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Mapping translocations *in the banana genome using* molecular cytogenetic techniques

Genetic studies on banana have been relatively limited to date. They have focused especially on assessing kinship between different wild varieties (ancestral diploid varieties) and current cultivated varieties (triploid).

Very little detailed information is currently available on the mechanisms of character transmission in banana. Breeding is thus still carried out using a traditional phenotypic approach based mainly on analyses to find interesting complementary traits derived from parents in different genomic configurations.

For over 15 years, CIRAD has been developing an original strategy geared towards obtaining new triploid varieties from natural or improved fertile diploids. A high number of progeny can thus be obtained (contrary to standard crossing strategies with current varieties that are mainly sterile), thus enabling selection and genetic analysis of characters.

New banana genetic improvement strategies based on genetic mapping and analysis of genetic factors determining traits of agronomic interest are being developed to overcome issues that hamper current improvement methods. One of the main problems concerns the presence of structural heterozygosity between homologous chromosomes (due to chromosome anomalies that occur during meiosis, i.e. so-called translocations) as well as the lack of knowledge on their impact on character transmission. The "banana cartogenetic mapping" project aims to determine the mechanisms underlying translocations from a formal genetics standpoint, and to ultimately transfer the research results for the benefit of banana breeders. •••

▷▷ Visiting scientist

Alberto Vilarinhos (EMBRAPA) was hosted by UMR PIA for 3 years, during his PhD training, with the aim of developing a BAC library to identify and locate translocation sites in the banana genome.

The agricultural research and engineering school of the French Ministry of Agriculture and Fisheries, Montpellier SupAgro, provided support for the PhD thesis of Alberto Vilarinhos.

▶ *'Dessert' banana plantation in Brazil*

CIRAD's goal was to streamline the banana breeding process. This project is being carried out within the framework of the Global *Musa* Genomic Consortium coordinated by Biodiversity International under the PROMUSA programme.

The research was aimed at identifying and characterizing translocations in banana so as to enhance genetic analyses and gain insight into the hereditary factors controlling target traits.

Plant material studied

Two banana cultivars were studied:

• **The Calcutta 4 clone *(Musa acuminata burmannicoides)*:** diploid (2n=2x=22) belonging to the North-A translocation group, structurally homozygous (no structural difference between homologous chromosomes), differing from the Central group by two translocations. Banana varieties were classified in different groups according to the chromosome pairing pattern observed during meiosis in hybrids. The absence of translocation or the presence of one to two translocations was typical in these groups.

• **The Madang clone (*Musa acuminata banksii*)**: diploid (2n=2x=22), structurally homozygous, belonging to the Central translocation group, i.e. the control group without any translocations.

Project goals

The project was aimed at developing tools to map translocations in banana and applying these tools to map translocations in order to differentiate Calcutta 4 and Madang clones.

Different technologies had to be developed and/or adapted to be able to meet these objectives, mainly:
• the construction of a library of large banana DNA fragments or a bacterial artificial chromosome (BAC) library;
• the development of cytogenetic mapping via *in situ* fluorescent hybridization of BAC clones for translocation mapping.

The project was also based on a genetic map constructed at CIRAD from an F2 population (second generation obtained by selfing) derived from a cross [Calcutta 4 x Madang] between two fertile and highly homozygotic varieties.

Banana

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▶ *Bunches of cv. Bluggoe (cooking) bananas that are triploid and interspecific* (M. acuminata/M. balbisiana)

Bananas are currently cropped in around 100 tropical and subtropical countries (India Uganda, Ecuador and Brazil are top producing countries). Banana is a food product (a low-fat nutritious edible fruit with a high sugar, potassium, vitamin A, B6 and C content) which produces fibres that are used industrially (paper, cardboard, rope) and generates considerable income due to the low production expenditures. Banana cropping consolidates the economy in European overseas regions (Guadeloupe, Martinique) where exports of bananas (over 80% cv. Cavendish) generate substantial income.

Bananas belong to the Musaceae family. They are high monocotyledon herbaceous plants that are monocarpic (only flower once) and the pseudostem is formed by the coiling of leaf sheaths around each other, with an edible flower (bunch) emerging from the centre. Banana breeding programmes have been set up to create new varieties, especially lines that are resistant to the many pest infestations affecting this fruit. Genetic variations concern the plant size, sucker vigour, number of hands per bunch, fruit size and sources of resistance to the main pests and diseases. Problems associated with typical characteristics of cultivated bananas must be overcome through the different breeding phases, i.e. sterility, triploidy (threefold more chromosomes than in common plants of close species) and the multispecific origin.

In banana, fertility has been counterselected by breeders for generations in order to obtain seedless and very pulpy edible fruits. Cultivated bananas are parthenocarpic (bulkier fruits develop without fertilized ovules or their transformation into embryonic seeds) and preserved cultivars therefore have to be vegetatively propagated from suckers. Cultivars are di-, tri- (the most numerous as they are the hardiest) or tetraploid and their genomes differ (denoted A and/or B). Most cultivars derive from haploid (11 chromosomes) wild species, i.e. *Musa balbisiana* (one B genome) and *Musa acuminata* (one A genome).

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Partnerships

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A wild banana (M. acuminata malaccensis) *bunch*

Research conducted

A BAC library of the Calcutta 4 (diploid AA) accession was constructed during the first phase of the project. BAC libraries now represent an essential genomic research tool. The first BAC library obtained consisted of a collection of large DNA fragments extracted from leaf cell nuclei from the *Musa acuminata* Calcutta 4 clone. It includes 55152 clones of fragments with a mean size of 100 kb spanning 9-fold the haploid nuclear genome (by virtual overlap of different DNA fragments).

Based on previous adjustments, two other BAC libraries were constructed by UMR PIA (attached to CIRAD/INRA/Montpellier SupAgro) for the *Musa* genus: from cv. *Grande Naine* (triploid AAA, belonging to the Cavendish subgroup); the second from the Pisang Klutuk Wulung (PKW) accession (diploid BB, belonging to *Musa balbisiana)*.

These BAC libraries are efficient tools that are now freely available for use by the international community involved in the Global Musa Genomics Consortium. They are essential for the development of different applications, e.g. gene cloning, genome sequencing, physical mapping, comparative genomics, etc. One new project undertaken using this tool involved comparing the banana genome organization with that of rice, and assessing their colinearity. Partial sequencing of the banana genome was thus initiated in this international setting.

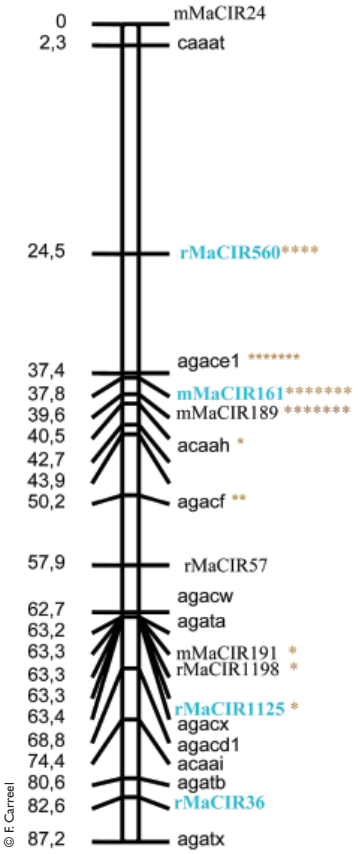
A second project was aimed at investigating and characterizing integration of the banana streak virus (BSV) in the banana genome and identifying the mechanisms responsible for activation of the integrated sequences. These BAC clones are also tools that can be effectively used to screen for single sequence repeat (SSR) microsatellite markers in specific genomic regions.

The second research phase involved the development of a BAC-FISH cytogenetic mapping technique. In the *Musa* family, cytogenetic studies are hard to carry out because the chromosomes are small (600 million bases for 11 chromosomes) and morphologically very similar. The fluorescent *in-situ* hybridization (FISH) method was used for cytogenetic mapping of BAC clones anchored to the genetic map via genetic markers *(cf. FISH hybridization technique)*. •••

▷▷ Publications

Vilarinhos A.D., 2004. PhD thesis in Integrative Biology at the *Université Montpellier II* – Montpellier SupAgro en biologie intégrative. "Cartographie génétique et cytogénétique chez le bananier : caractérisation des translocations".

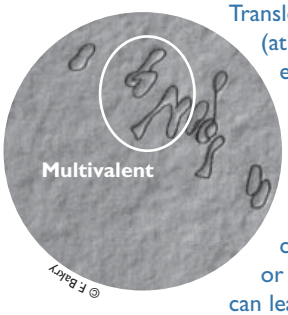
Vilarinhos A.D., Piffanelli P., Lagoda P., Thibivilliers S., Sabau X., Carreel F., D'Hont A., 2003. Construction and charactérization of a bactérial artificial chromosome library of banana (*Musa acuminata* Colla). Theor. Appl. Genet. 106:1102-1106.



Linkage group II on the genetic map for a Calcutta 4 x Madang cross

Translocation

Translocations are chromosome rearrangements involving the displacement, after cleavage, of one (or several) chromosome segment(s), followed by its (their) integration at a different site.



Translocations can spontaneously occur during gamete development (at meiosis) due, for instance, to accidental chromosome entanglement during the first phase of cell division, thus producing anomalies during the chromosome pairing phase. The most common so-called 'reciprocal' translocations correspond to an exchange between the extremities of nonhomologous (not belonging to the same pair) chromosomes. Many combinations are, however, possible and complex phenomena can involve over three breakages and/or more than two chromosome pairs. Recombination anomalies can lead to the formation of monovalent, trivalent (pairing of three homologous chromosomes instead of two in usual bivalent cases), or even hexavalent chromosome configurations during meiosis.

Translocations make it very hard to understand the key genetic factors underlying banana traits—these anomalies markedly complicate modelling of the transmission of characters of agronomic interest and their transfer to progeny.

Moreover, banana chromosomes are especially small, and standard cytogenetic procedures are not sensitive enough to identify chromosomes involved in translocation, or to explain the translocation features. It is thus important to develop a method to analyse these differences between chromosome structures. Translocations can be identified by *in situ* hybridization techniques.

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The anchor markers used were restriction fragment length polymorphism (RFLP) and SSR sequences, etc., associated with a locus.

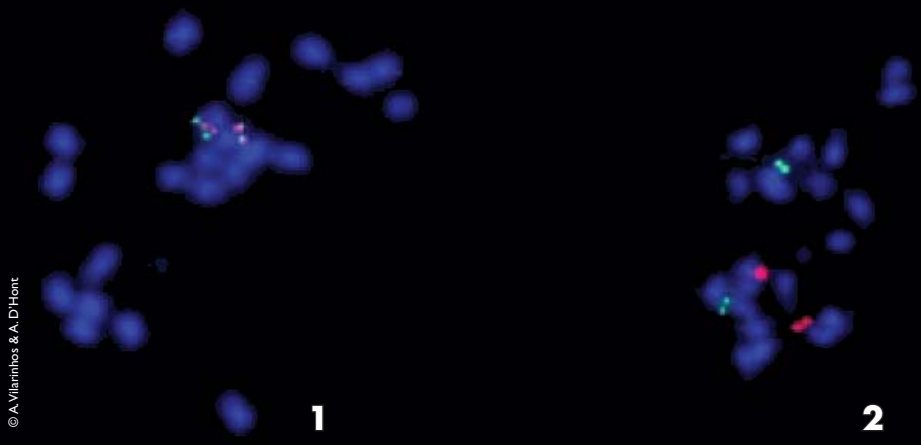
Loci to map were chosen according to the established genetic map. The study then focused on linkage group II, a potential bearer of translocations, based on the noted segregation distortion (linked with disturbances in the separation of chromatids during meiosis, likely due to structural differences between the parental genomes), analysis of the distribution of markers in linkage groups, and finally the comparison between genetic maps.

Some markers distributed on linkage group II were selected and used to screen the BAC library. Indeed, markers on the genetic map are too small to be used directly for *in situ* hybridization—it is thus essential to identify BAC fragments that contain them and then they can be utilized. The BAC-FISH results showed that linkage group II was derived from the artificial linkage of markers belonging to several chromosomes. These artificial linkages were due to the presence of at least two translocations in the Calcutta 4 clone as compared to the Madang clone, and these translocations likely involved three chromosomes. The present results highlight the importance of the translocation phenomenon in the banana genome structure.

The tools and techniques developed revealed the link between the genetic maps and the chromosomes. This translocation mapping and analysis of their impacts on recombination and segregation of involved regions will provide essential information on the choice of parents to be used in breeding programmes.

Transfer, development and project follow-up

This project led to the transfer of useful knowledge since the Brazilian researcher was trained on different library construction, genetic and cytogenetic mapping techniques and conducted theoretical research on the impact, in banana, of translocation on the allele distribution in the progeny. The results have already led to the publication of an article (a second one is in preparation), to a successfully defended PhD thesis, and the studies are still under way at CIRAD. A Brazilian researcher, Claudia Fortes Ferreira, also came to France to participate in the project in 2005. ■



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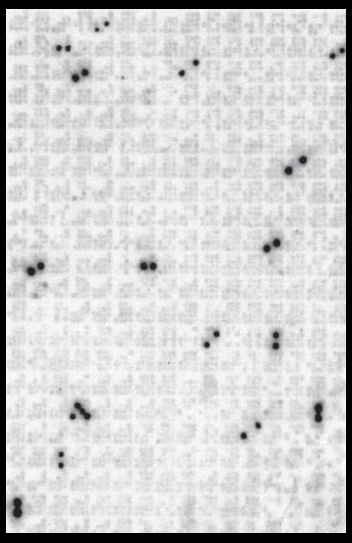
1. *Chromosomes of the Calcutta 4 (2n = 22) accession after FISH with BAC 52P01 (detected via FITC, green) and BAC54N07 (detected with Texas red staining, red)*

2. *Chromosomes of the Calcutta 4 (2n = 22) accession after FISH with BAC 52P01 (detected via FITC, green) and BAC59I20 (detected with Texas red staining, red)*

3. *Screening the BAC library with mapped probes*

Bacterial artificial chromosome-fluorescent *in-situ* hybridization (BAC-FISH) technique used with BAC library clones to identify 'labelled' sequences on banana chromosomes



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The fluorescent *in-situ* hybridization (FISH) technique is used to locate DNA sequences directly on chromosomes *(in situ)*. This technique is based on the capacity of two strands of complementary DNA to pair up and form double strands (pairing process). Target sequences are labelled with fluorochromes and can be detected on chromosomes under a fluorescence microscope.

FISH hybridization is a multistep procedure:
- labelling target sequences with a fluorochrome;
- plating chromosomes on a microscope slide;
- denaturation of DNA fragments (disassembly of two DNA strands);
- hybridization of the labelled sequence and chromosomes;
- detection of hybridization signals and location of the sequence on chromosomes.

Several probes are labelled with different fluorochromes so as to be able to detect several sequences simultaneously on one or several chromosomes.

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