonged geographic isolation.

From about 1300 BCE, colonization of the South Pacific consolidated the plantation of coconut. The fibre provided rope needed for construction of supple and durable boats. The immature fruits (for water) and mature fruits (for albumen) served as food supplies. The movement was gradual and uninterrupted. The two fruit forms, *Niu Vai* and *Niu Kafa*, coexisted and were probably selected for their own qualities. They were selected from an initially polymorphic stock, the genetic basis of which is similar to that of Southeast Asia and Papua New Guinea.

The populations of the Pacific group have the same markers, and the differences between the subregions pertain mostly to frequencies.

From 600 BCE, Malayan-Polynesian peoples migrated from Southeast Asia to Madagascar. The immigrants imported the coconuts with their costumes and their language, including the name of the plant (*voanio*). From there, the coconut reached East Africa and the Comoro Islands.

From 800 or a little earlier, the Arabs traded between India and East Africa (Ibn Battuta, 1351). They transported coconuts typical of these regions. Populations with intermediate genetic structure developed in East Africa by hybridization between the imported and local coconuts.

- The intermediate Indian Ocean group accumulates markers in its genome that characterize the Indo-Atlantic and the Pacific groups. From its distribution in two distinct geographic zones and the historical data, we can consider this intermediate group the result of a fusion between populations arising from the two original groups.

At an indeterminate period, flows were established from India towards Southeast Asia (perhaps through the Arab travellers). The populations of the Andamans, Indian islands to the northwest of Sumatra, thus have a genetic structure close to that of West Africa. Further east, from Indonesia to the Philippines, very limited exchanges occurred. An inverse exchange was the origin of the Kappadam Tall, which is distinguished from populations of the same region by its voluminous and round fruits.

- Three characteristic markers of the Indo-Atlantic populations were observed at a low frequency in Southeast Asia, but absent further east. They indicate a low genetic input of Indian origin.
- The Kappadam Tall has an exotic cytoplasmic marker and, in the nuclear markers, shows traces of introgression from the east, on a genetic basis that is typically Indo-Atlantic. The combination of these peculiarities may be explained as follows: this cultivar must have arisen from an introduction originating in Southeast Asia, followed by a selection of mother trees with round fruits during several successive generations. The open pollination, ensured for the most part by local populations, must in the long term have given it a nuclear genotype close to that of the Indo-Atlantic group. Towards 1498, in the wake of Vasco da Gama, the Portuguese circled the African continent and reached India by the sea route. The coconut was taken on the return voyage and established in West Africa. Between 1525 and 1550, directly or perhaps from West Africa (Cape Verde), it reached the Caribbean and the Atlantic coast of America. Strong historical arguments and the morphological resemblance between the coconuts of Brazil, the Caribbean (not included in this study), and Africa suggest that they belong to the same group.
- The similarity of RFLP profiles of the West African and Indian cultivars seems to confirm the results obtained by other methods. During the pre-Colombian era, the coconut was present on the Pacific coast of Panama, as related by Oviedo in 1535 in *La historia general y natural de las Indias, Islas y Tierra Firme del Mar Océano* (cited by Zizumbo and Quero, 1997), but absent in Mexico. The present-day populations could come from these coconuts. The distance between the Latin American coast and the Pacific islands, as well as the typically *Niu Vai* character of the local material, seem to exclude transportation by flotation. On the other hand, human transport seems possible, either by the Polynesians or by Southeast Asian populations, of which archaeological traces have been found as far as Ecuador (Langdon, 1995). During the 16<sup>th</sup> century, imports from Panama, the Philippines, and the Solomon Islands were reported in Mexico.
- The RFLP markers underline the specificities of the coconuts from Panama. All the evidence points to their origin in the Pacific group, although at present it cannot be decided whether they came through the Polynesian or the Southeast Asian route. Their considerable homogeneity and homozygosity are evidence of a strong foundation effect.
- Within the Panama origin, the Aguadulce population has, at low frequency, alleles specific to the Indo-Atlantic group, which is the highly probable signature of an introgression of genes coming from populations of the Atlantic coast. At a date that is difficult to fix, but after the separation of Pacific and Indo-Atlantic stocks, the Dwarfs appeared between the Southeast Asia

and Papua New Guinea populations, similar to those of the Philippines with respect to the number and diversity of Dwarfs found there. The underlying differentiation operates on a regional basis during the course of diffusion of this material, probably by intercrossing with the local Talls.

- Reliable indexes, linked to the number of alleles that they fix, are drawn from RFLP in favour of a single origin for Dwarfs, with the exception of *Niu Leka*. The Tall cultivars with an allelic composition most compatible with that of the Dwarfs are located in a zone ranging from Southeast Asia to Papua New Guinea.

At an indeterminate date, the *Niu Leka*, an allogamous Dwarf coconut, appeared in the region of Fiji, the Samoas, and Tonga.

- The resemblance between the RFLP profile of *Niu Leka* and that of the Tonga and Rotuma Talls originating from the same region testifies in favour of a local origin for this cultivar, and thus one independent of the origin of the other Dwarfs.

## An Approach to the Relation between Genetic Distance and Heterosis

In many plants, it can be observed that crosses between genetically distant populations are better than those with a narrow genetic base. This 'interpopulation heterosis' is widely exploited in recurrent reciprocal selection programmes. It is useful to verify whether such a phenomenon is observed in coconut. Table 1 shows the results of hybrid tests of Tall  $\times$  Tall as a function of the origin of parents. The intermediate Indian Ocean populations are represented by the Mozambique Tall.

In five trials comparing the Tall  $\times$  Tall hybrids, all the hybrids are superior to the West African Tall control. Among them, crosses between cultivars of

**Table 1. Production of copra of some Tall  $\times$  Tall hybrids classified according to the groups defined by RFLP: results of five assays**

Assay Year of plantation	PBGC 1 1965	PBGC 3 1969-70	PBGC 7 1971	PBGC 8 1972-73	PBGC 9 1971
Pacific $\times$ Indo-Atlantic	(2)* 130%	(2) 182%	(2) 174%	(3) 212%	(3) 182%
Indian Ocean $\times$ Pacific	(2) 124%	(2) 151%	(2) 153%		
Indian Ocean $\times$ Indo-Atlantic	(1) 136%				
Pacific $\times$ Pacific		(2) 138%	(1) 137%	(3) 142%	
Indo-Atlantic $\times$ Indo-Atlantic					(3) 142%
Control West African Tall (%)	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%
Production (kg/tree/year)	23.0	15.8	11.5	8.2	11.5
Self-fertilized control	(1) 88%				

\*For each assay, the number of crosses per type of recombination is given in parentheses.



the same group had the lowest yields, while crosses between the Indo-Atlantic and Pacific cultivars generally had the best production. The Indian Ocean group was represented by the Mozambique Tall. Its crosses with the cultivars of the two preceding groups had a performance between that of intragroup crosses and that of intergroup crosses.

On the other hand, even though the molecular data strongly suggest that the Dwarfs originate from the Pacific group, the latter may give excellent hybrids with the partners of either of the major groups defined above. This is the case, for example, of Malayan Yellow Dwarf  $\times$  West African Tall (PB121) and Malayan Red Dwarf  $\times$  Rennell Tall. Whatever the factors responsible for the 'interpopulation heterosis' mentioned above, the regime of autogamous reproduction that prevails in the Dwarfs seems to have induced a sufficiently significant genetic divergence in relation to their group of origin—due to the fixation of one allele per locus, rather than the appearance of new alleles—for heterosis to take place with these as well as with the Indo-African group.

## CONCLUSION

The results obtained with molecular markers largely agree with the results found earlier with other methods. However, they bring greater precision to the study of genetic relationships between populations. Studies of agronomic and morphological criteria are essential for characterization of the variability of a species such as coconut, to the extent that they provide elements essential for the use of cultivars studied in varietal improvement. The image of the genetic organization of populations drawn from only these criteria, however, is less precise than that made possible by molecular markers. On the one hand, environmental effects bias the comparisons when the studies are done in varying conditions of place or periods; on the other hand, natural or human selection may in some cases lead to similar phenotypes from populations of distinct origin. The similarity of phenotypes may conceal actual genetic complementarities. Finally, expression of the genetic value may be affected by the greater or lesser consanguinity of populations.

Polyphenol markers have proved to be highly effective in a preliminary study. However, the results obtained could not be reproduced in different environments. In plants, polyphenols are implicated in reactions to different stresses. It can thus be expected that the profiles obtained vary as a function of external stimuli. Finally, their implication in defence against stress suggests that they could not remain neutral with respect to selection. On the other hand, RFLP markers are chosen independently of the activity of the sequence concerned. Even though it is difficult to prove rigorously, the hypothesis of neutrality is more easily maintained.

Molecular markers offer several advantages for the improvement of coconut. They are precious tools in managing collections. In a perennial plant

such as the coconut, it is important to collect the widest possible genetic variability in a limited area. In certain situations, RFLP allows us to identify the varieties precisely. It is even possible, in certain populations, to detect the presence of genes of foreign origin and to explain the probable cause of them. Considering the small numbers used, this technique has proved to be very effective.

Moreover, the distribution of genetic stock of the species into two major geographical groups that have been subject to prolonged genetic isolation contributes a solid basis for the choice of a mechanism of recurrent reciprocal selection for the production of Tall  $\times$  Tall hybrids. It allows the prior assignment of a place to most of the cultivars in this scheme, in order to maximize the heterosis. The few intermediate cultivars located in East Africa and in South Asia and the Far East can be used to enrich the variability of one of two groups, especially the Indo-Atlantic, in which the genetic basis and phenotype variability is the least. Within each of the heterotic groups, it is possible to choose the cultivars that should be recombined on a priority basis to maximize the selectable variability.

Finally, molecular markers in some cases provide precise indexes on the history of plant material, which could be useful in the search for new sources of particular characters. The study of populations of the Pacific coast of Latin America with respect to tolerance to lethal yellowing in the Caribbean zone illustrates this field of application. The populations of Panama seem to have factors of tolerance to this disease. The RFLP profile of three of these populations suggests that they arise from a small initial population. Thus, the diversity and dynamics of populations in the Talls of the Pacific coast can be studied from a wider sample to enable more precise characterization of the nature of initial inputs and more certain identification of populations that can transmit disease tolerance. This broadening of the research could prove to be particularly useful in light of the fact that the Panama Tall is sensitive to *Phytophthora*, which is rampant in the same region.

## APPENDIX

### Plant Material

The leaf samples collected from 289 trees represent 26 Tall cultivars and 16 Dwarf cultivars from the collections of Côte d'Ivoire (Marc Delorme station), Vanuatu (Saraoutu station), and Jamaica. They cover most coconut cultivation zones, except the Caribbean zone and the east coast of America. The list of cultivars, with their geographic origin, is given in Table 2.

### RFLP Analyses

The extracts of total DNA are taken from lyophilised leaflet, taken from leaf no. 1 (youngest green leaf). The method used is that of CTAB (cetyl-trimethyl ammonium bromide), adapted on maize by Hoisington (1992). The restrictions are done using four enzymes: *EcoRI*, *EcoRV*, *BglII*, and *SstI*. The restricted DNA migrates in a 0.8% agarose gel in TAE buffer (tri-acetate EDTA), then it is transferred on a nylon membrane. The probes, used for the molecular hybridization following the protocol of Hoisington (1992), are marked with <sup>32</sup>P. The results are detected from autoradiograms.

### Origin of Probes

This study was done using 20 cDNA probes and a mitochondrial probe (Cox 1). Among the nuclear probes is a cDNA of coconut, the others being heterologous probes of rice, oil palm, and maize, the origin of which is cited in Lebrun et al. (1998b).

### Data Analysis

Each band is coded as a dominant marker: 10 for its presence and 01 for its absence. The binary matrix of bands × individuals thus obtained makes it possible to do a CA (Benzecri, 1973) using Addad software (Addad, 1983). The graphic representations of these multivariate analyses are used to describe the genetic structuration of the material.

Table 2. Number and geographic distribution of cultivars sampled

Origin	Talls	Code	No.*	Dwarfs	Code	No.*
West Africa	4 cultivars			2 cultivars		
● Côte d'Ivoire	West African Tall	GOA	5			
	Mensah West African Tall	GOA04	10			
● Benin	Ouidah West African Tall	GOA06	10			
● Cameroon	Kribi Cameroon Tall	GCA	5	Cameroon Red Dwarf	NRC	5
● Ghana				Ghana Yellow Dwarf	NJG	7
East Africa	2 cultivars					
● Comoro Islands	Moheli Comoro Tall	GCO	5			
● Mozambique	Mozambique Tall	GMZ	5			
South Asia	4 cultivars			1 cultivar		
● India	Micro Laccadives Tall	GND07	5			
	Kappadam Tall	GND05	5			
	Ordinary Andaman Tall	GND02	4			
● Sri Lanka	Sri Lanka Tall	GSL	5	Sri Lanka Green Dwarf	NVS	5
Southeast Asia	8 cultivars			7 cultivars		
● Thailand	Thailand Tall	GTH	5			
● Philippines	Baybay Tall	GPH04	5	Catigan Green Dwarf	NVP02	5
	Tagnanan Tall	GTN	5	Pilipog Green Dwarf	NVP05	5
				Tacunan Green Dwarf	NVP03	5
● Cambodia	Cambodia Tall	GCB	10			
● Indonesia	Tenga Tall	GDO02	5	Ternate Brown Dwarf	NBO	5
	Palu Tall	GDO03	5			
	Takome Tall	GDO04	5			

(Contd.)

(Table 2. Contd.)

Origin	Talls	Code	No.*	Dwarfs	Code	No.*
● Malaysia	Malaysia Talls	GML	11	Malayan Yellow Dwarf Malayan Green Dwarf Malayan Red Dwarf	NJM NVM NRM	15 5 5 + 5**
South Pacific	9 cultivars			5 cultivars		
● Papua New Guinea	Karkar Tall	GNG01	5	Madang Brown Dwarf	NBN	5
● New Guinea	Markham Valley Tall	GNG03	5			
	Gazelle Tall	GNG04	5 + 5**			
● Solomon Islands	Rennell Tall	GRL	7 + 5**			
	Solomon Tall	GSL	6			
● French Polynesia	Polynesia Rangiroa Tall	GPY01	5	Polynesian Red Dwarf	NRY	5
● Fiji	Rotuma Tall	GRT	5	<i>Niu Leka</i> Dwarf	NNL	7
● Tonga	Tonga Tall	GTG	5			
● Vanuatu	Vanuatu Tall	GVT	5	Vanuatu Red Dwarf Kiribati Green Dwarf	NRV NVT	5** 5**
Latin America	3 cultivars			1 cultivar		
● Panama	Panama Tall	GPA	10***			
	Panama Tall (Aguedulce)	GPA01	6			
	Panama Tall (Monagre)	GPA02	6			
● Brazil				Brazilian Green Dwarf	NVB	5

\*All samples from Côte d'Ivoire, unless otherwise mentioned.

\*\*Sampled at Vanuatu.

\*\*\*Sampled at Jamaica.



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