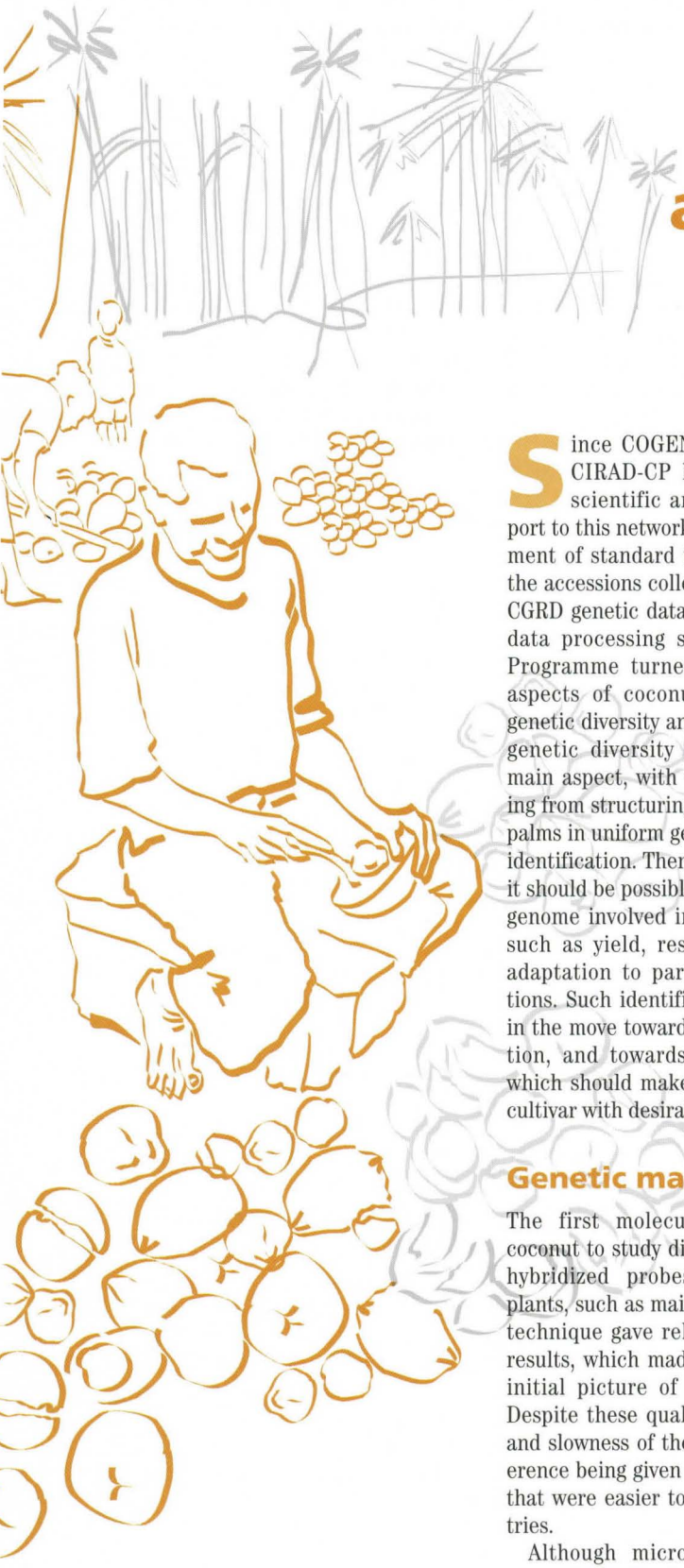
Molecular markers: recent progress, and soon an identification kit



Since COGENT was set up in 1992, CIRAD-CP has been providing its scientific and methodological support to this network. Following the development of standard tools for characterizing the accessions collected (Stantech manual, CGRD genetic database and the CDM field data processing software), the Coconut Programme turned its attention to two aspects of coconut molecular genetics: genetic diversity and genome mapping. The genetic diversity study is currently the main aspect, with various objectives ranging from structuring a collection of coconut palms in uniform genetic groups, to cultivar identification. Then, with genome mapping, it should be possible to identify areas of the genome involved in the variation of traits such as yield, resistance to diseases or adaptation to particular climatic conditions. Such identification is the first stage in the move towards marker-assisted selection, and towards genetic modification, which should make it possible to enrich a cultivar with desirable traits.

Genetic markers used

The first molecular markers used on coconut to study diversity were RFLP, with hybridized probes usually from other plants, such as maize, rice or oil palm. This technique gave reliable and reproducible results, which made it possible to gain an initial picture of the species' diversity. Despite these qualities, the laboriousness and slowness of the operations led to preference being given to new types of markers that were easier to use in producing countries.

Although microsatellite markers are expensive to develop, they are reproducible and simple to use. They are abundant, and have numerous codominant alleles well distributed on the genome. Their only major disadvantage lies in the cost of obtaining them, though the cost of using them remains competitive. A clone-enriched library containing the motif GA (guanine-adenine) has been produced. It was obtained by the magnetic bead capture method and represents around 800 clones, 80% of which contain a microsatellite motif. After sequencing part of the 140 positive clones, then elimination of sequences unsuitable for defining primers (microsatellite motif too close to the edge of the insert, redundant clone, impossible to define a primer in the sequence flanking the microsatellite) around a hundred clones are now available. Their readability, reproducibility, and their degree of polymorphism (between 2 and 17 alleles per marker), were checked using a sample of 31 palms representing coconut diversity (figure 1).

The oil palm is another source of microsatellite markers since a third of them are usable on the coconut palm.

Although their polymorphism is relatively low in coconut, AFLP markers provide further information that is useful for mapping. The difficulty in reading them, and problematic reproducibility, led to their being ruled out for diversity studies.

Genetic mapping of the coconut palm

In order to produce the genetic map of a species, the coefficient of recombination is calculated between the different markers studied on the progeny of a cross. The lower the coefficient, the more the corres-

ponding loci are genetically linked. This calculation can only be carried out if the parents are heterozygotes. The coconut palm is not a particularly prolific species, and crosses generally have few progenies. Appropriate populations are rare. For instance, the only genetic map of the coconut palm currently published by the Neiker team (Spain) uses a cross represented by 52 progenies, carried out especially for that purpose. The observations available on this cross are still very limited.

A new map was undertaken in collaboration with Neiker (Spain) and Long Ashton Research Station (UK) to search for quantitative trait loci (QTL). The chosen population had been planted in a genetic trial for 15 years at the M. Delorme station in Côte d'Ivoire, and therefore benefited from several years of individual observations. It was a cross between a Rennell Tall parent (PO 2664) and 12 Cameroon Red Dwarfs. This latter cultivar is self-fertilizing and can be considered as a pure line. Its uniformity was confirmed by microsatellite markers and RFLP. Only a few low intensity AFLP bands proved to be variable between the different Cameroon Red Dwarfs and were removed from the analysis. In the progeny of the cross, 2 out of 69 individuals revealing alleles that were absent from the parents were considered illegitimate and discarded. The genetic map of the Rennell Tall parent was constructed using heterozygous markers present in that individual and, for the AFLP markers, absent from the Dwarf parents.

The Rennell parent proved to be less heterozygous than expected. Of the 64 primer combinations available in the laboratory (8 Eco R1 and 8 Mse1), only 21 varied in the progeny. However, 190 polymorphic markers, 78 of which were obtained at CIRAD (11 microsatellites, 6 RFLP and 61 AFLP), were positioned on 16 linkage groups. Few markers were not assigned to any group. The longest linkage group, LG3, measured 202 centimorgans and contained 22 markers; the shortest, LG 16, only contained 5 markers distributed over 34 centimorgans. Most of the markers were regularly distributed over the linkage groups, apart from a cluster on GL 3 containing 11 markers over 29 centimorgans (figure 2).

The 16 linkage groups identified most probably corresponded to the 16 chromosomes of the coconut palm. The results obtained were an initial stage in obtaining a saturated genetic map. Its construction, along with the QTL search, will be carried out under an INCO project selected by the European Union in 2001.

Coconut genetic diversity

The initial diversity analyses carried out on 26 Tall cultivars (191 individuals) and 16 Dwarf cultivars (98 individuals), using RFLP, revealed that all the coconut palms divided into three genetic groups (figure 3):

- the "Indo-Atlantic" group, which contained 5 cultivars from West Africa, India and Sri Lanka,
- the "Indian Ocean" group, which contained 3 cultivars,
- the "Pacific" group, which contained all the Dwarf cultivars, those of Southeast Asia, Papua New Guinea and the Pacific islands.

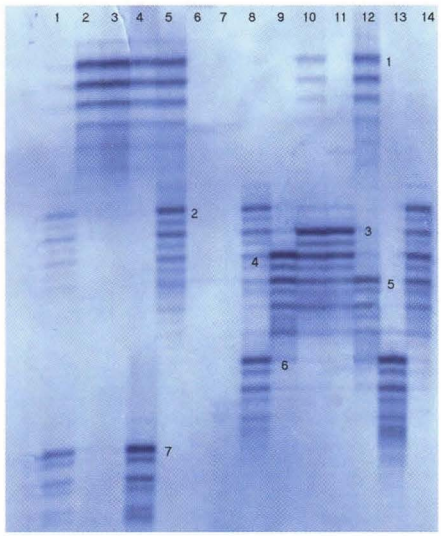


Figure 1. Microsatellite marker CnCirC5 with 7 alleles.

An analysis of gene flows between cultivars enabled us to define smaller, uniform units within the large Pacific group. Given the laboriousness of operations with RFLP markers, it was worth finding out whether such results could be obtained with markers that were easier to use, such as microsatellites. The speed with which they evolve is an important difference between the two types of markers. Microsatellites, which evolve much faster than the other types of markers, are very

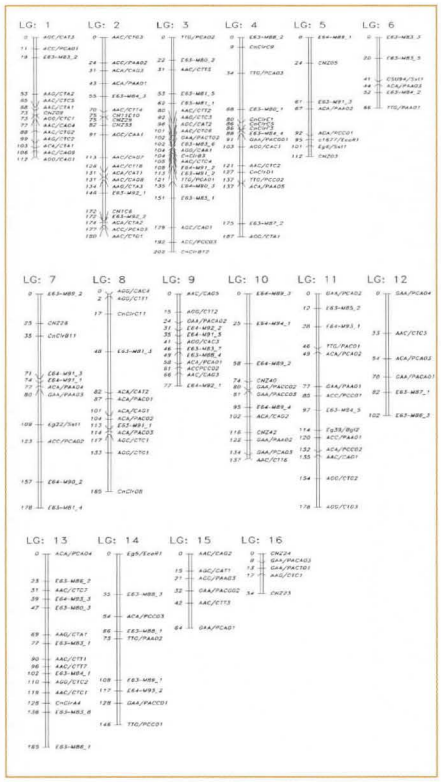


Figure 2. Beginnings of a coconut genetic map.

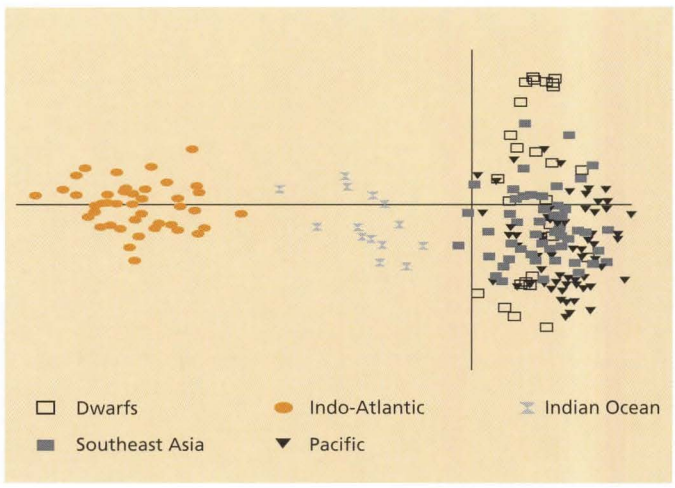


Figure 3. Factorial analysis of correspondences carried out using RFLP data.

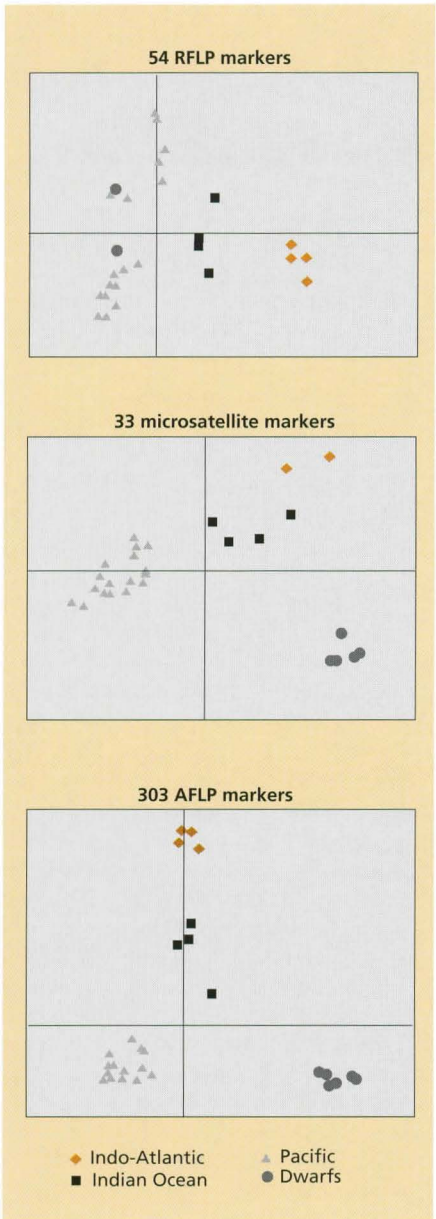


Figure 4.
Image of diversity obtained with the three types of marker.

suitable for shedding light on microevolution processes, whereas RFLP markers can be used to monitor macroevolution.

This study was carried out on a subsample of 31 individuals, chosen to represent the overall diversity of the species. Fifteen cultivars belonging to the three genetic groups were represented by 2 or 3 individuals. The AFLP and microsatellite molecular analyses were carried out at IACR's Long Ashton station, the RFLP at CIRAD. The results for the three techniques were represented by principal components analyses. The three major groups were clearly found: the Pacific, the Indian Ocean and the Indo-Atlantic group (figure 4).

Prospects and applications

The experience acquired during this research led to CIRAD-CP being chosen by IPGRI and the COGENT network to produce a microsatellites kit for the identification of coconut cultivars. This kit needs to be as discriminant as possible, and easy and cheap to use. The first requirement for success is to have a sufficient number of markers: around 130 microsatellite markers developed at CIRAD and at the Long Ashton research station are available. Only ten or so of these will be selected for their sufficient number of clearly readable alleles, and their high discriminant power, which assumes that polymorphism occurs between cultivars rather than within the cultivar.

In order to evaluate the discriminant power, it is necessary to test these microsatellites on an appropriate representation of diversity on a world scale: leaf samples from 400 individuals, representing more than 80 cultivars, have been collected. It will then be necessary to use an identification method adapted to the genetic structure of the populations in question. The Tall coconut palm is cross-fertilizing, and in traditional cultivars all individuals differ from each other: identification will therefore have to be based on the probability of belonging, and make use of allelic frequencies. A statistical identification method (Bayesian method) is currently being developed and has already given some promising results.

This kit will be used to describe coconut diversity in Papua New Guinea (on a sample of 29 Tall cultivars and 3 Dwarfs), in Vanuatu (on a dozen populations collected during participatory surveys) and in four archipelagos of the Pacific: Cook, Tuvalu, Kiribati and Marshall. In each case, the populations will be positioned in relation to worldwide diversity. The polymorphism existing between the populations and within them will be assessed. The results will make it possible to more effectively orient the strategy for the collection and conservation of diversity, and its use in breeding. ■

List of publications

Lebrun P., N'Cho Y.P., Seguin M., Grivet L. Baudouin L., 1998. Genetic diversity in coconut (Cocos nucifera L.) revealed by restriction fragment length polymorphism (RFLP) markers. Euphytica 101 :103-108.

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Teulat, B, Aldam C., Trebin R., Lebrun P., Barker J.H.A., Arnold G.M., Karp A., Baudouin L., Rognon F., 2000. An analysis of genetic diversity in coconut (Cocos nucifera) populations from across the geographic range using sequence-tagged microsatellites (SSRs) and AFLPs. Theor. Appl. Genet. 100 : 764-771.