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GeneTrop
SCIENTIFIC PAPERS

Third Edition : December 2000

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GeneTrop

Scientific Papers

Third Edition : December 2000

Report prepared by Carole BESSIERE

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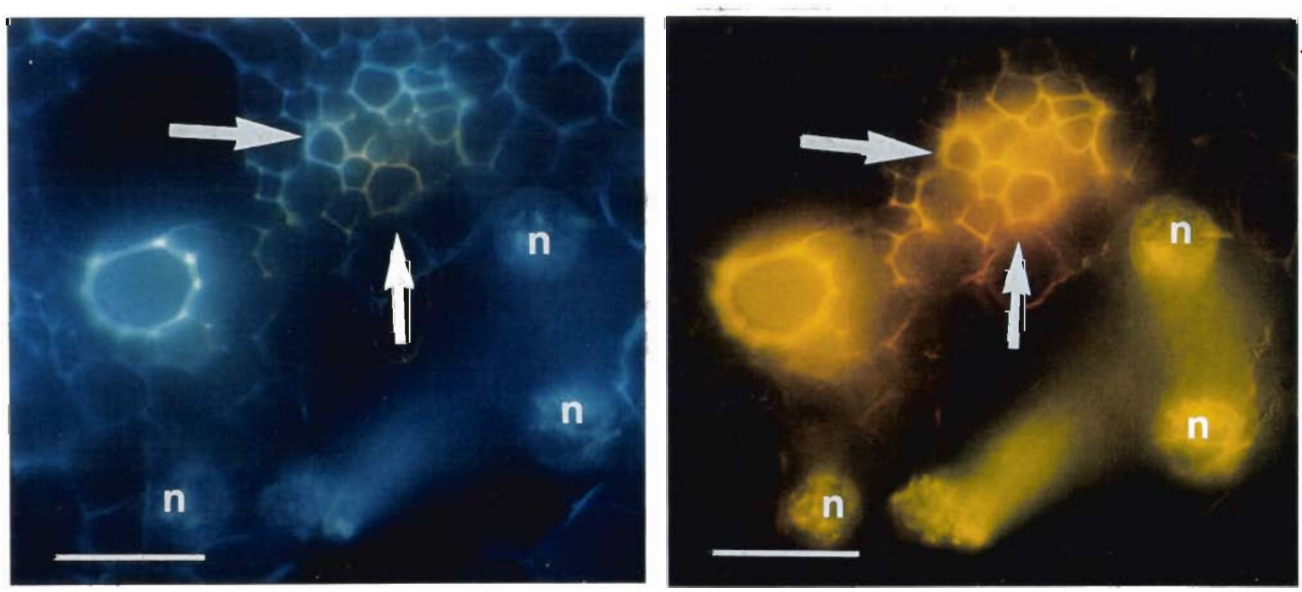
Genetic and diversity of coffee

Histochemical and cytochemical investigations of phenols in roots of banana infected by the burrowing nematode Radopholus similis.

C. Valette, C. Andary, J.P. Geiger, J.L. Sarah, and M. Nicole.

Phytopathology, 1998, 88: 1141-1148.

The burrowing nematode Radopholus similisis one of the most damaging pathogens on banana plantations. The role of phenolics in plant defense responses to the nematode was histochemically and ultrastructurally investigated in susceptible and partially resistant cultivars. Histochemical observations of healthy roots revealed that high levels of lignin, flavonoids, dopamine, cafeic esters, and ferulic acids were associated with a very low rate of root penetration in the resistant cultivar. The presence of lignified and suberized layers in endodermal cells contributed to limit invasion of the vascular bundle by the pathogen. After infection, flavonoids were seen to accumulate early in walls of cells close to the nematode-migrating channel in both cultivars, and in all tissues of the infected resistant roots, including the vascular tissues. The labeling pattern obtained with the gold-complexed laccase and with anti-pectin monoclonal antibodies showed that phenolics were distributed in a loosened pectin-rich material surrounding the nematode. This study provides indications that constitutive phenolics in banana roots are associated with the limitation of host penetration and colonization by R. similis. Accumulation of flavonoids in response to infection was detected in the vascular tissues of susceptible plants and in all root tissues in the partially resistant plants.



Production of flavonoids close to the migration channel of the nematode (n) in a susceptible banana cultivar. Those phenolics are identified using the Neu's reagent by a yellow-orange pale colour under UV illumination (left, arrowss) or lemon-yellow orange under blue light (right, arrows).

Apoplastic NADH-peroxidase generates superoxide anions in cells of cotton cotyledons undergoing the hypersensitive reaction to Xanthomonas campestris pv malvacearum race 18.

Martinez, C., Montillet, J.L, Bresson, E, Agnel, J.P., Dai, G.H., Daniel, J.F., Geiger, J.P et Nicole, M.

Molecular Plant-Microbe Interaction, 1998, 11, 1038-1047.

Cotton cotyledons displayed a hypersensitive reaction (HR) in the resistant variety Reba B50 after infiltration with the avirulent race 18 of *Xanthomonas campestris* pv *malvacearum* (Xcm). Generation of active oxygen species during the HR was studied biochemically and cytochemically. $O_2^{\cdot -}$ was detected in cotyledon discs by the cytochrome c reduction assay three hours after infection (Figure 1). This activity was inhibited by SOD and by the peroxidase inhibitors, SHAM and KCN, but not by the NADPH oxidase inhibitor DPI. Correlatively, a strong NADH oxidation activity that was assessed three hours after infection in crude extracts or in the apoplastic washing fluid, dramatically decreased after treatment with SHAM or KCN, and was activated by 2,4 dichlorophenol and $MnCl_2$. The increase in the activity of cationic peroxidase isozymes (pI 9.5) was detected by isoelectrofocusing three hours after infection of resistant cotyledons. Activities of apoplastic peroxidase(s) and H_2O_2 accumulation were observed cytochemically (Figure 2), three and four hours post infection, respectively. When digitonin, a $O_2^{\cdot -}$ elicitor, was infiltrated within cotyledons from the resistant and the susceptible varieties Acala 44, generation of $O_2^{\cdot -}$ radicals was shown to be reduced by SOD and inhibited by SHAM, KCN, or DPI. These results strongly suggest that cotton cotyledons contain two $O_2^{\cdot -}$-generating enzymes. But challenged cells that trigger the HR in response to an avirulent race of Xcm produce $O_2^{\cdot -}$ mediated by an apoplastic peroxidase, despite the presence of a NADPH oxidase.

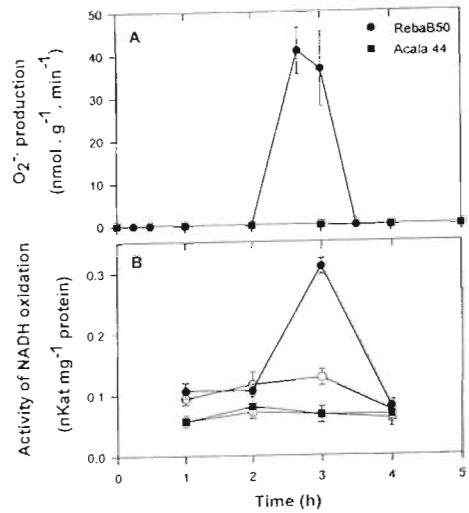


Figure 1 : **(A)** Time course changes in $O_2^{\cdot -}$ production ; values of inoculated plants were substracted from the corresponding water infiltrated controls.
(B) activity of NADH oxidation of crude enzymatic extracts of Reba B50 (l, m) and Acala 44 (n, p) inoculated with Xam, race 18. $O_2^{\cdot -}$ production ; open symbols correspond to NADH oxidation activity of water controls. Values are means ± SD of 2 separate experiments with 5 replicates each.

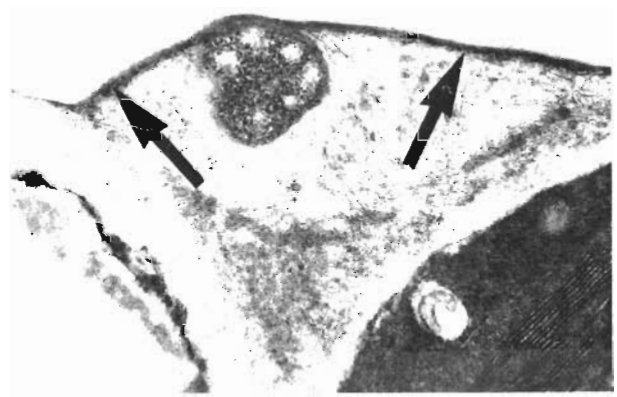


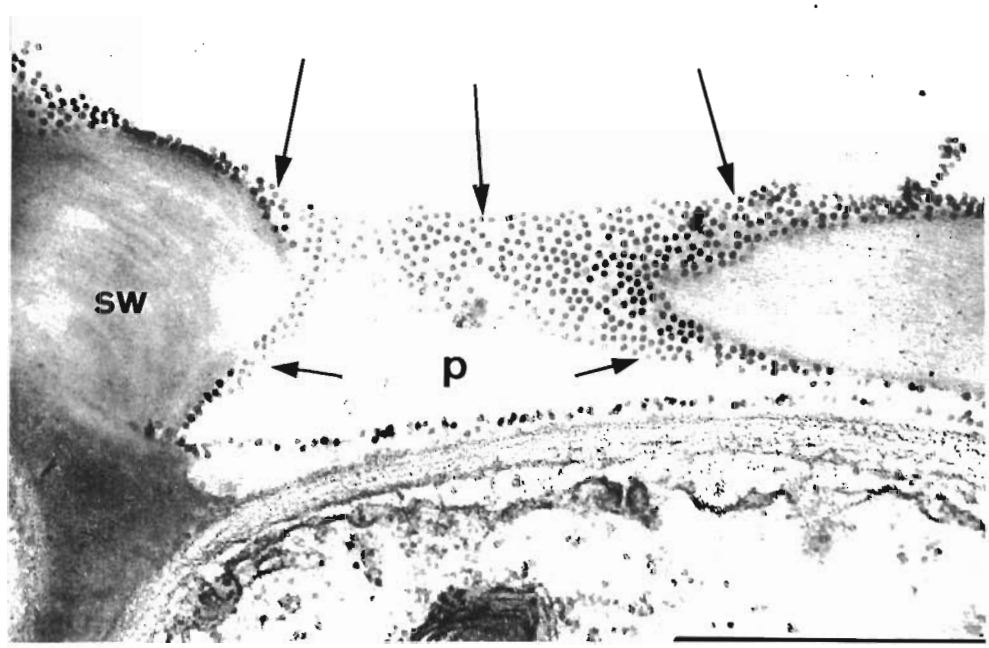
Figure 2 : Electron-dense middle lamella showing peroxidase activity (arrows) surround the site of attachement of the bacteria close to the cell wall (w) (Bar = 0.3µm).

Movement of rice yellow mottle virus between xylem cells through pit membranes.

Opalka N., Brugidou C., Bonneau C., Nicole M., Yeager M., Beachy R.N. and Fauquet C.

Proceedings of the National Academy of Science, 1998, 95 : 3323-3328.

The translocation of rice yellow mottle virus (RYMV) within tissues of inoculated and systemically infected *Oryza sativa* L. leaves was characterized by Western immunoblotting, Northern blotting, and electron microscopy of thin sections. In inoculated leaves, RYMV RNA and coat protein first were detected at 3 and 5 days postinoculation, respectively. By 6 days postinoculation, RYMV had spread systemically to leaves, and virus particles were observed in most cell types, including epidermal, mesophyll, bundle sheath, and vascular parenchyma cells. Most of the virions accumulated in large crystalline patches in xylem parenchyma cells and sieve elements. Colocalization of a cell wall marker for cellulosic β-(1-4)-D-glucans and anti-RYMV antibodies over vessel pit membranes suggesis a pathway for virus migration between vessels. We propose that the partial digestion of pit membranes resulting from programmed cell death may permit virus migration through them, concomitant with autolysis. In addition, displacement of the Ca^{2+} from pit membranes to virus particles may contribute to the disruption of the pit membranes and facilitate systemic virus transport.



Vascular localization of RYMV particles (arrows) in a rice plant close to avessel secondary cell wall (sw) in a pit area (p) (x 60000).

Hyaline mutants from *Verticillium dahliae*, an example of selection and characterization of strains for host-parasite interaction studies

Daayf F., Nicole M., Bélanger R.R. and Geiger J.P

Plant Pathology, 1998, 47 : 523-529.

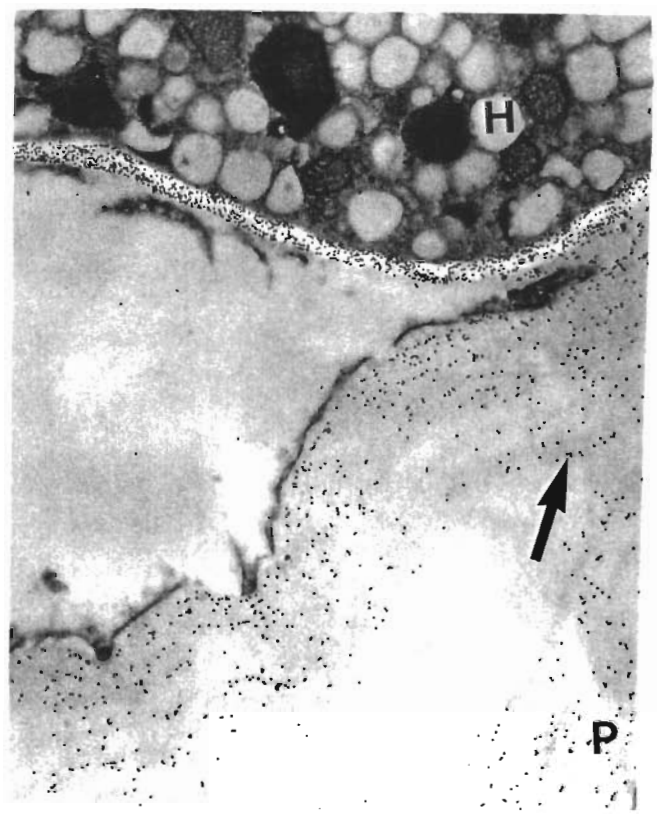
The objective of the present work was to select stable well-characterized strains of *Verticillium dahliae* that could be used as biological tools in genetic and plant-microbe interaction studies. Hyaline mutants, known for their stability in pathogenicity were chosen for the study. Diversity in pathogenicity was found among hyaline subclones obtained from a defoliating wild-type clone, but not within those from nondefoliating ones. Most subclones from the defoliating clone had parental pathotypes, but one (V7-2) exhibited weak pathogenicity. This subclone (V7-2), together with a highly virulent one (V7-7) deriving from the same defoliating parent clone (7), were selected for further characterization, because of their differences in pathogenicity. When studied on the basis of their growth requirements, the two subclones expressed marked differences. V7-7 grew better than V7-2 over a wider range of temperature conditions. Both subclones grew similarly in media supplemented with NH_4 as nitrogen source, but in those with $N0_3$, V7-7 grew more vigorously than V7-2 and only the former could grow when $N0_2$ was used. In spite of these differences, the two subclones were found to belong to the same vegetative compatibility group, confirming their genetic proximity. These results highlight the physiological and genetic complexity inherent in V. dahliae. In view of their characteristics, the clones obtained in this study should prove to be valuable tools in furthering the understanding of genetic and host-*V. dahliae* interactions.

A comparative study of carrot root tissue colonization and cell wall degradation by *Pythium violae* and *Pythium ultimum*, two pathogens responsible for cavity spot

Campion C., Vian B., Nicole M. and Rouxel F.

Canadian Journal of Microbiology, 1998, 44 : 221-230

The process of infection of carrots by *Pythium violae* and *Pythium ultimum*, two causes of cavity spot, is described. The first species causes limited root necrosis, the second progressive root rot. Colonization by both species was intracellular and limited within the tissues. Modes of cell wall degradation were studied by staining (PATAg test) and labeling techniques. Pectins were labeled with monoclonal antibodies and cellulose with an exoglucanase-gold complex. Cell wall polysaccharides were degraded differently by the two species. *Pythium violae* was responsible for degradations, which could be noticeable, especially for high methylesterified pectins, but which occurred after colonization and were localized near the hyphae. The conservation fo integrity of diseased tissue was apparently due to the absence of degradation away from the hyphae. In contrast, *P. ultimum* was responsible for more extensive degradation fo pectins and cellulose, which occurred at a relatively greater distance from the hyphae. Degradation of pectins was always more rapid in the cell walls than in the intercellular junctions. This phenomenon led to loss of tissue integrity and could explain the tissue maceration caused by *P. ultimum* infection. These differences in infection process are discussed in connection with the enzymic potential for degradation of cell wall polysaccharides.



Cellulose labelling using an exoglucanase-gold probe. Over the plant cell wall (P) close to the fungus (H), gold particles distribution is reduced (arrow) (bar = 1µm).

Etude de la compatibilité végétative chez des souches de *Fusarium oxysporum* isolées dans la région Ouest de lAlgérie.

J.E., Henni, Z. Fortas and J.P. Geiger

Pytopathologia Mediterranea, 1998, 37, 69-74.

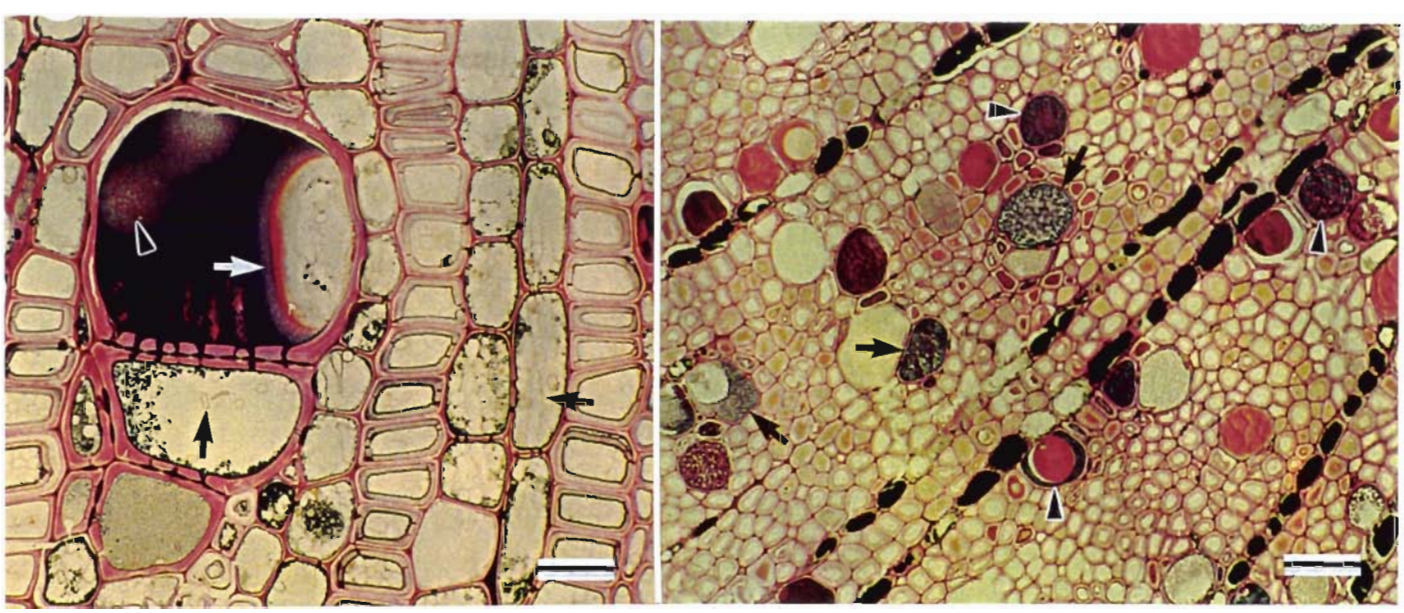
Fusarium oxysporum strains originating from the region of Oran (Algeria) were isolated either from wilted tomatoes (*F. o.* f. sp. *lycopersici*) or from the rhizosphere. This collection of strains was analysed on the basis of vegetative compatibility, using *nit* mutants. *NitM*, *nit1* and *nit3* mutants could be selected from each strain. It has been demonstrated that the rhizosphere strains were self-incompatible, and incompatible with all the other strains, while the tomato-virulent strains (*Fol*)were shown to be self-compatible and compatible with each-other. Thus all the virulent strains grouped within a unique VCG, demonstrating the homogeneity of the *Fol* population in the Oran region regarding vegetative compatibility.

Immunocytochemical Evidence that Secretion of Pectin Occurs During Gel (Gum) and Tylosis Formation in Trees

Rioux D., Nicole M., Simard M. and Ouellette G.B.

Phytopathology, 1998, 88 : 494-505

During gel (Gum) formation in angiosperm trees, fibrillar material accumulated in protective layers of xylem parenchyma cells before being secreted across half-bordered pit membranes into vessel elements. Immunogold labeling demonstrated that this fibrillar material was mainly composed of partially esterified pectic polysaccharides. The primary wall of expanding tyloses, an extension of the parenchyma protective layer, secreted similar pectic substances to completely block vessel elements.
In most studies, these occluding structures were reported to be formed in response to causative factors such as aging processes, injuries, or infections. Current observations support the view that partial to complete embolism, which almost always accompanies these factors, might be the main cause triggering the formation of vessel occlusions. Whereas pectin seems to be the basic component of gels (gums) ans of the external layer of tyloses, other substances, such as phenols, were also detected either as a part of these plugs or as accumulations beside them in vessels. Finally, it is proposed that the term 'gel' instead of 'gum' be used in future studies to describe the occluding material secreted by ray and paratracheal parenchyma cells.



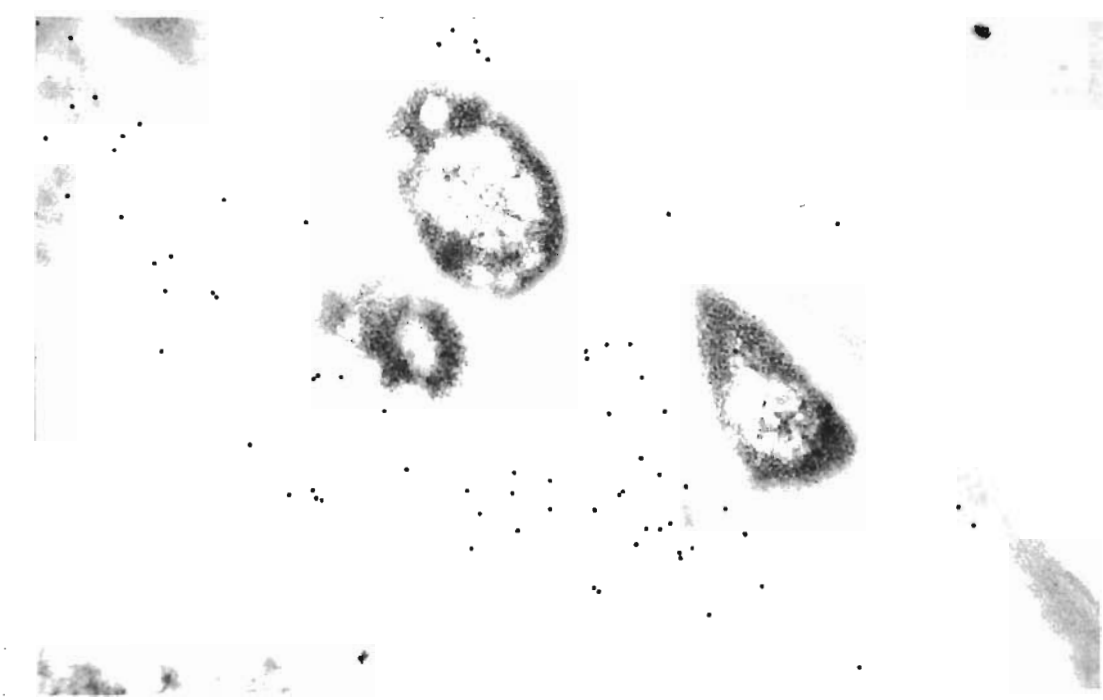
Light microscope observations of sections stained with safranin and toluidine blue. **A**, Most of the gels formed in vessels of *Prunus pensylvanica* are stained red, some appear predominantly blue (arrows), and others disclose both colors (arrowheads) (28 days postinoculation). Bar = 30 µm. **B**, A tylosis (white arrow) and a gel completely occlude this vessel element in *Hevea brasiliensis*. The gel is mainly stained blue, but some parts appear mostly red (arrowhead). Fungal cells with poorly stained cytoplasm can be seen everywhere in this section (black arrows). Bar = 25 µm.

Generation of the oxidative burst - scavenging for the truth

Bestwick C., Bolwell P., Mansfield J., Nicole M. and Wojtaszek P.

Trends in Plant Science, 1999, 4 : 89-90.

The generation of reactive oxygen species (ROS) by plant cells in response to microbial challenge continues to be a controversial subject. Multiple consequences of ROS accumulation have been described including the direct or indirect induction of host cell death (the hypersensitive reaction), induction of the synthesis of antimicrobial compounds (phytoalexins), structural re-inforcement of the plant cell wall, antimicrobial activity and participation in the development of systemic acquired resistance. Although not the only proposed sources of ROS, a membrane associated neutrophil-like NADPH oxidase complex and apoplastic peroxidase activity have emerged as generators of superoxide and H2O2 in a number of plant-pathogen/elicitor interactions. It should be remembered that the oxidative burst, when discovered in mammalian neutrophils, was originally called "the respiratory burst" and defined as "... sudden increase in the consumption of oxygen by the phagocytes that was resistant to inhibitors like *azide and cyanide*." Search for a similar burst in suspension-cultured bean cells was undertaken, but found elicited increases in *oxygen uptake* to be cyanide sensitive and, therefore, clearly not associated with an NADPH oxidase system. Additional evidence for a role of peroxidase in bean comes from modelling of ROS generating systems *in vitro* using components isolated from the plant cell wall, and involving reconstitution of the cross-linking of extracellular proteins without added H2O2. Further support is provided from immunolocalisation studies of peroxidase in bean leaves responding to bacterial infection. Similar correlative biochemical and ultrastructural evidence for the involvement of peroxidase in the oxidative burst in response to bacterial infection has been demonstrated in lettuce. The major importance is that it was the first description of the localisation of H2O2 by electron microscopy in cells undergoing the HR. Martinez *et al.* have demonstrated the existence of at least two different systems for ROS generation in cotton leaves. Similar duality in ROS generation has been found recently in soybean roots. The lack of a requirement for membrane-bound NADPH oxidase for ROS generation is also clearly demonstrated by the activity found in purified cell wall preparations.



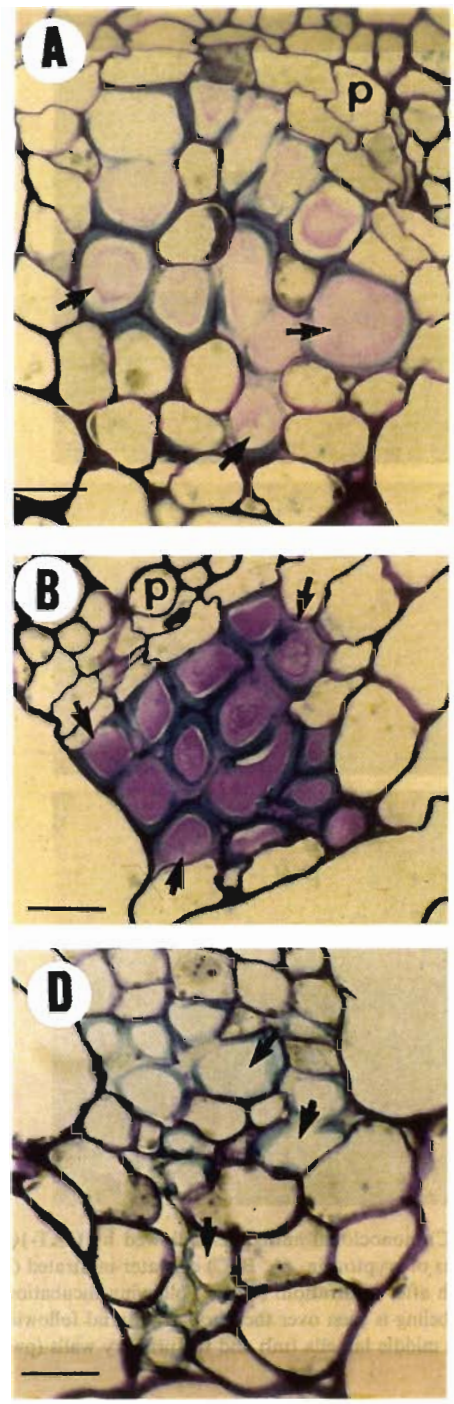
Immunolocalisation à l'or colloidal d'une peroxydase de cotonnier, au voisinage de bactéries (b), 3h après infection de cotyledons par *Xanthomonas campestris* pv. *malvacarum* (x 45000).

Leptosphaeria maculans and cryptogein induce similar vascular responses in tissues undergoing the hypersensitive reaction in *Brassica napus*

Roussel S., Nicole M., Lopez F., Ricci P., Geiger J.P., Renard M. and Brun H.

Plant Sciences, 1999, 144 : 17-28.

Cotyledons of *Brassica napus* cultivars displayed an hypersensitive reaction (HR) after either inoculation with an avirulent isolate of *Leptosphaeria maculans* (*Lm*), the fungus responsible for blackleg of crucifers, or infiltration with an elicitin, the cryptogein. In both cases, ultrastructural observations revealed that the lumen of vessels located in the HR areas was occluded by a fibrillar-like material. This material was labeled with anti-pectin antibodies, but not with antibodies specific for cellulose, callose, hemicellulose, or hydroxyproline-rich glycoprotein. Xylem and phloem parenchyma cells displayed ultrastructural features including morphological changes of nuclei and coagulation of the cytoplasm, reminiscent of cell death. None of these reactions were observed in plants infected with a virulent isolate of *Lm* or in controls. The data show that (1) responses of vascular parenchyma cells are associated with the HR induced either by an avirulent isolate of *Lm* or cryptogein, (2) cryptogein can mimic effects of an avirulent isolate of *Lm* at the vascular level. It is suggested that cryptogein and an avirulent isolate of *Lm* may activate similar signalling pathways leading to vascular reactions in the HR.



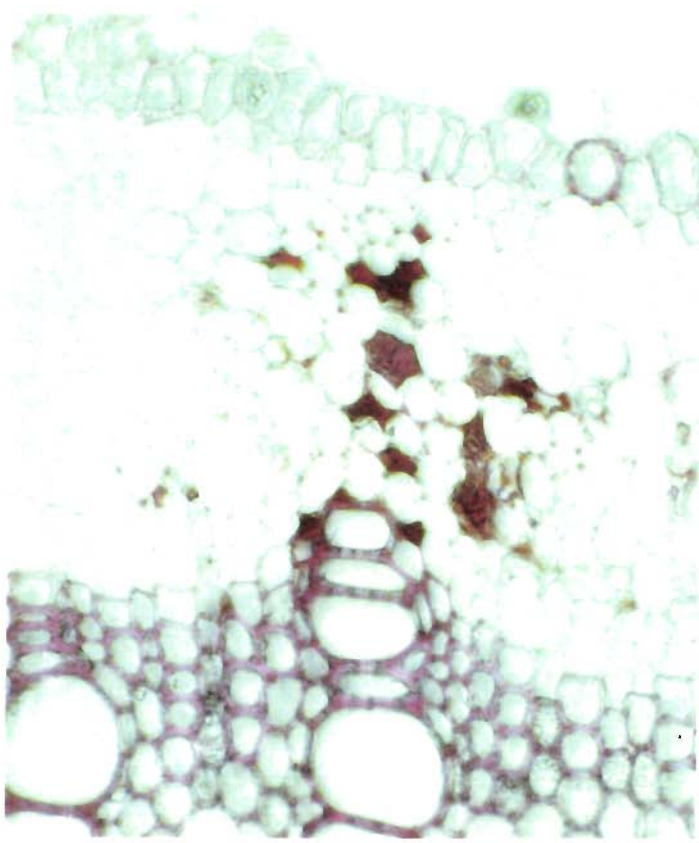
Immunolocalization of pectin using the JIM 5 monoclonal antibody followed by incubation with GAT-10 secondary antibodies conjugated to gold and by silver enhancement. Observation under epifluorescence polarized fluorescence microscope. (A) Twenty four hours after infiltration, the material present in the lumen of vessels stained pink (arrowheads). (B) Pink staining is more intense 72 than as 24 h after infiltration (arrowheads). (C) The material present in the lumen of vessels stained pink (arrowheads) 72 h after inoculation by *L. maculans*. No material is observed in the control (D) 72 h after treatment. The blue staining of secondary walls of xylem vessels indicates the occurrence of lignin.

Cell biology of plant immunization against microbial infection: the potential of induced resistance in controlling plant diseases

Benhamou N. and Nicole M.

Plant Physiology and Biochemistry, 1999, 37 : 703-719.

In the past decade, the advances in molecular biology and plant transformation, culminating with the accumulation of tremendous information on the signal transduction pathways linked between perception of pathogen attack and elaboration of plant defense responses, have opened novel avenues for biotechnological applications in agriculture. From these fundamental studies, it has become more and more realistic that sensitizing a plant to respond more rapidly to infection could confer increased protection against virulent pathogens. One important facet in ascertaining the significance of defense molecules in plant disease resistance is the exact knowledge of their spatio-temporal distribution in stressed plant tissues at or near the sites of attempted pathogen penetration. In an effort to understand the process associated with the induction of plant disease resistance, the effect of microbial and chemical elicitors on the plant cell response during attack by fungal pathogens was investigated and the mechanisms underlying the expression of resistance to bacteria and nematodes studied by both histo- and cytochemistry. Evidence is provided that the disease-resistance response correlates with changes in cell biochemistry and physiology that are accompanied by marked structural modifications including the formation of callose-enriched wall appositions and the infiltration of phenolic compounds at sites of potential pathogen penetration. Activation of the phenylpropanoid metabolism and accumulation of phenolics at strategic sites are crucial phenomena involved in pathogen growth restriction and host cell survival under stress conditions. Ultrastructural and cytochemical approaches have the potential to significantly improve our knowledge of how plants defend themselves and how plant disease resistance is expressed at the cell level. These approaches will be active areas for future research in the development of biological control alternatives.



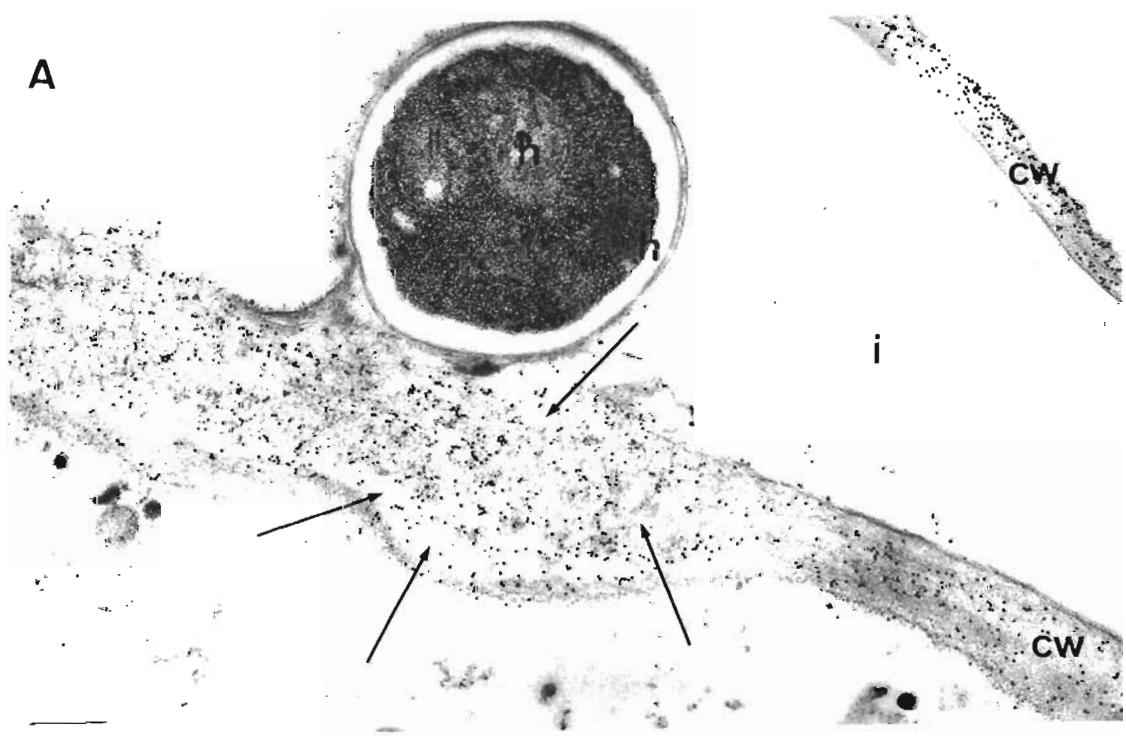
Réaction de lignification (flèches) dans le phloème de racines de manioc infectées par *Xanthomonas campestris* pv. *manihotis* (x 250).

Cytological investigation of resistance to *Leptosphaeria maculans* conferred to *Brassica napus* by introgressions originating from *B. juncea* or *B. nigra* B genome

Roussel S., Nicole M., Lopez F., Renard M., Chèvre A.M. and Brun H.

Phytopathology, 1999, 89 : 1200-1213.

Introgressions into *Brassica napus* from the B genome, either the *B. nigra* chromosome B4 or the *B. juncea* fragment carrying the Jlm1 gene, have given rise to the B. napus-B. nigra addition line (LA4+) and the *B. napus-B. juncea* recombinant line (MXS), respectively. The resistance of these two lines to *Leptosphaeria maculans* is characterized by a hypersensitive reaction (HR) on both the cotyledons and leaves, while the collar displays a high degree of resistance. Responses induced in cotyledons of the two lines by *L. maculans* inoculation were investigated with emphasis on cytological events underlying the HR. and on host defense reactions. Features of host cell changes including condensation and lobbing of nuclei, fragmentation of chromatin, disruption of the nuclear membranes, and plasma membrane withdraw were reminiscent to HR cell death in MXS and LA4+ plants. Strict restriction of the pathogen growth to the infection areas in LA4+ line was correlated to reinforcement of cell wall barriers. In MXS line, the lower expression of resistance is associated with delay in plant responses. Mechanisms underlying the HR in the *B. napus* recombinant and addition lines are differently controlled according to the introgressed genes.



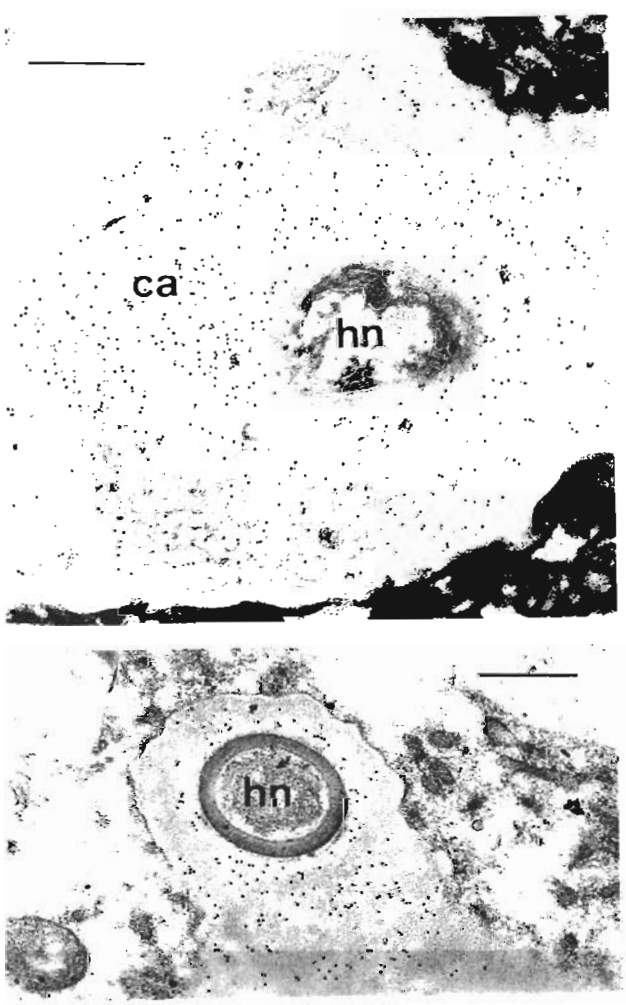
Lyse (flèches) de la paroi cellulaire (cw) au contact du champignon (h);
la cellulose est marquée à l'or colloidal (échelle = 0,7 µm).

Cytochemical aspect of the plant-rust fungus interface during the compatible interaction*Coffea arabica* (cv Caturra) - Hemileia Vastatrix (RACE III)

Silva M.C., Nicole M., Rijo I., Geiger J.P. and Roddrigues C.J.

International Journal of Plant Sciences, 1999, 160 : 79-91.

The infection process of *Hemileia vastatrix* within leaf tissues of susceptible *Coffea arabica* plants was investigated ultrastructurally and cytochemically and also by light microscopy. An exoglucanase-gold complex, anti-galacturonic acid monoclonal (JIM7), anti ß-1,3-glucans polyclonal antibodies, and a WGA-ovomucoid-gold complex were used to localize ß-1,4-glucans, pectins, callose, and chitin, respectively. After urediospore germination and appressoria differentiation on leaf stomata, the fungus penetrated and colonized the mesophyll tissues unter-and intracellularly. The intercellular hyphae, including haustorial mother cells and haustoria, contrained ß-1,3-glucans and chitin in their walls. The interface of the pathogen with the host cell wall was characterized by the occurrence of adhesive material that was labeled of pectin. Also, labeled plant cell wall frangmets for ß -1,4-glucans were detected close to the intercellular fungal cell wall. Plant cell wall degradation during haustorium formation was restricted only to the site of host cell penetration as judged by the use of the exoglucanase-gold complex. In advanced stages of the infection process, haustoria were encased by a material that positively reacted fo callose and ß-1,4-glucans. Pectins were not detected in the encasement material around the haustorial body but only in the encasement material around the penetration peg. This haustorium encasement is a plant defense reponse but occurred too late to be efficient in preventing fungal growth and sporulation.



Immunogold labelling of callose deposits within material encasing the haustorium (hn) in a coffe mesophyll cell (bars = 0.5µm)

Salicylic Acid mediated by the Oxidative burst is a key molecule in Local and Systemic Responses of Cotton Challenged by an Avirulent Race of *Xanthomonas campestris* pv. *malvacearum*

Martinez C., Baccou J.C., Bresson E., Baissac Y., Assigbetsé K., Daniel J.F., Jalloul A., Montillet J.L., Geiger J.P. and Nicole M.

Plant Physiology, 2000, 122 : 1-10.

Production of reactive oxygen species, accumulation of salicylic acid (SA) and peroxidase activity (Pox activity) were analysed during the incompatible interaction between cotton cotyledons of the cultivar Reba B50 / *Xanthomonas campestris* pv *malvacearum* (Xcm) race 18. SA was detected in petioles of cotyledons 6h after infection, and 24h post-inoculation in cotyledons and in non treated leaves. The first peak of SA occurring 3h after generation of O2.-, was inhibited by infiltration of catalase. Pox activity and accumulation of SA increased in petioles of cotyledons and leaves following H2O2 infiltration of cotyledons from 0.85 to 1mM. Infiltration of 2mM SA increased Pox activity in treated cotyledons and in the first leaves. But most of the infiltrated SA is rapidly conjugated within the cotyledons. When increasing concentration of SA were infiltrated 2.5h post-Xcm inoculation at the beginning of the oxidative burst, activity of the apoplastic cationic O2.--generating Pox decreased in a dose-dependent manner. We have shown that during cotton HR to Xcm H2O2 is required for local and systemic accumulation of SA, which may locally control generation of O2.-. Detaching cotyledons at intervals after inoculation demonstrated that the signal leading to systemic accumulation of SA was emitted around 3h post-inoculation, associated with the oxidative burst. SA produced 6h post-infection at HR sites was not the primary mobile signal diffusing systemically from infected cotyledons.

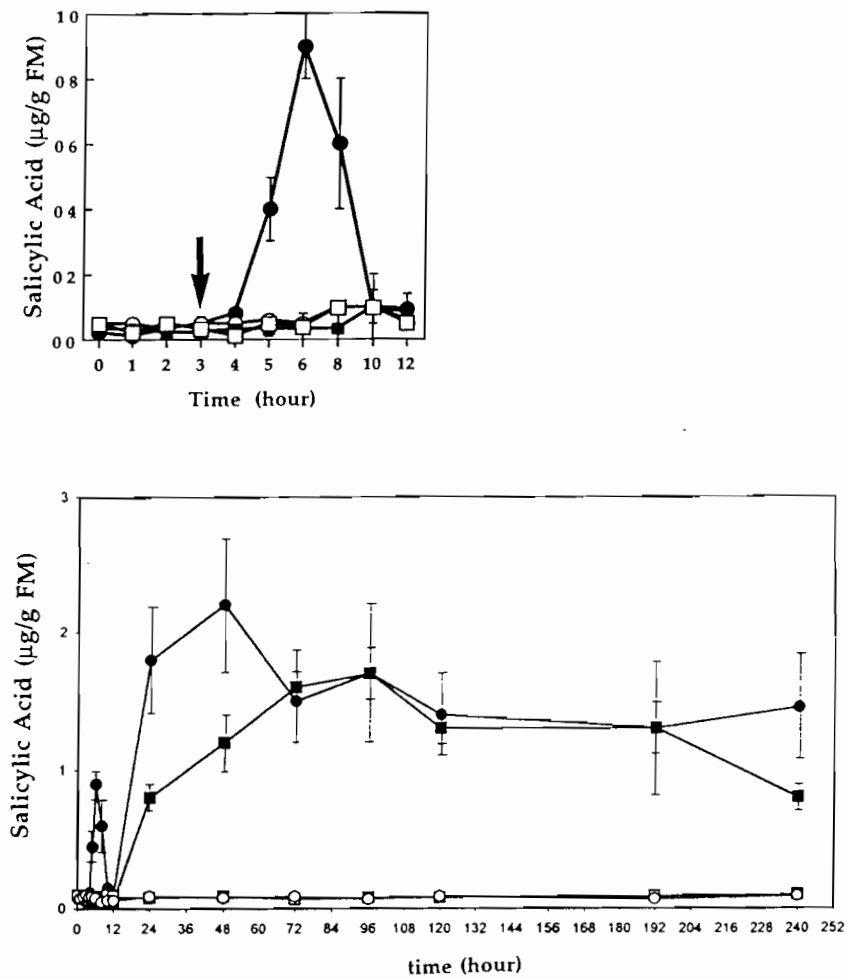


Figure 1. Effect of Xcm race 18 on the endogenous level of free SA in relationship to time (A, 0–12 h; B, 0–240 h) in the petioles of infected cotton cotyledons (●; ○) and the upper untreated leaves (■; □) of the cv Reba B50 (●; ■) and cv Acala-44 (○; □) lines. The cotyledons of each variety were inoculated, and samples of petiole phloem exudate were collected at various times following infection. SA was separated by HPLC and measured as described in "Materials and Methods." Each value is the mean ± SE of 10 replicates from different plants. The arrow in A indicates the time at which the oxidative burst occurs. SA content is expressed as $\mu g\ g^{-1}$ fresh matter (FM).

Functional organization of the cassava vein mosaic virus (CsVMV) promoter

Verdaguer B., de Kochko A., Fux C.I., Beachy R.N. and Fauquet C.

Plant Molecular Biology, 1998, 37 (6) : 1055-1067.

Cassava vein mosaic virus (CsVMV) is a pararetrovirus that infects cassava plants in Brazil. A promoter fragment isolated from CsVMV, comprising nucleotides - 443 to C 72, was previously shown to direct strong constitutive gene expression in transgenic plants. Here we report the functional architecture of the CsVMV promoter fragment. A series of promoter deletion mutants were fused to the coding sequence of *uidA* reporter gene and the chimeric genes were introduced into transgenic tobacco plants. Promoter activity was monitored by histochemical and quantitative assays of --glucuronidase activity (GUS). We found that the promoter fragment is made up of different regions that confer distinct tissue-specific expression of the gene. The region encompassing nucleotides - 222 to - 173 contains *cis* elements that control promoter expression in green tissues and root tips. Our results indicate that a consensus as1 element and a GATA motif located within this region are essential for promoter expression in those tissues. Expression from the CsVMV promoter in vascular elements is directed by the region encompassing nucleotides - 178 to - 63. Elements located between nucleotides - 149 and - 63 are also required to activate promoter expression in green tissues suggesting a combinatorial mode of regulation. Within the latter region, a 43 bp fragment extending from nucleotide - 141 to - 99 was shown to interact with a protein factor extracted from nuclei of tobacco seedlings. This fragment showed no sequence homology with other pararetrovirus promoters and hence may contain CsVMV-specific regulatory *cis* elements.

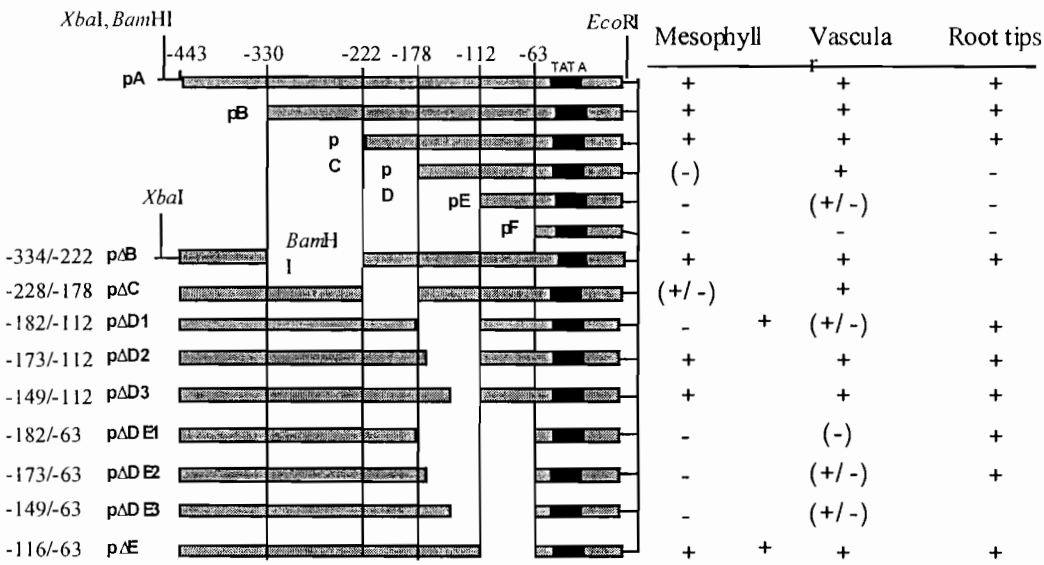


Figure 1. Schematic representation of the deleted CsVMV promoter:*uid*A fusion genes and a summary of histochemical detection of GUS activity in transgenic tobacco plants.
The names of the different plasmids and the end points of the 5' and internal deletions are indicated. Internal deletions are designated by the symbol Δ. The construct pA contains a full-length CsVMV promoter. All promoters deleted from the 5' end have a *BamH*I site at their 5' ends. Internal deletions were created by *Bam*HI ligation of the promoters truncated from the 5' end with those truncated from the 3' end (See Materials and Methods). The data summarize the observations performed on ten different transgenic R1 tobacco plants for each promoter construct. The transgenic plants were analyzed for GUS expression in mesophyll cells, vascular tissues and root tips. GUS expression is scaled in each tissue according to 4 levels. +: no visible difference with the full length promoter; (+/-) : lower staining than with the full length promoter; (-) : very low expression; - : no detectable staining.

Rice Tungro Bacilliform Virus ORF 3 encodes a single 37 kDa coat protein

Marmey Ph., Bothner B., Jacquot E., de Kochko A., Ong C. A., Yot P., Siuzdak G., Beachy R. N. and Fauquet C.

Virology, 1999, 253(2) : 319-326.

Rice tungro bacilliform virus (RTBV) is a plant pararetrovirus and a member of the *Caulimoviridae* family, and closely related to viruses in the *Badnavirus* genus. The coat protein of RTBV is part of the large polyprotein encoded by open reading frame (ORF) 3. ORF3 of an RTBV isolate from Malaysia was sequenced (#AF076470) and compared to published sequences for the region that encodes the coat protein(s). Molecular mass of virion proteins was determined by mass-spectrometry (MALDI-TOF) performed on purified virus particles from three RTBV isolates from Malaysia. The N- and C- terminal amino acid sequences of the coat protein were deduced from the mass spectral analysis, leading to the conclusion that purified virions contain a single coat protein of 37 kDa. The location of the coat protein domain in ORF3 was reinforced as a result of immunodetection reactions using antibodies raised against 6 different segments of ORF3 using western immunoblots following SDS-PAGE and isoelectrofocusing of proteins purified from RTBV particles. These studies demonstrate that RTBV coat protein is released from the polyprotein as a single coat protein of 37 kDa. The regions of P3 upstream and downstream of the CP do not contain CP sequences and would be similar in molecular mass (about 20 kDa), and charge (pI 4.5). To date, proteins of this size and charge have not been identified in RTBV particles or infected cells; we suggest that these regions are in some way involved in the activity of the protease in releasing the 37 kDa CP.

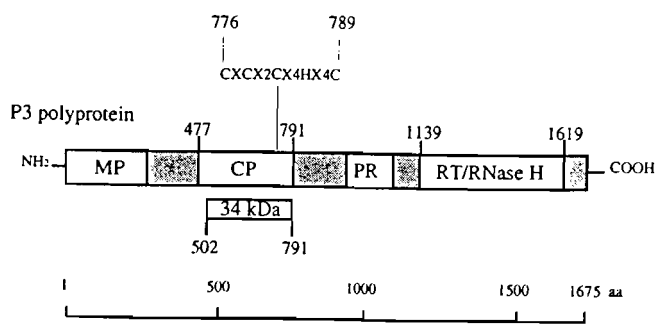


Figure : Schematic organization of CP domain within P3 polyprotein. Positions of amino- and carboxy-termini of the 37 kDa CP are 477 and 791, respectively. The 34 kDa peptide CP is positioned below the polyprotein with its amino- and carboxy- termini corresponding to aa 502 and 791, respectively.

Transgenic rice plants (*Oryza sativa* L.) that containing rice tungro spherical virus (RTSV) coat protein transgenes are resistant to virus infection.

Sivamani E., Huet H., Shen P., Ang Ong C., de Kochko A., Fauquet C. and Beachy R.N.

Molecular Breeding, 1999, 75(2) : 177-185.

The three coat protein (CP) genes, CP1, CP2 and CP3, of rice tungro spherical virus (RTSV) were introduced individually or together to indica and/or japonica rice cells by particle bombardment and transgenic plants were produced. Plants derived from selfed progeny of the primary transformants were subjected to virus inoculation via leafhoppers, the natural vector of the virus. Sixteen out of the nineteen selected transgenic plant lines, as well as their R1, R2 and/or R3 progeny that contained the target gene, accumulated transcripts of the chimeric CP gene(s) by RNA blot analysis. We obtained evidence of moderate levels of protection to RTSV infection, ranging from 17 to 73% of seedlings that escaped infection and a significant delay of virus replication under greenhouse conditions in plant lines that expressed the RTSV-CP1, CP2 and CP3 genes singly or together. There was not an additive effect on resistance when more than one CP gene is expressed. This study is the first to report pathogen-derived resistance to infection by RTSV, one of the two viruses that are involved in rice tungro disease. It is also the first example of CP-mediated protection against a virus that contains more than one CP gene from the same virus.

Near immunity to Rice tungro spherical virus (RTSV) achieved in rice by a replicase-mediated resistance strategy.

Huet H., Mahendra S., Wang J., Sivamani E., Ong C.A., Chen L., de Kochko A., Beachy R.N. and Fauquet C.

Phytopathology, 1999, 89 (11) : 1022-1027.

Rice tungro disease is caused by Rice tungro bacilliform virus (RTBV) which is responsible for the symptoms, and Rice tungro spherical virus (RTSV) which assists transmission of both viruses by leafhoppers. Transgenic rice plants (*Oryza sativa* L., sp. *japonica*) were produced containing the RTSV replicase (Rep) gene in the sense or antisense orientation. Over 70% of the plants contained 1 to 5 copies of the Rep gene with integration occurring at a single locus in most cases. Plants producing antisense sequences exhibited significant but moderate resistance to RTSV (60%); accumulation of antisense RNA was substantial, indicating that the protection was not of the homology-dependent type. Plants expressing the full length Rep gene, as well as a truncated Rep gene, in the (+) sense orientation were 100% resistant to RTSV even when challenged with a high level of inoculum. Accumulation of viral RNA was low leading us to conclude that RTSV Rep-mediated-resistance is not protein-mediated, but of the co-suppression type. Resistance was effective against geographically distinct RTSV isolates, and in addition RTSV-resistant transgenic rice plants were unable to assist transmission of RTBV. Such transgenic plants could be used in an epidemiological approach to combat the spread of the tungro disease

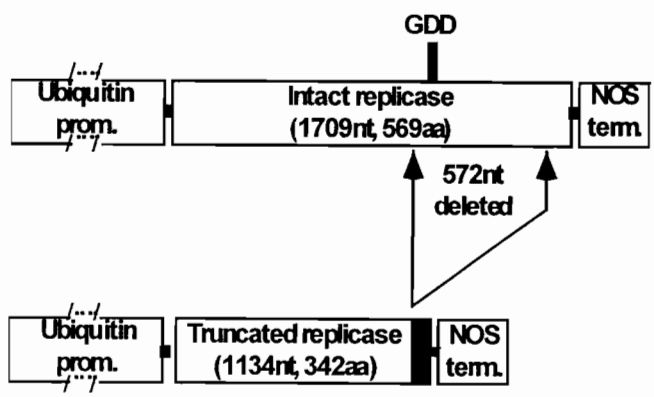


Figure 1. Schematic representation of the intact and truncated form of the RTSV-replicase gene as transfered to TP309 rice plants. The replicase gene is driven by the maize ubiquitin promoter (ubiquitin prom.) and terminated by the nopaline synthase terminator (NOS term.). ☐ means that this region was not properly translated due to a frame shift introduced by the deletion.

Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon

Fondong V.N., Pita J.S., Rey M. E. C., de Kochko A., Beachy R. N. and. Fauquet C. M

J. Gen. Virology, 2000, 81: 287-297.

Stem cuttings were collected in Cameroon from cassava plants displaying cassava mosaic disease (CMD) symptoms. The nature of the viruses present was determined by using the PCR with primers specific for the coat protein (CP) genes of African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV). All samples were infected by ACMV and eight of the 50 samples were infected by both ACMV and an EACMV-like virus. The complete nucleotide sequences of DNA-A and -B of representative ACMV and EACMV-like viruses were determined. The DNA-A component of the EACMV-like virus contained evidence of recombination in the AC2–AC3 region and DNA-B also contained evidence of recombination in BC1. However, both components retained gene arrangements typical of bipartite begomoviruses. When Nicotiana benthamiana plants were doubly inoculated with these Cameroon isolates of ACMV and EACMV (ACMV/CM, EACMV/CM) by using sap from cassava plants or infectious clones, the symptoms were more severe than for plants inoculated with either virus alone. Southern blot analysis of viral DNAs from infected plants showed that there were significantly higher levels of accumulation of both ACMV/CM components and, to a lesser extent, of EACMV/CM components in mixed-infected plants than in singly infected plants. These results strongly suggest the occurrence of a synergistic interaction between the two viruses.

Figure 1. (a) Symptoms of cassava mosaic virus disease on a cassava plant doubly infected by ACMV/CM and EACMV/CM in the south-west rainforest region of Cameroon. In such severe cases there is complete defoliation of the stem shoot. (b) In the main epidemic area severely affected plants resulting from dual infection by ACMV/CM and EACMV/CM can be found growing next to mildly affected plants that result from infection by ACMV/CM alone. (c) The differences in symptom severity between doubly infected cassava plants and those singly infected by ACMV/CM or EACMV/CM are also observed in the greenhouse, (d) when inoculated mechanically on *N. benthamiana* with sap from infected cassava plants and (e) when inoculated mechanically with cloned components of ACMV/CM and EACMV/CM. ACMV/CM on the left, EACMV/CM on the right and the doubly infected plants in the center

Detection of the Cassava bacterial blight pathogen, *Xanthomonas axonopodis* pv. *manihotis*, by polymerase chain reaction

V. Verdier, Masquera G. and Assigbetsé K.

Plant disease, 1998, 82 : 79-83.

Cassava bacterial blight, caused by *Xanthomonas axonopodis* pv. *manihotis*, is of significant concern wherever cassava is grown. The movement of infected, asymptomatic stems is a major means of pathogen dispersal. A reliable and sensitive diagnostic procedure is necessary for the safe movement of cassava planting material. We used a cloned and sequenced pathogenicity gene of *X. axonopodis* pv. *manihotis* to develop a polymerase chain reaction (PCR) test for this pathogen. A set of primers directed the amplification of an 898-hp fragment in all 107 pathogenic strains of *X. axonopodis* pv. *manihotis* tested. PCR products were not observed when genomic DNA was tested for 27 strains of other xanthomonads, for saprophytic bacteria, or for five nonpathogenic strains of *X. axonopodis* pv. *manihotis*. The primers worked well for pathogen detection in direct PCR assays of *X. axonopodis pv. manihotis* colonies grown on liquid medium and in PCR assays of extracts from leaf and stem lesions. The minimum number of cells that could be detected from cassava stem and leaf lesions was 3102 to 10^4 CFU/ml. The PCR assays proved to be relatively sensitive and could become very useful in detecting the pathogen in cassava planting material.

Fot 1 Insertion in the _Fusarium oxysporum_ f. sp. _albedinis_ genome provide diagnostic PCR targets for detection of the Date Palm pathogen.

Fernandez D., Ouinten M., Tantaoui A., Geiger J.P., Daboussi M.J. and Langin T.

Applied and Environmental Microbiology, 1998, 64 (2), 633-636.

Populations of *Fusarium oxysporum* f. sp. *albedinis* (*Foa*), the causal agent of Bayoud disease of date palm, are derivatives of a single clonal lineage and exhibit very similar *Fot* 1 hybridization patterns. In order to develop a sensitive diagnostic tool for *Foa* detection, we isolated several DNA clones containing a copy of the transposable element *Fot* 1 from a genomic library of the date palm pathogen. Regions flanking the insertion sites were sequenced, and these sequences were used to design PCR primers that amplify the DNA regions at several *Fot* 1 insertion sites. When tested on a large sample of *Fusarium* isolates, including 286 *Foa* isolates, 17 other special forms, non pathogenic *F. oxysporum* isolates from palm grove soils, and 8 other *Fusarium* species, the primer pair TL3-FOA28 allowed amplification of a 400-bp fragment found only in *Foa*. Sequence analysis showed that one of the *Fot* 1 copies was truncated, lacking 182 bp at its 3′ terminus. The primer pair BIO3-F0A1 amplified a 204-bp fragment which overlapped the *Fot* 1 truncated copy and its 3′ site of insertion in the *Foa* genome and identified 95 % of the isolates. The primer pairs BIO3-FOA1 and TL3-FOA28 used in PCR assays thus provide a useful diagnostic tool for *Foa* isolates.

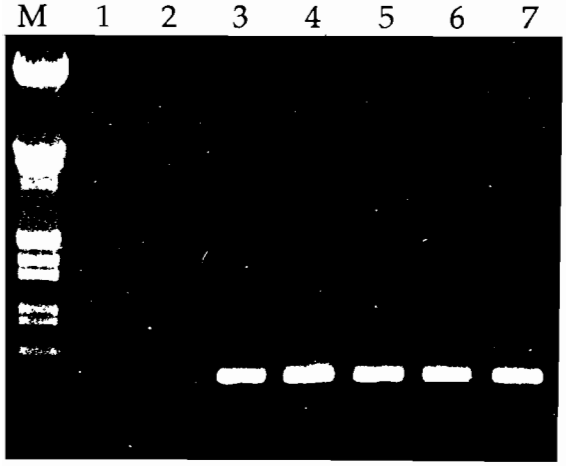
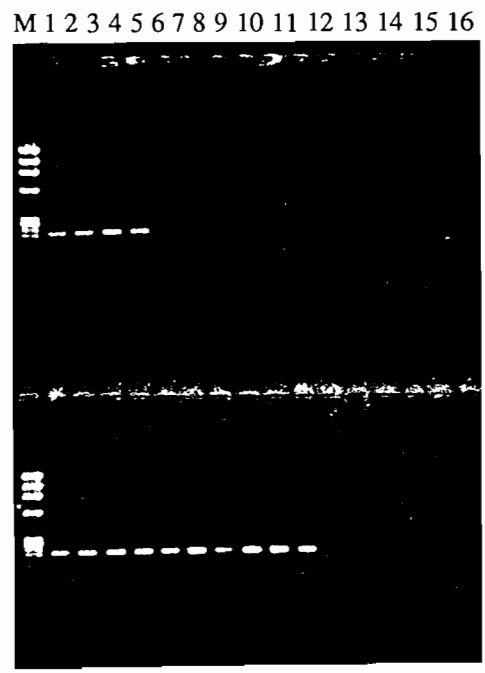


Fig. 1



Ethidium bromide-stained gel showing results of PCR amplification with the :
Fig. 1 : primer pair TL3/FOA28. Lanes 1 to 7 contain amplification products from *F. o.* f. sp. *vasinfectum*, *F. o.* f. sp. *melonis* and five distinct *F. o.* f. sp. *albedinis* (*Foa*) isolates. Lane M, molecular markers. Note the selective amplification of a 400-bp fragment from *Foa* isolates only.
Fig. 2 : primer pair BIO3/FOA1. **Fig. 2A** : lanes 1 to 4 contain amplification products from 4 distinct *Foa* isolates; lanes 5 to 16 contain amplification products from 6 *F. o.* f. sp. *vasinfectum*, 1 *cubense*, 1 *lycopersici*, 1 *melonis*, 1 *Fusarium avanaceum* and 2 *F. o.* f. sp. *elaeidis*. **Fig. 2B** : lanes 1 to 10 contain amplification products from 10 distinct *Foa* isolates; lanes 11 to 15 contain amplification products from 5 *F. o.* (soil); lane 16 was the "negative" control. Lane M : molecular marker. Note the selective amplification of a 200-bp fragment from *Foa* isolates only.

Molecular characterization of the incitant of cowpea bacterial blight and pustule, *Xanthomonas campestris* pv. *vignicola*

Verdier V., Assigbétsé K., Khatri-Chhetri G., Wydra K., Rudolph K., Geiger J-P.

European Journal of Plant Pathology,1998, 104 : 595-602 .

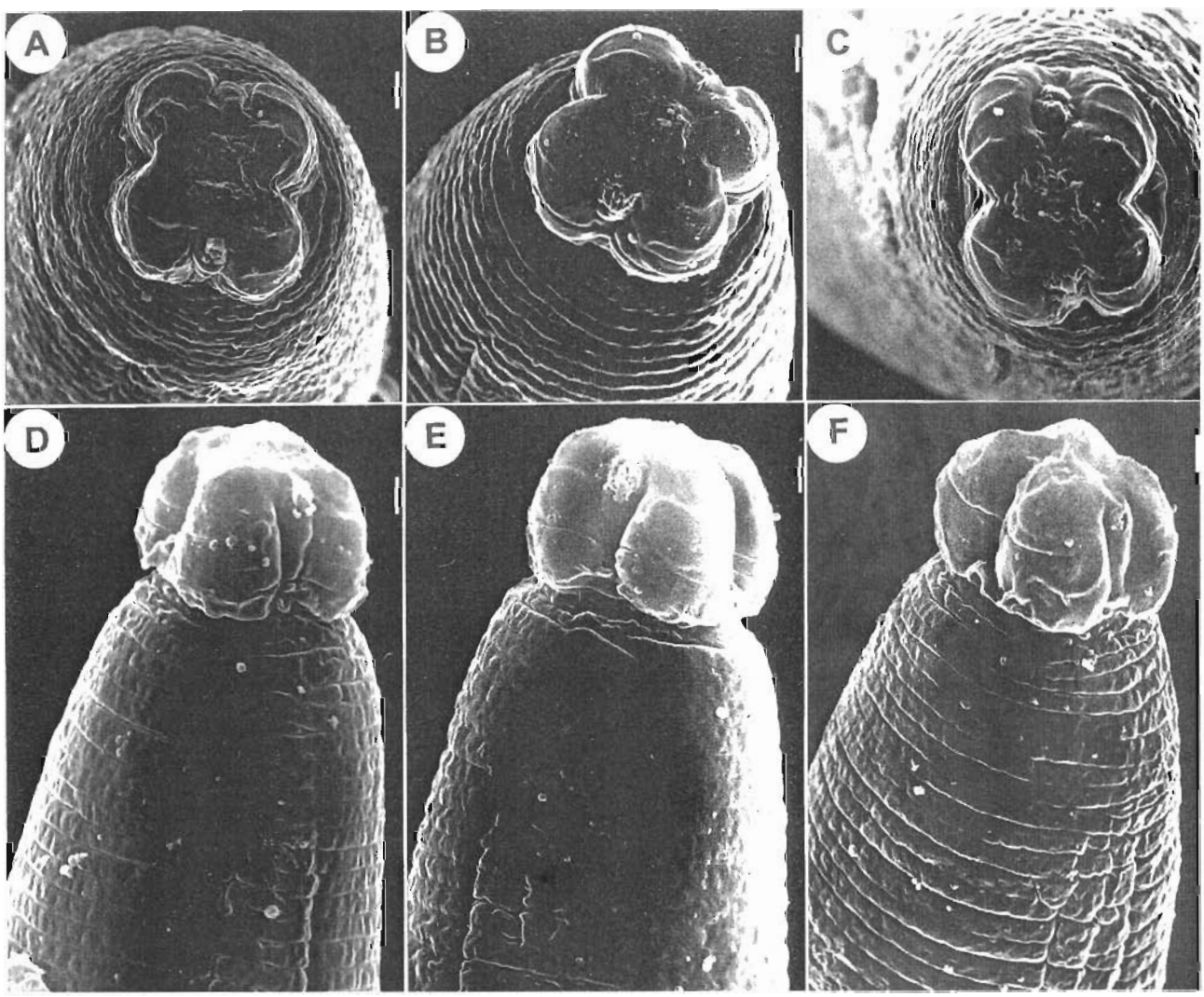
Strains of *Xanthomonas campestris* pv. *vignicola* (Xcv), isolated from cowpea leaves with blight or minute pustules and collected from various geographic areas, were selected on the basis of pathological and physiological features. All strains were analyzed for genotypic markers by two methods : ribotyping with *Eco*RI endonulease, and RFLP analysis with a plasmid probe (*pth*B) containing a gene required for pathogenicty from *Xanthomonas campestris* pv. manihotis. Ribotyping revealed a unique pattern for all the strains that corresponded to the previously described ribotype rRNA7. Based on polymorphism detected by *pth*B among Xcv strains, nine haplotypes were defined. The observed genetic variation was independent of the geographic origin of the strains and of pathogenic variation. Some haplotypes were widely distributed, whereas others were localized. In some case, we could differentiate strains isolated from blight symptom and pustules according to haplotypic composition. However, in most cases, no significant differences were observed. Our results- and the previous pathogenic and biochemical characterization suggest that the strains isolated from leaves with blight symptoms or minute pustules belong to the same pathovar. We provide information on pathogen diversity that can be used to identify and characterize resistant germplasm.

Scanning electron microscope study of two African populations of *Radopholus similis* (*Nematoda : Pratylenchidae*) and proposal of *R. citrophilus* as a junior synonym of *R. similis*

Valette C., Mounport D., Nicole M., Sarah J.L. and Baujard P.

Fundamental and Applied Nematology, 1998, 21 : 139-146.

Cuticular structures of two African populations of *Radopholus similis* from the Ivory Coast and Guinea Bissau were observed under the scanning electron microscope. These two populations have similar cuticular structures, which exhibited a range of variation overlapping that described in American population of *R. similis* and *R. citrophilus*. Consequently, *R. citrophilus* is proposed as a junior synonym of *R. similis*. Additional data are provided on female tail shape and lateral fields and male head and bursa shape.



Observation intrastructurale de la cuticule (sèche) de *Radopholus similis*
Coupe transversale x 30 000

Cassava vein mosaic virus (CsVMV), type species for a new genus of plant double stranded DNA viruses?

A. de Kochko, B. Verdaguer, N. Taylor, R. Carcamo, R. N. Beachy, and C. Fauquet

Archives of Virology, 1998, 143 (5): 945-962.

The complete sequence of 8159 nucleotides of the double stranded DNAgenome of cassava vein mosaic virus (CsVMV) was determined (# U59751) and revealed a significant difference in genome organization when compared with a previous report (# U20341). When transferred to cassava plants by microbombardment, the full length CsVMV clone was infectious, confirming the genome organization here described. Sequence comparisons between CsVMV and members of the genera *Caulimovirus* and *Badnavirus* revealed high homolo-gies between consensus sequences of several proteins that are indispensable for virus replication, including a potential transactivator factor not reported previ-ously. The presence of a sequence complementary to a plant Met tRNA confirms that CsVMV is a plant pararetrovirus and is most closely related to members of the genus *Caulimovirus* as previously assessed. However, differences in genome organization, number and size of the ORFs, in addition to sequence compar-isons with other plant pararetroviruses, shows that either the genetic variability of caulimoviruses is much greater than previously thought, or that CsVMV is the unique representative of a new genus within the *Caulimoviridae* family. On the basis of this study, it is proposed to upgrade the floating genus *Caulimovirus* to the family level and to divide the *Caulimoviridae* family into at least three genera with CsVMV being the type member of a new genus.

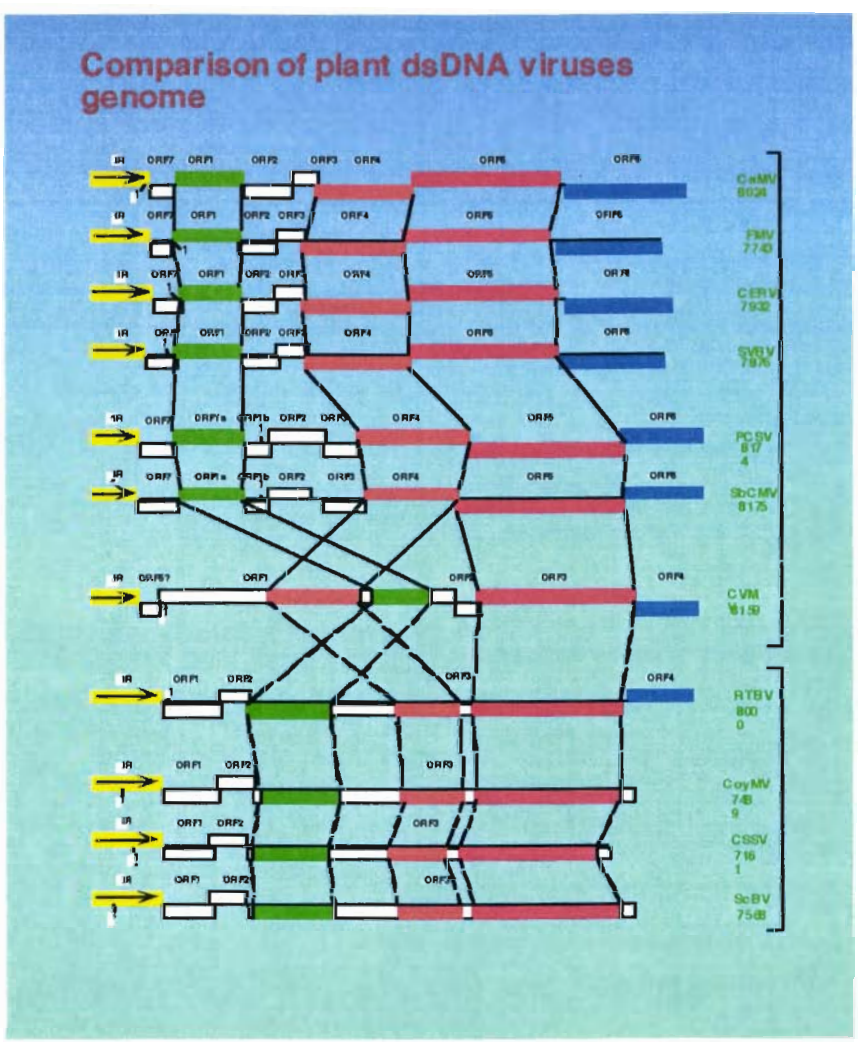


Figure 2: Comparison of the genomic organization of CsVMV with the caulimoviruses and badnaviruses. ORFs or ORF segments encoding similar putative function are linked by vertical lines; the number 1 indicates the origin of DNA replication. ♣: MP active site, *: RNA binding site, ◊: PR active site, ♦: RT active site, ♠: TAV active center, [RNAse H consensus sequence. All maps commence at the beginning of the intergenic region because it is a common region defined just by the sequence and produces a coherent figure. Starting at the site of DNA replication will give a scrambled figure as this site varies considerably among all the viruses. As the initiation of transcription is not known for all of the viruses it cannot be used as starting point.

Trifling variation in truffles

Bertault G., M. Raymond, A. Berthomieu, G. Callot and D. Fernandez.

Nature, 1998, 394, 734-734.

Of the ten species of European truffle (fungi of the genus Tuber, phylum Ascomycota), some have economic value because of their organoleptic properties (taste and perfume), in particular the black truffle (*Tuber melanosporum* Vitt.), and the burgundy and summer truffles. The black truffle is mainly found in Spain, France and Italy (Fig. 1a), and it shows variation in several traits, including in its famous organoleptic properties, across this geographical range. In an attempt to explain the variation in *T. melanosporum* and to study the distribution of the genetic variability within and among populations, we analysed fruiting bodies (ascocarps) from different populations in France and Italy for Random Amplified Polymorphic DNA polymorphism (RAPD) and microsatellite polymorphism.
We found an extremely low level of polymorphism over the whole study area for both types of markers (Fig. 1b,c). The low level of genetic diversity of the black truffle surely cannot be explained by its current population size.A population bottleneck probably occurred during the last – and coldest glaciation, during which the broadleaved forest was considerably reduced, mainly to the Mediterranean coastal zone. The present low level of genetic variability in the black truffle populations is consistent with such a severe bottleneck 10,000 years ago, followed by a rapid colonisation of south-western Europe, also explaining the absence of phylogeographic signal expressed by the few polymorphic markers found.

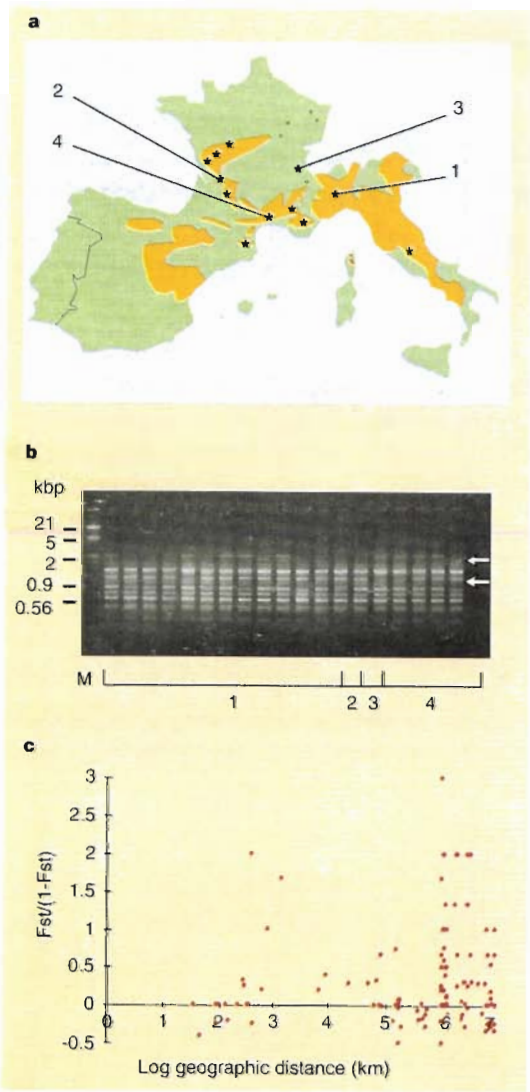


Figure 1 Geographic and genetic characteristics of the black truffle *Tuber melanosporum* Vitt. **a,** The western European geographical range (shaded area) of *Tuber melanosporum* and sampling localities (stars); numbers refer to samples shown in **b**. All truffles (n=208) were collected in natural habitats, except one sample which was collected in an artificial 'truffle-field'. **b,** Example of RAPD patterns obtained with the OPF-14 primer (Operon Technologies, Alameda) for truffles from four locations (numbers refer to **a**); arrows indicate polymorphic bands used. Sizes on left are in kilobase pairs. **c,** Analysis of isolation by distance according to ref. 7, using six RAPD loci (generated by primers OPF-11, OPF-13, OPF-14 and OPB-2) and the only two polymorphic microsatellite loci out of the nine assessed, $(GAGT)_{14}$ and $(GTTA)_9$, showing two alleles each. Computations were performed with Genepop version 3.1b (ref. 8). The increase in genetic differentiation with geographic distance was not significant (Mantel test, 10^5 permutations, P>0.10).

Our study shows that the morphological and organoleptic differences over the geographical range of the black truffles can probably be explained by environmental variation rather than by genetic factors. Research is needed to identify the environmental variables that affect the black truffle's perfume and taste, which are the objects of intense human interest.

Diversité génétique des populations de truffe

Fernandez D. et Raymond M., 1999.

In : "**La truffe, la terre, la vie**", G. Callot coord., Paris, INRA Eds. : 78-84.

La classification des espèces du genre *Tuber* a été traditionnellement basée sur des critères morphologiques et plusieurs clés de détermination ont été proposées à partir de l'observation macro et microscopique des ascocarpes ou des mycorhizes. Cependant, parmi la vingtaine d'espèces de *Tuber* actuellement reconnues en Europe, quelques-unes demeurent difficiles à séparer sur ces critères. Les techniques d'analyse moléculaire développées récemment ont toutes mis en évidence une diversité génétique au sein du genre *Tuber*, permettant d'une part, de séparer clairement les espèces qui, jusqu'à présent, étaient difficilement identifiables, et, d'autre part, de mettre au point des outils rapides d'identification (de type sonde ADN ou oligonucléotides PCR).

Concenant la truffe noire du Périgord (*T. melanosporum*), un niveau de variabilité relativement faible a été observé au sein de l'espèce. Il est toutefois possible de détecter du polymorphisme, aussi bien au niveau des bandes RAPD que des locus microsatellites, ce qui a permis d'appréhender la structure des populations de truffe à une échelle réduite. Au sein d'un brûlé, il semble clair qu'un même arbre puisse être colonisé par plusieurs génotypes de *T. melanosporum*, mais un génotype est toujours majoritaire en général. De plus, si l'on analyse les ascocarpes sur plusieurs années consécutives de production, on peut trouver des génotypes identiques à l'intérieur d'un même brûlé, résultant probablement du développement d'un même mycélium qui subit une autofécondation lors de la mise en place de chaque ascocarpe. D'autre part, au niveau des foyers de production que l'on observe généralement au sein d'une truffière, on n'observe pas de regroupement d'un génotype particulier. La présence de foyers de production au sein d'une truffière ne peut donc pas être attribuée à la colonisation, suivie d'une diffusion focale, d'un génotype de *T. melanosporum* particulier. Ce résultat indique donc que les différences de productivité que l'on peut observer à une petite échelle sont davantage à mettre au compte d'une variabilité de terrain que de la présence d'un « écotype » particulier de champignon.

La faible variabilité génétique présente chez *T. melanosporum* indique que cette espèce n'a pas divergé d'une région à l'autre, et que l'on ne peut donc pas différencier des sous-espèces géographiques. Les différences (morphologiques, gustatives, etc.) que l'on peut donc observer entre ascocarpes récoltés dans des régions différentes n'ont certainement pas une base génétique. En d'autres termes, il existe un effet environnemental certain sur les qualités gustatives des truffes et la notion de « terroir » peut être appliquée à l'espèce *T. melanosporum*.

La truffe, la terre, la vie par Callot G. coord.

La production de truffières sauvages, comme celle des truffières implantées, a considérablement diminué depuis le début du siècle. Selon les auteurs, ce phénomène est lié à la dégradation biologique du milieu et en particulier à la disparition progressive de la faune du sol, indispensable pour décompacter et aérer le sol.

A partir de l'analyse détaillée de truffières pilotes et d'études de laboratoire utilisant les méthodes les plus modernes, l'importance du facteur sol est réellement mise en évidence. L'ouvrage s'adresse aux enseignants et chercheurs en science du sol et en biologie, mais aussi aux trufficulteurs et à toute personne curieuse de mieux connaître ce mystérieux champignon.

Rapid Detection of the *Fusarium oxysporum* lineage containing the Canary Island Date Palm wilt pathogen.

Plyler T. R., Simone G. W., Fernandez D. and Kistler H. C.

Phytopathology, 1999, 89 : 407-413.

Fusarium oxysporum f. sp. *canariensis* causes Fusarium wilt disease on the Canary Island Date Palm (*Phoenix canariensis).* In order to facilitate disease management, a polymerase chain reaction (PCR) diagnostic method has been developed to rapidly detect the pathogen. A partial genomic library of *F. oxysporum* f. sp. *canariensis* isolate 95-913 was used to identify a DNA sequence diagnostic for a lineage containing all tested isolates of *F. oxysporum* f. sp. *canariensis*. Two oligonucleotide primers were designed and used to amplify a 567 base pair fragment with *F. oxysporum* f. sp. *canariensis* DNAs. DNA from 61 outgroup isolates did not amplify using these primers. Once the primers were shown to amplify a 0.567 kb fragment from DNA of all the *F. oxysporum* f. sp. *canariensis* isolates tested, a rapid DNA extraction procedure was developed which led to the correct identification of 98% of the tested *F. oxysporum* f. sp. *canariensis* isolates.

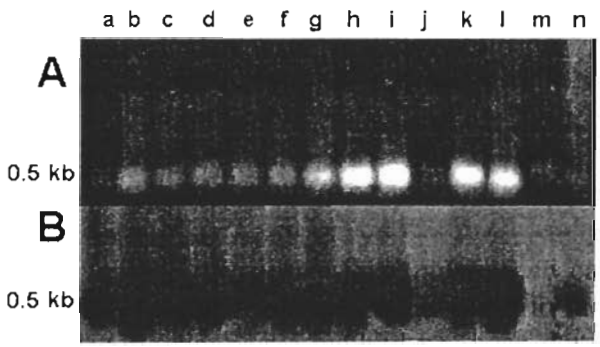


Fig. 2. A, Polymerase chain reaction (PCR) amplification of DNA from isolates of *Fusarium oxysporum* f. sp. *canariensis* at a 60°C annealing temperature using primers HK66 and HK67. The isolates (beginning in lane a) are 84-01b, 94-841, 95-1007, 84-02b, 84-305, 84-01a, 95-1001, 84-207, 95-1006, 95-2122, 95-914, and 84-104. DNA from strains of *F. oxysporum* f. sp. *raphani* (699) and *F. oxysporum* f. sp. *cubense* (3S1) are in lanes m and n, respectively. B, Southern blot of PCR products in A probed with clone C26.

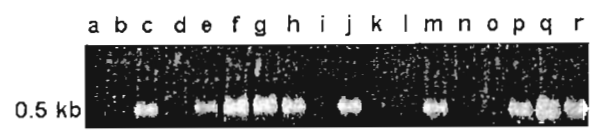


Fig. 3. Polymerase chain reaction (PCR) amplification of DNA from isolates of *Fusarium oxysporum* f. sp. *cubense* at a 62°C annealing temperature using primers HK66 and HK67. Lane a, PCR-negative control consisting of reaction mix without DNA template. Lane b, DNA from a strain of *F. oxysporum* f. sp. *raphani* (699). Lanes c to o, Amplification products from isolates of *F. oxysporum* f. sp. *cubense* (SABA, 3S1, A2, PW7, GM, F9127, STPA, F0001, B2-1, DAVAO, 22615, STNP1, and STA2). Lanes p to r, *F. oxysporum* f. sp. *canariensis* isolates (94-2140, 95-1004, and 95-1004).

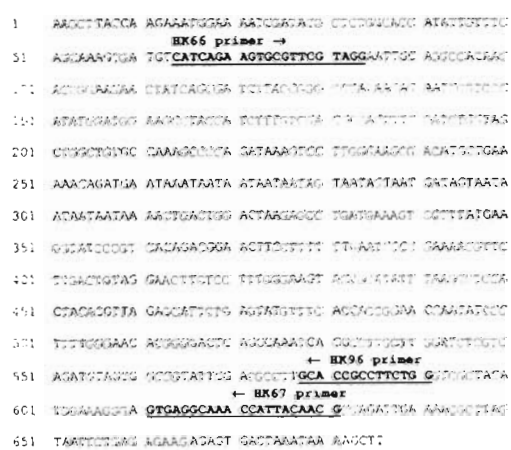


Fig. 1. Sequence and primer selection for clone C26. C26 is a clone from the partial library of *Fusarium oxysporum* f. sp. *canariensis* isolate 95-913. The GenBank accession number for C26 is AF118442.

Genetic diversity among isolates of *Fusarium oxysporum* f. sp. *canariensis*.

Plyler T. R., Simone G. W., Fernandez D. and Kistler H. C.

Plant Pathology, 2000, 49 : 155-164.

Fusarium oxysporum f. sp. *canariensis* causes vascular wilt disease of *Phoenix canariensis*, the Canary Island date palm. Seventy-two isolates of this fungus were obtained from diverse geographic locations including France, Japan, Italy, the Canary Islands, California, Florida, and Nevada. The isolates were tested for vegetative compatibility and for similarities based on mitochondrial DNA (mtDNA), single-copy sequences, and repetitive DNA (pEY10) polymorphisms. Seventy-one percent of the isolates belonged to a single vegetative compatibility group (VCG 0240) and four closely related mitochondrial RFLP patterns were found. A subset of the isolates was further tested for single-copy RFLPs and repetitive DNA fingerprints. Only four single-copy RFLP haplotypes were found among 25 representative isolates of *F. oxysporum* f. sp. *canariensis* tested, using nine polymorphic single-locus probe/enzyme combinations. Finally, 32 different pEY10 DNA fingerprints were found out of 57 isolates examined. Overall the results indicate that *F. oxysporum* f. sp. *canariensis* is a single lineage with a low to moderate level of genetic diversity.

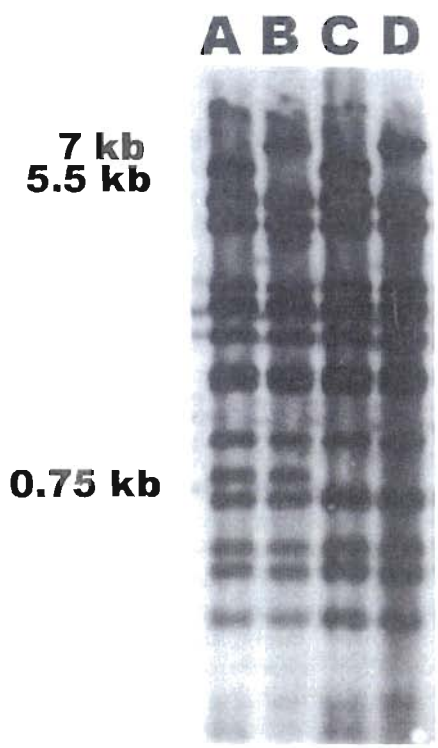


Figure 3 Mitochondrial haplotypes generated from total *F. oxysporum* f. sp. *canariensis* DNA digested with *Hae*III and probed with mitochondrial DNA from *F. oxysporum* f.sp. *cubense* isolate 3S1. Letters A -D correspond to the mitochondrial haplotype designation shown in Table 1.

Fusarium wilt disease of Canary Island date palm (photo G.Simone)

Widespread occurrence of the *Fot 1* transposon within the *Fusarium oxysporum* species.

Langin T., Davière J-M., Fernandez D. and Daboussi M-J.

Gene, 2000, (in press).

Fotl, first identified in the plant pathogenic fungus *Fusarium oxysporum,* is a member of the new *pogo* superfamily of transposable element, distributed in insects, human and fungi. To evaluate the evolutionary forces that shape the distribution of this element within the *F. oxysporum* species, a combination of three different techniques were used, *i. e.* Southern blot, PCR amplification and sequencing. The analysis of more than 270 strains, revealed thad *Fot 1* elements are present in most of the 12 *formae speciales* analyzed, essentially as complete copies. The partial sequencing of several *Fot 1* copies from different strains belonging to four *formae speciales* revealed that *Fot 1* family is made of functional and nonfunctional elements presenting a very low level of intraspecitic polymorphism (<2%). Phylogenetic analysis identified at least three different subfamilies that can coexist in the same strain or in the same *forma specialis*. All these results suggest that *Fot 1* represents an ancient component of the *F. oxysporum* genome . The copy number of *Fot 1* varies tremendously in *F. oxysporum* strains (0 to more than 100) . The strains containing more than 100 copies reflects the existence, in natural conditions, of a burst of amplification, triggered by unknown mechanisms. The existence of several strains and *formae speciales*, devoid of *Fot1* copy, suggest that active or inactive copies of this element can be eliminated by stochastic loss events. At the opposite, the spotty distribution of *Fot1* in the *F. oxysporum* f. sp. *vasinfectum* could reflect a recent introduction of this element by horizontal transfer.

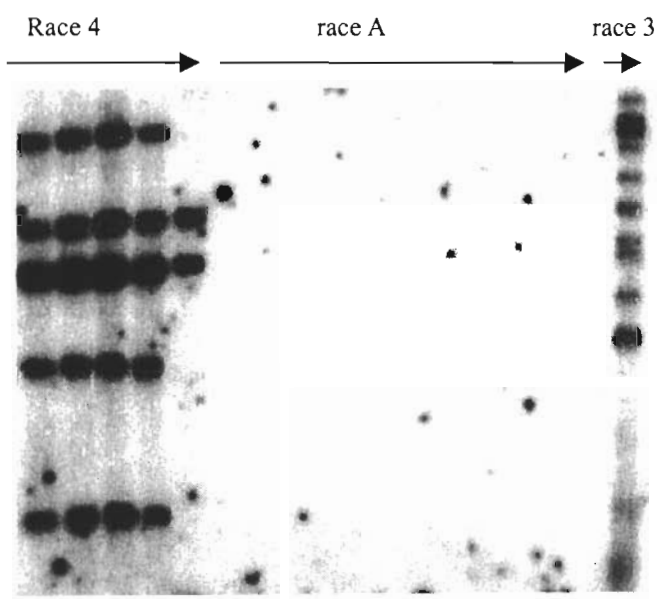


Fig 4 : distribution of the transposable element *Fot1* in the *F. oxysporum* f. sp. *vasinfectum* races.

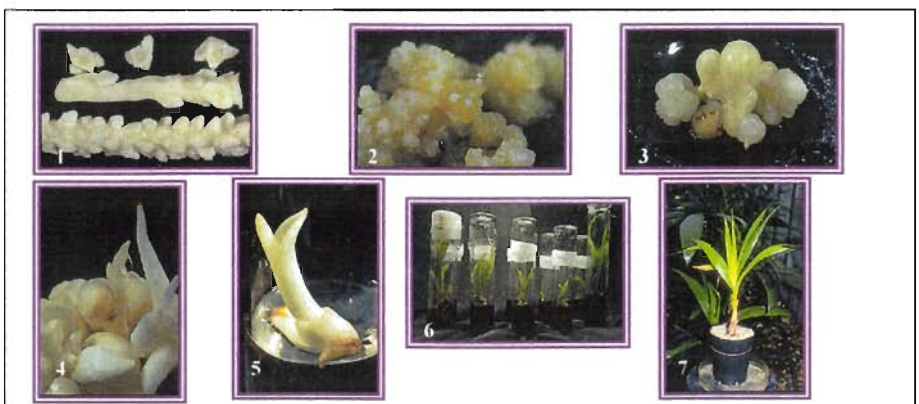
International co-operation for the development of *in vitro* vegetative propagation in coconut (*Cocos nucifera* L.)

HOCHER V., VERDEIL J.L. et al.., *Cahiers Agricultures, 1998, 7 : 505-509.*

This paper discusses how an international collaboration was established to overcome difficulties encountered in vitro coconut regeneration; The main results obtained during the collaborative work carried out by the different partners of this international EU-funded project funded are also presented.

1) Why is coconut regeneration warranted? Coconut palm (*Cocos nucifera* L.) is a major agricultural crop in tropical areas. It is a major oil-crop on an industrial scale, and an important subsistence and cash crop for smallholders. However, the coconut sector is burdened with a number of problems that affect its productivity, particularly the widespread use of unimproved planting material, the old age of existing plantations and the prevalence of various pests and diseases, for which no chemical treatment is currently available. Since coconut palm is generally cross-pollinated and heterozygous, seed propagation gives rise to high variability in hybrid progeny. *In vitro* vegetative multiplication, through somatic embryogenesis, of high performance individuals thus offers the only short and medium-term hope for the production of homogenous planting material and for substantial improvement in the plantation productivity. Cloning should also allow rapid multiplication of selected individuals with resistance or tolerance to serious diseases and to harsh growing conditions. Unfortunately, coconut is a highly recalcitrant species with respect to tissue culture.

2) International collaboration potential. In 1993, several groups (ORSTOM/CIRAD-CP, France; IDEFOR/DPO, Côte d'Ivoire; Wye College, United Kingdom; the Hanover University, Germany; PCA, Philippines and CICY, Mexico) involved in coconut regeneration research joined forces for the first time to overcome major difficulties encountered in coconut regeneration. This collaboration was made possible by EU funding within the STD-3 programme « Coconut : development of methods for the clonal propagation of élite, disease resistant palms by somatic embryogenesis » (ERBTS3*CT940298) which was initiated in January 1995. After accurately identifying the problems (intense browning of the tissue, heterogeneity tissue response, slow *in vitro* process, low somatic embryos rates, weakness of somatic plantlets, the different participants of the project have set up a work programme for each team and many exchanges (information, protocols, material and people) have been planned.



1- Explant preparation : spike of coconut immature inflorescence. 2- Coconut embryogenic callus. 3- Coconut embryo structures, globular type. 4- Clump of coconut somatic embryos during maturation phase. 5- Coconut germinating somatic embryo. 6- Coconut somatic plantlets. 7- Coconut somatic plantlet after weaning in the greenhouse

3) Results obtained *via* the *in vitro* protocol. The micropropagation process developed by the different team includes four steps: callogenesis, embryogenesis induction, maturation and embryo germination. The ORSTOM/CIRAD protocol is presented in *photos 1 to 7*. In this project, there has been solid progress in controlling coconut regeneration in the last 3 years. The partners have exchanged information and techniques, thus enabling them to improve their protocols. Clonal plantlets are now produced in most of the participating laboratories. The results of the different studies undertaken through this collaboration have considerably advanced the research.

4) Other strategies developed through the project. Different phytohormone analyses were developed by ORSTOM/CIRAD in media culture and by Wye college and CICY in tissue culture. This has led to improved management of 2,4-D and activated charcoal concentrations used for coconut regeneration.

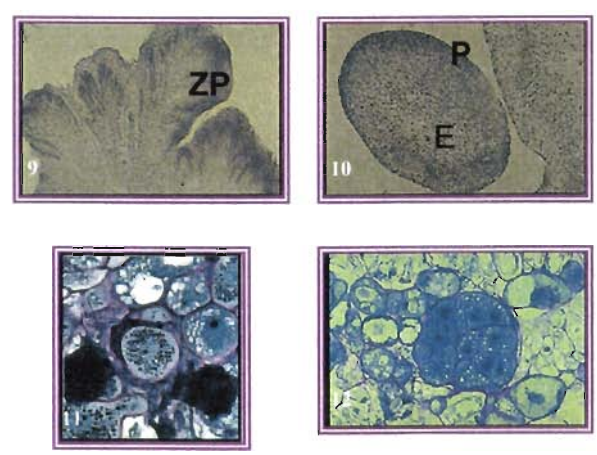
As coconut regeneration is a slow process, the use of histology is essential for controlling somatic cultures. The embryogenic steps were accurately defined by each team and it was found that the events were the same, irrespective of the system and / or the team involved. Two somatic embryo origins were noted: a pluricellular pathway (figure 9 and 10) and a unicellular pathway (figure 11 and 12) and we have now precise criteria for the characterisation of cultures. The German team has also developed an approach for investigating early protein markers using antibodies specific to embryogenesis induction and of cell division. Another important aspect of this project has involved application of the protocol to different cultivars. This was facilitated by IDEFOR's participation in the project and by between-partner exchanges of different types of material. Work is now focused on embryo germination and growing micropropagated plantlets in the greenhouse, which is still a bottleneck. The results of a study of photosynthesis during *in vitro* development of zygotic embryos suggest that a nutritional deficiency could be responsible for the slow development of somatic plantlets. A study on nutrition during coconut germination is under way at ORSTOM/CIRAD, CICY and PCA and the role of haustorium (cotyledon) is currently being investigated.

9-:Coconut embryogenic callus giving rise to somatic structures by fragmentation of the pseudo-cambial zone (ZPC)

10- Coconut somatic proembryo from pluricellular origin. E : embryo, P : protoderm

11- Coconut embryogenic cell, isolated by a specific cell wall, which will give a somatyic embryo.

12- Coconut somatic proembryo from unicellular origin.



5) Conclusion. We agree with Georges and Sherrington that coconut is one of the most difficult species for *in vitro* regeneration, even though considerable progress has been made through international collaboration between many research teams in the STD3 project. Some difficulties have been overcome (callogenesis, induction of embryogenesis, development of complete embryos) but we are still cautious as coconut germination and regeneration remains a difficult challenge. Nevertheless, the promising results of this project clearly indicate that is essential to continue this type of interchange, which would not be possible without EU funding. Continued research on coconut regeneration is crucial, and the future for coconut smallholders will hopefully be enhanced through such international co-operation initiatives.

Suitability of RAPD analysis for the detection of somaclonal variants in oil palm (*Elaeis guineensis* Jacq).

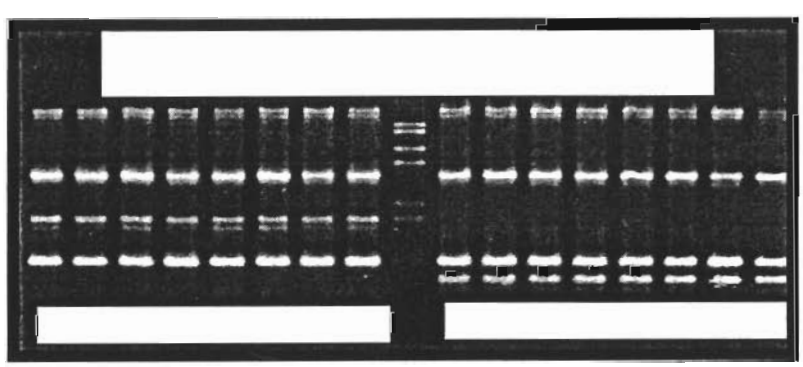
Rival A., Bertrand L., Beulé Th., Combes M.C., Trouslot P. and Lashermes P.

Plant Breeding, 1998, 117 : 73-76.

RAPD analysis using arbitrary 10-mer oligonucleotide primers was employed in order to investigate the genetic fidelity of somatic embryogenesis-derived regenerants of oil palm (*Elaeis guineensis* Jacq). Regenerant palms bearing the «mantled» phenotype were identified in the field and the utility of RAPD markers to distinguish these variants from palms of the normal type was assessed. Of the 387 primers, 259 (67%) were successfully used to amplify oil palm DNA genomic fragments with consistently reproducible banding.

Of these 387 primers, 73 (19%) primers enabled the identification of polymorphism between clones. 24 of these 73 primers were chosen for use in a larger experiment aimed at compairing firstly the mother palm genome with that of its clonal offspring and secondly true-to-type and variant regenerants. No intraclonal variability and no differences between mother palms and regenerants could be identified using the total number of scored markers of 8,900. Thus, the regeneration protocol based on somatic embryogenesis set up for oil palm clonal propagation does not induce any gross genetic changes. The results obtained revealed however that the RAPD approach is not suitable for the detection of the « mantled» variant phenotype.

The use of RAPD markers for the detection of somaclonal variation in oil palm is discussed and alternative molecular approaches are proposed.



Gel electrophoresis of RAPD fragments obtained with somatic embryogenesis-derived adult oil palms from two different clonal lines : LMC51 and LMC 63 : [N], normal; [AN], abnormal «mantled» variants. Primer OPJ 024 (Operon Technologies).

Growth and carboxylase activities in *in vitro* micropropagated oil palm plantlets during acclimatisation : comparison with conventionally germinated seedlings.

Rival A., Beulé T., Lavergne D., Nato A. and Noirot M.

Advances in Horticultural Sciences, 1998, 3 : 111-117.

In order to characterise the physiological phenomena which occur during acclimatisation of oil palm (*Elaeis guineensis* Jacq) plantlets produced through somatic embryogenesis, a comparison of the growth and carboxylase activities of *in vitro* propagated plants and seedlings was carried out over a 100 day period. Growth parameters (total FW, relative foliar FW and the number of expanded leaves) and biochemical characteristics (soluble protein and chlorophyll content, specific PEPC, RubisCO activities and relative RubisCO content) were studied. Oil palm *in vitro* propagated plants were found to undergo an original pattern of acclimatisation, as their PEPC/RubisCO ratio was not affected during transplanting to the greenhouse environment and remained at the same level (ca. 0.05) as was measured in *in vitro* growing leaves. At about D_{60} after sowing (or *ex vitro* transplanting) the main physiological characteristics (chlorophyll and soluble protein contents, and PEPC/RubisCO ratio) were similar in both seedlings and *in vitro* propagated plants, but growth characteristics were markedly different. Rocket immuno-electrophoresis revealed that relative RubisCO amounts were in a comparable range (ca. 230 $mg.g^{-1}_{prot.}$) in leaves from *in vitro* grown and already acclimatised *in vitro*-propagated plants and were found to be lower than in greenhouse-cultivated adult oil palms (350 $mg.g^{-1}_{prot.}$).

Changes in the PEPC/RubisCO ratio in oil palm seedlings and in vitro grown plantlets.

Time after transplanting (days)	PEPC/RubisCO ratio (in vitro grown plantlets)	PEPC/RubisCO ratio (seedlings)
0	0.05[d]	38.53[a]
20	0.44[d]	8.21[b]
30	0.30[d]	0.93[c]
50	0.03[d]	0.18[d]
80	0.02[d]	0.12[d]
100	0.07[d]	0.02[d]

The data are the means of four replicate samples. Means followed by the same letter are not significantly different as determined by the Newman and Keuls' test.

Key words : Arecaceae, autotrophy, carbon metabolism, Elaeis guineensis Jacq, PEPC, RubisCO

Accumulation of storage protein and 7S globulins during zygotic and somatic embryo development *Elaeis guineensis*

Morcillo F., Aberlenc-Bertossi F., Hamon S. and Duval Y.

Plant Physiology and Biochemistry, 1998, 36 : 509-514.

The 7S globulins are the storage proteins which predominate in oil palm (*Elaeis guineensis* Jacq.) zygotic embryos. The accumulation of these proteins was studied in embryos during seed development and in single somatic embryos cultured *in vitro*. Antibodies raised against these proteins were used for their detection by Western-blot and quantification with E.L.I.S.A.. In zygotic embryos, the 7S globulins were deposited mainly between the 14th and the 17th post-anthesis week, corresponding to the end of the embryo growth. This represented 10% of the dry weight and 50% of soluble proteins. The amount of soluble proteins and 7S globulins in somatic embryos increased rapidly during the early stage of development, but were almost 80 times lower than in zygotic embryos. In somatic embryos, 7S globulins represented 0.3% of dry weight and 4% of soluble proteins. After 22 days development, the protein content declined slowly, suggesting a lack of embryo maturation and a early germination.

Table 1. Total soluble protein and 7S globulin accumulation kinetics in zygotic embryos.

	Post-anthesis weeks			
	14	17	21	24
Soluble proteins (mg per 50 embryos)	6.105 [a]	15.570 [b]	16.940 [b]	17.840 [b]
7S globulin equivalents (mg per 50 embryos)	2.406 [a]	6.682 [b]	8.437 [c]	7.209 [b]
7S globulin equivalents/ total soluble proteins ($mg.g^{-1}$)	394.1 [a]	429.2 [a]	498.1 [a]	404.1 [a]

Table 2. 7S globulin accumulation kinetics in somatic embryos.

	Days culture				
	15	18	22	25	29
Soluble proteins (mg per 50 embryos)	0.794 [a]	2.803 [b]	2.894 [b]	2.353 [bc]	1.995 [c]
7S globulin equivalents (mg per 50 embryos)	0.018 [a]	0.084 [b]	0.109 [c]	0.098 [d]	0.087 [b]
7S globulin equivalents/ total soluble proteins ($mg.g^{-1}$)	22.5 [ab]	30.5 [abc]	37.4 [bcd]	42.7 [cd]	43.4 [cd]

BA enhances the germination of oil palm somatic embryos derived from embryogenic suspension cultures

Aberlenc-Bertossi F., Noirot M. and Duval Y.

Plant Cell, Tissue and Organ Culture, 1999, 56 : 53-57.

Embryogenic suspension cultures of oil palm (*Elaeis guineensis* Jacq.) allow mass propagation of somatic embryos; however regeneration rates are low. Histological observations have revealed that shoot development might be limited by the absence of a caulinary meristem. The addition of 6-benzyladenine during development was found to induce shoot apex differentiation and thus increased germination rates, by up to 70 %. However, multiple shoot formation was a consequence of a longer period of cytokinin supply during the development of the embryo. In contrast, a short period of culture on medium with 6-benzyladenine at the begining of embryo development was found to result in single shoot production.

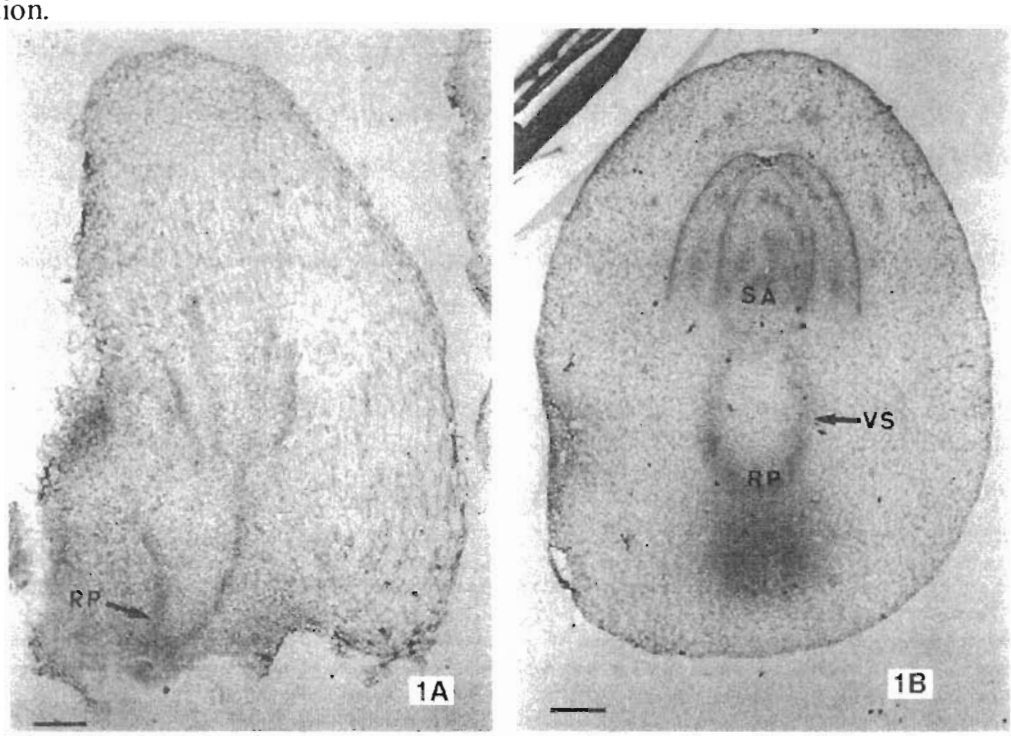


Fig. 1. Oil palm somatic embryos cultured on hormone-free medium (A) (R.P: root pole ; Bar=250 µm) and on BA containing medium (B) (S.A: shoot apex, V.S: vascular strand ; Bar=300 µm).

Table 1 Effects of culture on media with various concentrations of BA on the percentage of single shoot produced by somatic embryos after 2 months germination. Four clones were cultured over a period of 4 weeks.

BA	Single shoot (%)			
(µmol)	clone 121	clone 123	clone 221	clone 87
0	22 a	7 a	47 a	22 a
1	60 b	67 b	73 b	33 a
5	29 a	56 b	64 ab	29 a
10	33 a	42 b	56 ab	20 a

BA effect : F (3,32)=27.13 ; p<0.000 ; *** ; Clone effect : F (3,32)=27.72 ; p<0.000 ; ***
Interaction : F (9,32)=3.73 ; p=0.0027 ; **.

DIFFERENTIAL EFFECTS OF GLUTAMINE AND ARGININE ON 7S GLOBULINS ACCUMULATION DURING THE MATURATION OF OIL PALM SOMATIC EMBRYOS

Morcillo F., Aberlenc-Bertossi F., Noirot M., Hamon S. and Duval Y.

Plant Cell Reports, 1999, 18 : 868-872.

The low vigour of plantlets resulting from oil palm somatic embryos may be due to insufficient levels of deposited storage proteins. Thus, in order to improve embryonic maturation and the vigour of regenerated plantlets, we investigated the effects of modifying culture conditions with respect to the accumulation of the major oil palm storage proteins, the 7S globulins. In this study, the effect of arginine and glutamine on globulin accumulation was studied using somatic embryos of two different genotypes. Arginine and glutamine were both found to enhance protein accumulation but in different ways which were best illustrated by measurements of soluble proteins per embryo and 7S globulin content per dry weight. Arginine increased the level of soluble proteins by 46% irrespective of the clone, and glutamine by 19 and 63% depending on the clone. The clone which accumulated the least protein in the presence of glutamine was that which contained the more protein initially. Only arginine favoured the accumulation of 7S globulin content per dry weight, irrespective of the clone considered (+ 26%). This study will enable further investigations of specific storage proteins as potential markers for regenerated plantlets vigour.

Table 1: Intra-clone effects of glutamine and arginine on maturation somatic embryos. The data are given as the mean of six independent experiments

Treatment Arg (mM)	Treatment Glu (mM)	No. of embryos		Dry weight (mg) / 50 embryos		Soluble proteins (mg) / 50 embryos		Soluble proteins / Dry weight (mg/g)		7S globulins (mg) / 50 embryos	
		121	221	121	221	121	221	121	221	121	221
0	0	203.67^{a}	179.80^{a}	47.42^{a}	49.80^{a}	2.40^{a}	3.90^{a}	51.2^{a}	82.00^{ab}	0.052^{a}	0.261^{a}
0	20	188.09^{a}	151.52^{b}	65.43^{b}	65.24^{b}	4.13^{b}	6.01^{b}	62.73^{b}	96.76^{abc}	0.070^{b}	0.360^{b}
5	0	195.38^{a}	159.57^{ab}	51.21^{a}	57.27^{c}	3.73^{b}	6.05^{b}	72.10^{c}	110.70^{cd}	0.076^{b}	0.390^{b}
5	20	186.50^{a}	153.73^{b}	78.24^{c}	75.20^{d}	6.04^{c}	8.00^{c}	76.55^{c}	111.5^{bcd}	0.11^{c}	0.520^{c}

The intra-clone means followed by the same letter are not significantly different at the 0.05 probability threshold determined by the Newman and Keuls test.

Treatment Arg (mM)	Treatment Glu (mM)	7S globulins / Dry weight (mg/g)		7Sglobulins/Soluble proteins (mg/g)	
		121	221	121	221
0	0	1.06^{a}	5.34^{a}	21.00^{a}	65.25^{a}
0	20	1.04^{a}	5.66^{a}	16.71^{a}	60.38^{a}
5	0	1.48^{b}	7.00^{b}	20.76^{a}	64.79^{a}
5	20	1.38^{b}	6.89^{b}	18.26^{a}	65.13^{a}

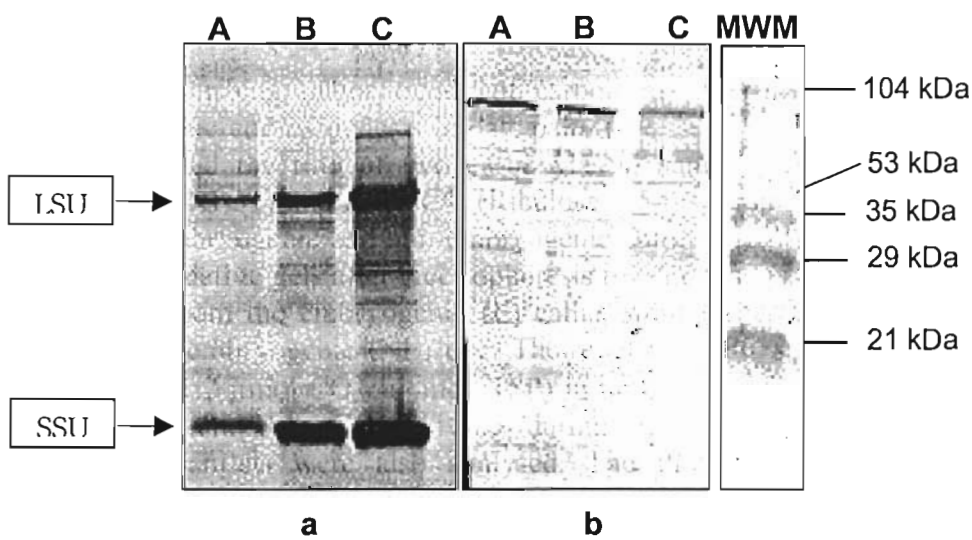
The intra-clone means followed by the same letter are not significantly different at the 0.05 probability threshold determined by the Newman and Keuls test.

Carbon metabolism in *in vitro* cultures of date palm : *Phoenix dactylifera* L. : the role of carboxylases (PEPC and RubisCO).

Masmoudi R., Rival A., Nato A., Lavergne D., Drira N. and Ducreux G.

Plant Cell, Tissue and Organ Culture, 1999, 57 : 139-143.

In the aim of describing major trends of carbon metabolism during the initiation and expression of somatic embryogenesis in date palm (*Phoenix dactylifera* L., cv Deglet Nour), we have investigated the role of two carboxylases, namely PEPC (Phosphoenolpyruvate carboxylase, EC 4.1.1.31) and RubisCO (Ribulose 1,5-bisphosphate carboxylase/oxygenase, EC 4.1.1.39), in embryogenic and non-embryogenic cultures. The detection of PEPC activity on polyacrylamide native gels after electrophoresis revealed the presence of 3 active isoforms in crude extracts from the embryogenic (E) callus strain, whereas only a single band was present in the non-embryogenic (NE) one. The level of PEPC specific capacity was of the same order (3.9 ± 1.2 µmol CO_2 $.h^{-1}.mg^{-1}$ TSP) in both types of cultures. Further changes in carboxylase (PEPC and RubisCO) activities during the growth and development of somatic embryo–derived plantlets were also analysed. The PEPC/RubisCO ratio was found to progressively decrease (from 17.7 to 0.2) throughout the *in vitro* development of plantlets, due to a substantial depletion of PEPC activity, which decreased from 5.3 to 1.2 µmol $CO_2.h^{-1}.mg^{-1}$ $TSP_{.}$. Concomitantly, RubisCO assumed greater importance (from 0.3 to 5.3 µmol $CO_2.h^{-1}.mg^{-1}{}_{TSP}$) and became the main route for inorganic carbon fixation. Western blot analysis using polyclonal antibodies raised against PEPC and RubisCO purified from tobacco leaves confirmed this trend in terms of relative enzyme abundance.



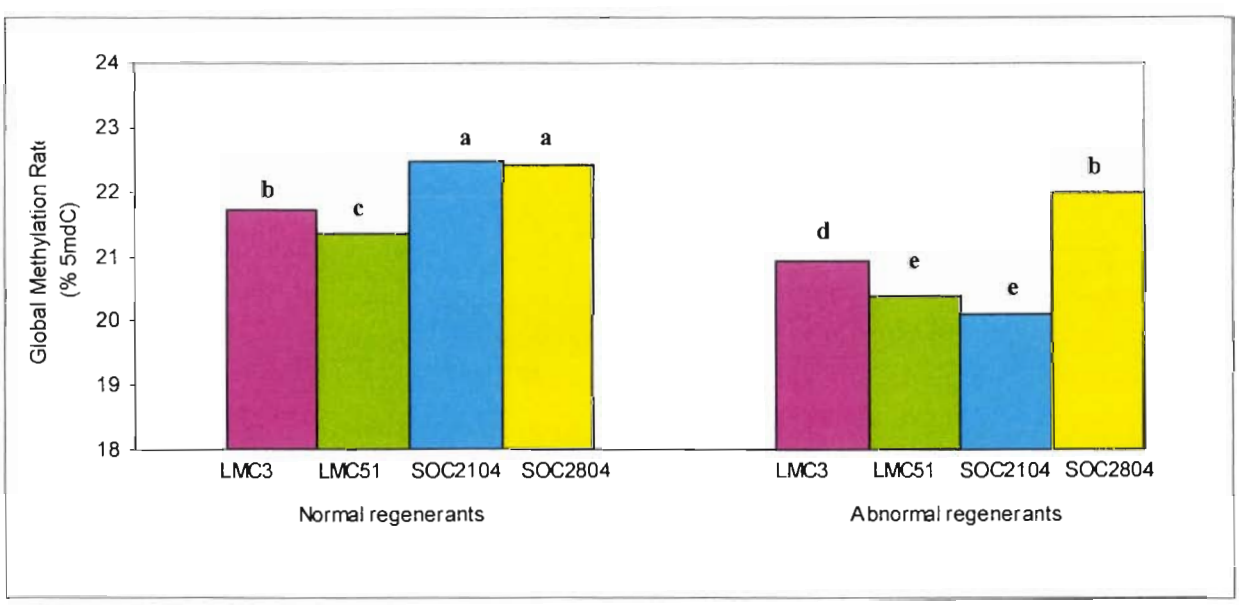
Western blot analysis after SDS-PAGE of RubisCO (a) and PEPC (b) carboxylases from date palm crude extracts sampled at various stages of somatic embryos development.
Lane A: young somatic embryos; Lane B: plantlets (1 leaf); Lane C: plantlets (2-3 leaves).
MWM : Molecular weight markers.
TSP amounts in wells were 1µg for RubisCO detection and 30µg for PEPC detection.

Somaclonal variation in Oil Palm (*Elaeis guineensis* Jacq.): The DNA methylation hypothesis

Jaligot E., Rival A., Beulé Th., Dussert S. and Verdeil J.L.

Plant Cell Reports, 2000, 19 : 684-690.

The occurrence of somaclonal variants (*ca* 5%) among populations of somatic embryo-derived oil palms (*Elaeis guineensis* Jacq.) currently hampers the scaling-up of clonal plant production. In order to investigate the relationship between the „mantled" somaclonal variant and possible alterations in genomic DNA methylation rate, two complementary approaches have been used. HPLC quantification of relative amounts of 5-methyl-deoxycytidine has shown that global methylation in leaf DNA of abnormal regenerants is 0.5 to 2.5% lower than in their normal counterparts (20.8% versus 22%, respectively). When comparing Nodular Compact Calli (NCC) and Fast Growing Calli (FGC), yielding respectively 5% and 100% of „mantled" plantlets, this decrease was up to 4.5% (from 23.2 to 18.7%). An alternative method, the *Sss*I-Methylase Accepting Assay (*Sss*I-MAA), based on the enzymatic saturation of CG sites with methyl groups, gave convergent results. This work demonstrates that a correlation exists between DNA hypomethylation and the „mantled" somaclonal variation in oil palm.



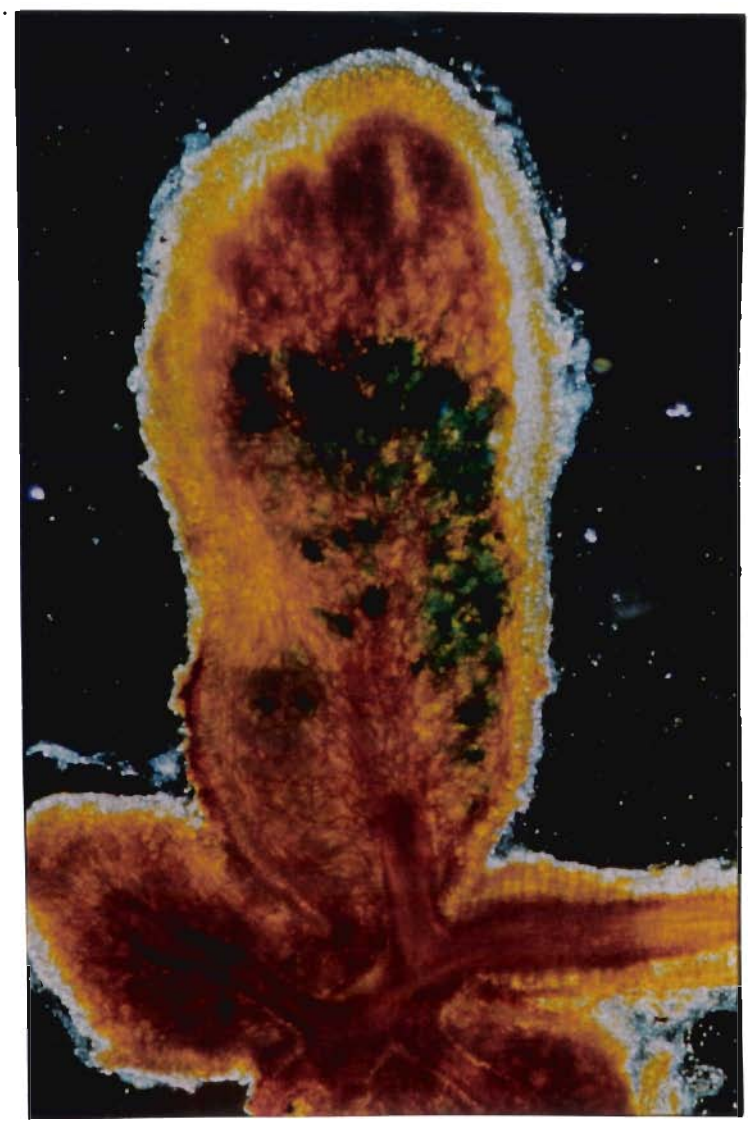
Methylation rates in genomic DNA from leaves of adult regenerant palms. Each value is the mean of three independent measurements performed on seven different palms (i.e. 21 measurements). Data followed by the same letter are not significatively different at the 1‰ level.

The soybean (*lbc3*), *Parasponia* and *Trema* hemoglobin gene promoters retain their symbiotic and nonsymbiotic specificity in transgenic *Casuarinaceae*. Implications for the evolution of hemoglobin genes and root nodule symbioses.

Franche C., Diouf D., Laplaze L., Auguy F., Frutz T., Rio M., Duhoux E. and Bogusz D.

Molecular Plant Microbes Interaction, 1998, 11 : 887-894.

The purpose of this study is to compare the control of expression of legume and non-legume hemoglobin genes. We used the *Casuarina glauca* and *Allocasuarina verticillata* transformation system to examine the properties of the soybean (*lbc3*), *Parasponia* and *Trema* hemoglobin gene promoters in actinorhizal plants. Expression of the hemoglobin promoters *Gus* genes was examined by fluorometric and histochemical assays. The fluorometric assays in various organs showed that the soybean and *Parasponia* promoters were most active in nodules whereas the *Trema* promoter gave a very high activity in roots. The histochemical study showed that GUS activity directed by the soybean and the *Parasponia Gus* chimeric genes appeared mainly confined to the infected cells of the *Casuarinaceae* nodules. The *Trema* hemoglobin promoter was primarily expressed in the root's cortex and vascular tissue. The results indicate that the soybean, *Parasponia* and *Trema* hemoglobin promoters retain their cell specific expression in transgenic *Casuarinaceae,* suggesting a close relationship between legume, *Ulmaceae* and actinorhizal hemoglobin genes. The conservation of the mechanism for nodule specific expression of soybean, *Parasponia* and *Casuarinaceae* hemoglobin genes is discussed in view of recent molecular phylogenetic data that suggest a single origin for the predisposition to form root nodule symbioses.



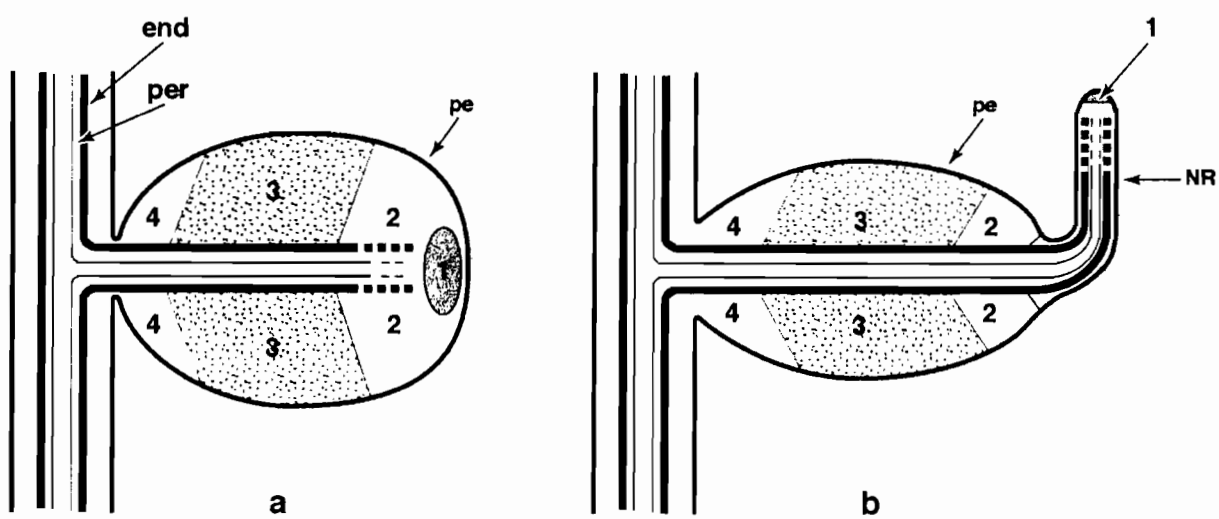
Expression of soybean leg hemoglobin promoter in transgenic *Casuarina glauca* nodule lobe.

Actinorhizal Symbioses: Recent Advances in Plant Molecular and Genetic Transformation Studies

Franche C., Laplaze L., Duhoux E. and Bogusz D.

Critical Reviews in Plant Sciences, 1998, 17 : 1-28.

Infection of actinorhizal plants roots by the actinomycete *Frankia* leads to the formation of a nitrogen-fixing root nodule (actinorhiza) consisting of multiple lobes, each of which is a modified lateral root. Actinorhiza development involves several specific steps, e.g. root hair infection, prenodule formation, and initiation of lobe primordia from root pericycle. This paper summarizes the latest development in the isolation and characterization of nodule-specific and -enhanced transcripts isolated from actinorhiza. The amino acid sequence derived from the nucleotide sequence of the cDNAs, in combination with localization data, showed that gene products are involved in nitrogen, carbon and oxygen metabolism. Furthermore, some transcripts represented encoded gene products that might be part of infection and senescence mechanisms in actinorhiza. The paper also reviews experiments designed to establish genetic transformation systems for actinorhizal plants. This research has led to obtainment of transgenic plants of the *Casuarinaceae* family by using *A. rhizogenes* and *A. tumefaciens*. These new findings are discussed in view of future studies on actinorhizal symbiosis. Since molecular and cellular studies on *Casuarinaceae* and *Betulaceae* are more advanced than on the other six actinorhizal plant families, we will concentrate primarily on species within these two families.



Structure of actinorhizal nodule lobe :
end : endoderme; per : pericycle; pe : periderme.
1 : meristem; 2 : infection zone; 3 : fixation zone; 4 : senescence zone; NR : nodule root.
a- *Alnus* type.
b- *Myrica* or *Casuarina* type. Each nodule consists of multiple lobes, each of which is a modified lateral root.

cDNA Sequence for an Acyl Carrier Protein from actinorhizal Nodules of *Casuarina glauca* (Accession No. Y10994) (PGR98-066).

Laplaze L., Gherbi H., Franche C., Duhoux E. and Bogusz D.

Plant Physiology, 1998, 116 : 1605.

Acyl carrier protein (ACP) is a component of the plant chloroplast fatty acid synthetase (Somerville and Browse, 1991). It binds acyl groups convalently via the prosthestic group, 4-phosphopantetheine. ACP can also serve as an acyl carrier for other reactions than fatty acid biosynthesis (Somerville and Browse, 1991).

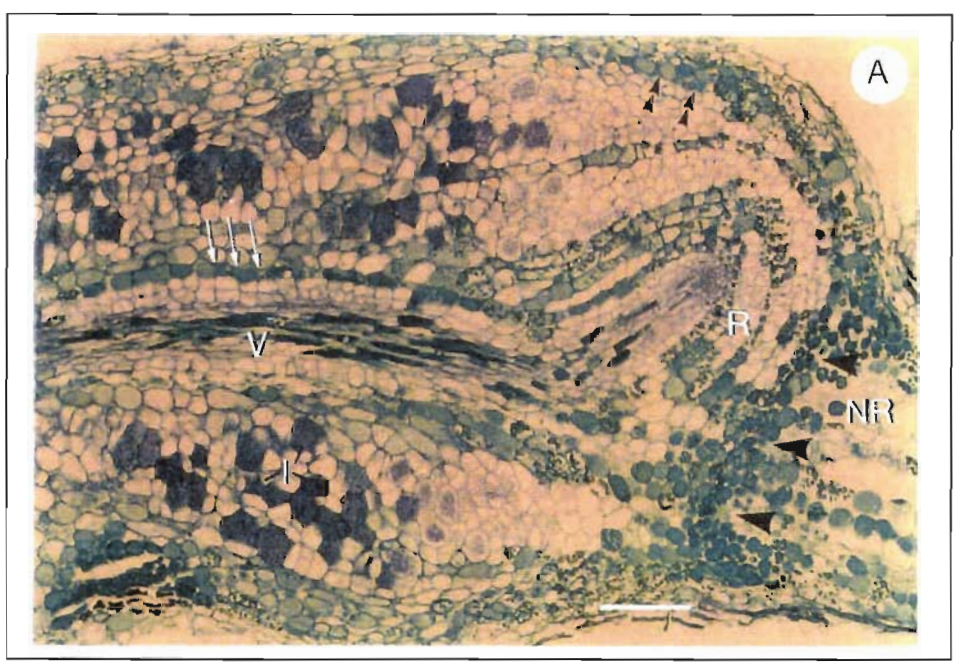
Actinorhizal nodules are organized structures which develop in the root of the so-called actinorhizal plants following the interaction with the actinomycete Frankia. The establishment and functionning of this nitrogen-fixing symbiosis involve modifications of plant gene expression (Franche *et al.*, 1998; Pawlowski and Bisseling, 1996). Cytological and ultrastructural analyses have shown that during infection and in mature infected cells, symbiotic Frankia are surrounded by host-derived wall material whithin the invagined plasma membrane of the host plant. Thus, this suggests that membrane synthesis occurs during the symbiotic process. Here, we report the isolation of a cDNA clone for ACP (cgACP1) isolated from root nodules of the actinorhizal tropical tree *Casuarina glauca*. cgACP1 cDNA is 738 bp long and contains an open reading frame of 411 bp starting at position 118 and ending at position 528. The corresponding polypeptide is 137 amino acids long and its calculated molecular weight and pII are 14,3 kDa and 5,17 respectively. It is very homologous to plant ACP precursors from various plants. The predicted protein contains a putative chloroplast transit peptide clivage-site motif (VCCA; Gavel and von Heijne, 1990) from aa 50 to aa 53 (cleavage before ala 53) and its amino terminus shows high homology to chloroplast transit peptide from other plant ACPs, suggesting that this protein is targeted to the chloroplast. A putative phosphopantetheine attachment site is found from aa 86 to aa 99 (ser 91). Southern blot experiments showed that, like ACP genes from other plants (Somerville et Browse, 1991), cgACP1 belongs to a small multigene family.

Flavan-containing cells delimit *Frankia* infected compartments in *Casuarina glauca* nodules

Laplaze L., Gherbi H., Frutz Th., Pawlowski K., Franche C., Macheix J.J., Auguy F., Bogusz D. and Duhoux E.

Plant Physiol., 1999, 121 : 113-122.

We investigated the involvment of polyphenols in the *Casuarina glauca* - *Frankia* symbiosis. Histological analysis revealed a cell-specific accumulation of phenolics in *C. glauca* nodule lobes creating a compartmentation in the cortex. Histochemical and biochemical analyses indicated that these phenolic compounds belong to the flavan class of flavonoids. We showed that the same compounds were synthesized in nodules and uninfected roots. However, the amount of each flavan was dramatically increased in nodules compared to uninfected roots. The use of *in situ* hybridization established that chalcone synthase transcripts accumulate in flavan-containing cells at the apex of the nodule lobe. Our findings are discussed in view of the possible role of flavans in plant-microbe interactions.



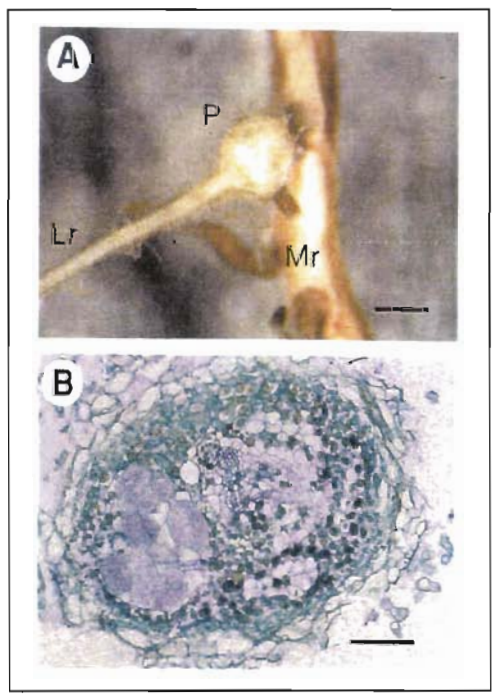
Localization and histochemical characterization of *C. glauca* nodule phenolics. A, longitudinal section of a nodule lobe stained with toluidine blue showing a central vascular bundle (V), a ramification of the nodule lobe (R) and the basis of the nodular root (NR). *Frankia*-infected cells (I) are visible as purple cells. Phenolics (green color) accumulate in the endoderm (white arrows), below the periderm (double black arrow heads) and between the nodule lobe and the nodule root (black arrow heads); bar= 100 μm.

Actinorhizal Prenodule cells display the same differentiation as the corresponding nodule cells

Laplaze L., Duhoux E., Franche C., Frutz Th., Svistoonoff S., Bisseling T., Bogusz D. and Pawlowski K.

Mol. Plant-Microbe Interact., 2000, 13 : 107-112.

Recent phylogenetic studies have implied that all plants able to enter root nodule symbioses with nitrogen fixing bacteria go back to a common ancestor (D. E. Soltis, P. S. Soltis, D. R. Morgan, S. M. Swensen, B. C. Mullin, J. M. Dowd, and P. G. Martin, 92:2647-2651, Proc. Natl. Acad. Sci. USA, 1995). However, nodules formed by plants from different groups are distinct in nodule organogenesis and structure. In most groups, nodule organogenesis involves the induction of cortical cell divisions. In legumes these divisions lead to the formation of a nodule primordium, while in non-legumes, they lead to the formation of a so-called prenodule consisting of infected and uninfected cells. Nodule primordium formation does not involve prenodule cells, and the function of prenodules is not known. Here, we examined the differentiation of actinorhizal prenodule cells in comparison to nodule cells with regard to both symbionts. Our finding indicate that prenodules represent primitive symbiotic organs whose cell types display the same characteristics as their nodule counterparts. The results are discussed in the context of the evolution of root nodule symbioses.



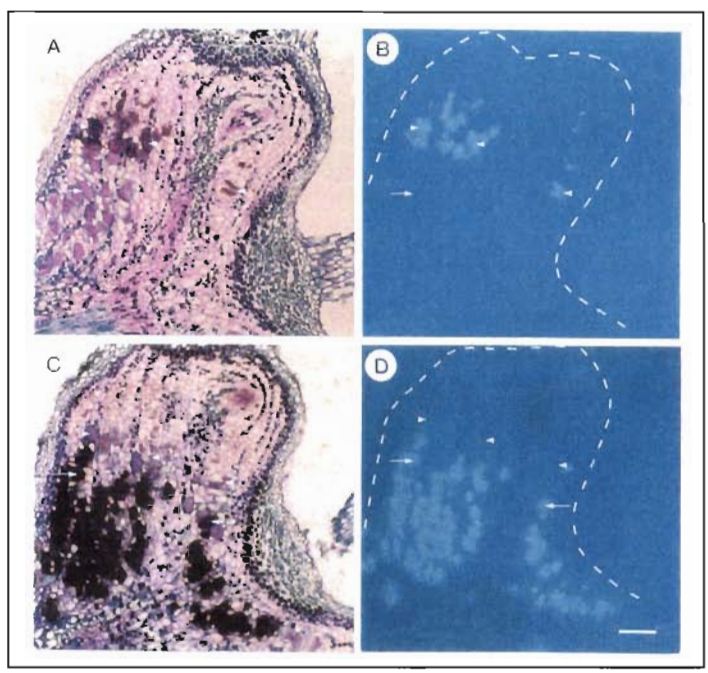
A, Prenodule on a lateral root of *Casuarina glauca*. Divisions and enlargement of cortical cells in the proximity of the infected root hair form the prenodule (P) which appears as a small protuberance. Lr, lateral root; Mr, main root. Bar= 500 μm. **B,** Transversal section through a lateral root showing a prenodule, stained with toluidine blue. The prenodule consists of large *Frankia* infected cells and uninfected cell. Accumulation of phenolics (green color) is observed in response to *Frankia* infection. Bar= 100 μm.

Characterization of a *Casuarina glauca* nodule-specific subtilisin-like protease gene, a homolog of *Alnus glutinosa ag12*

Laplaze L., Ribeiro A., Franche C., Duhoux E., Auguy F., Bogusz D. and Pawlowski K.

Mol. Plant-Microbe Interact., 2000, 13 : 113-117.

In search for plant genes expressed during early interactions between *Casuarina glauca* and *Frankia*, we have isolated and characterized a *C. glauca* gene which has strong homology to subtilisin-like protease gene families of several plants including the actinorhizal nodulin gene *ag12* of another actinorhizal plant, *Alnus glutinosa*. Based on the expression pattern of *cg12* in the course of nodule development, it represents an early actinorhizal nodulin gene. Our results suggest that subtilisin-like proteases may be a common element in the process of infection of plant cells by *Frankia* in both *Betulaceae* (*Alnus glutinosa*) and *Casuarinaceae* (*Casuarina glauca*) symbioses.



Localization of *cg12* transcripts in mature nitrogen-fixing nodules of *C. glauca*. In **A** and **C**, bright-field microscopy was used ; silver grains denoting hybridization appears as black dots. In **B** and **D**, dark-field microscopy and epipolarized light were used ; silver grains are visible as yellow dots. **A** and **B**, expression of *cg12* in a longitudinal section of a *C. glauca* nodule lobe. **C** and **D**, expression of *Frankia nifH* in an adjacent longitudinal section of the same nodule lobe. Arrowheads point at infected cortical cells of the infection zone where high levels of *cg12* expression are found (**A**, **B**) but *Frankia* does not yet express *nifH* (**C**, **D**). Arrows point at infected cortical cells of the fixation zone where *Frankia nifH* is expressed but *cg12* expression has decreased dramatically. No signal was found in control hybridizations using sense RNA as a probe. Bar = 100 μm.

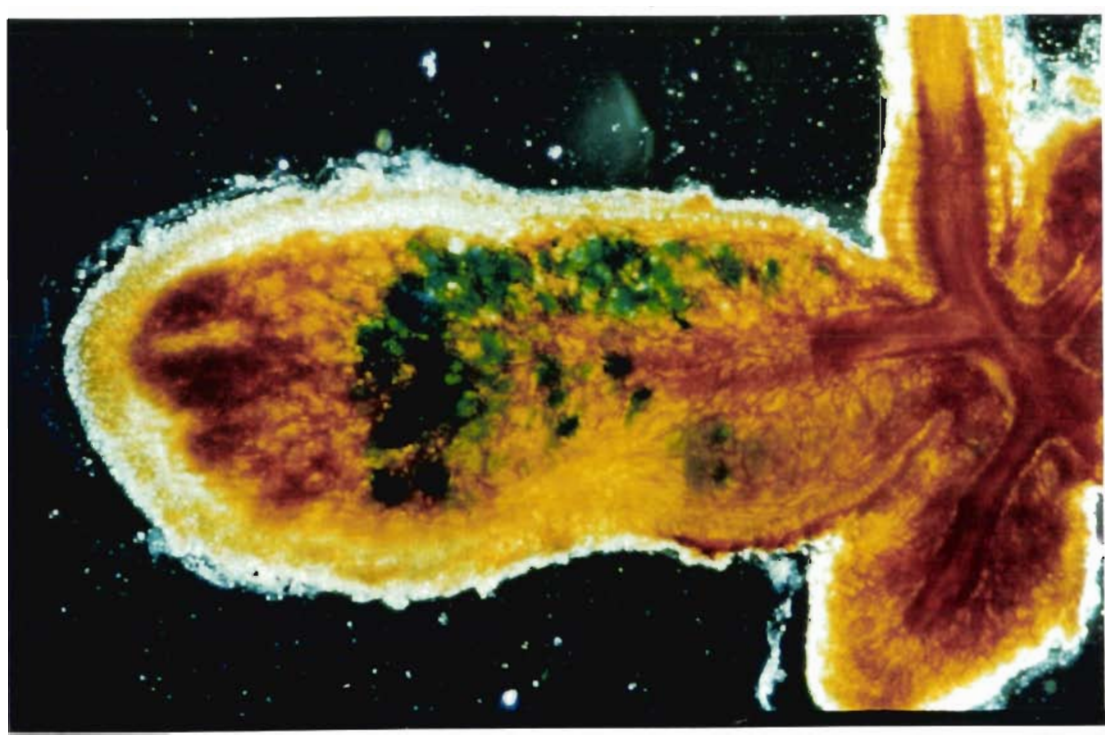
Molecular biology of tropical nitrogen-fixing trees in the *Casuarinaceae* family

Laplaze L., Bon M.C., Sy M.O., Smouni A., Allonneau C., Auguy F., Frutz T., Rio M., Ghermache F., Duhoux E., Franche C. and Bogusz D.

Molecular Biology of Woody Plants, 2000, 1 : 269-285.

Bacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Azorhizobium*, interact with legume plants (with the exception of *Parasponia* from the *Ulmaceae* family; Trinick, 1979), whereas the soil actinomycete *Frankia* establishes a root nodule symbiosis with non-leguminous shrubs and tree species from eight angiosperm families and twenty four genera, collectively called actinorhizal plants (Benson & Silvester, 1993).

Considering the numerous physiological, biochemical, and morphological studies that have been carried out on the genus *Casuarina* (Pinyopusarerk & House, 1993) and the numerous uses in tropical and subtropical agroforestry, we have chosen the *Casuarinaceae* family as a model family to study actinorhizal symbioses. The aim of this review is to report on the most recent advances on molecular biology of the symbiotic interaction between *Casuarinaceae* and *Frankia*. We will describe the isolation and characterization of plant genes involved in *Casuarina glauca* nodule formation and functioning. Besides, we will show how heterologous gene expression studies in transgenic *Casuarinaceae* might contribute to the analysis of both evolution of nitrogen-fixing root nodule symbioses and actinorhizal nodule development.



Expression of the cell-cycle promoter *cdc2* from *Arabidopsis thaliana* in transgenic *A. verticillata* nodule lobe. GUS staining is limited to the phellogen (arrowheads)

Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTLs identification and relationship between resistance and plant morphology

Albar L., Lorieux M., Ahmadi N., Rimbault I., Pinel A., Sy A.A., Fargette D. and Ghesquière A.

Theoretical and Applied Genetics, 1998, 97 : 1145-1154.

Rice yellow mottle virus (RYMV) resistance QTLs were mapped in a doubled-haploid population of rice, IR64/Azucena. Disease impact on plant morphology and development, expression of symptoms and virus content were evaluated in field conditions. Virus content was also assessed in growth chamber. RYMV resistance was found to be under a polygenic determinism, and 15 QTLs were detected on seven chromosomal fragments. For all the resistance QTLs detected, the favourable allele was provided by the resistant parent Azucena. Three regions were determined using different resistance parameters and in two environments. On chromosome 12, a QTL of resistance that had already been detected in this population and another *indica/japonica* population, was confirmed both in the field and in controlled conditions. Significant correlations were observed between resistance and tillering ability, measured on control non-inoculated plants. In addition, three genomic fragments involved in resistance were also involved in plant architecture and development. In particular, the semi-dwarfing gene *sd-1*, on chromosome 1, provided by the susceptible parent, IR64, mapped in a region where resistance QTLs were detected with most of the resistance parameters. In contrast, the QTL of resistance mapped on chromosome 12 was found to be independent of plant morphology.

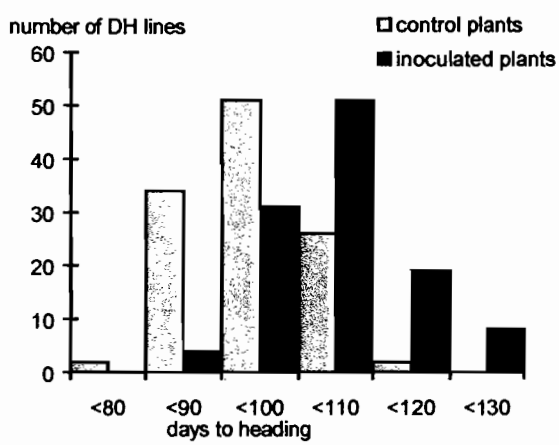
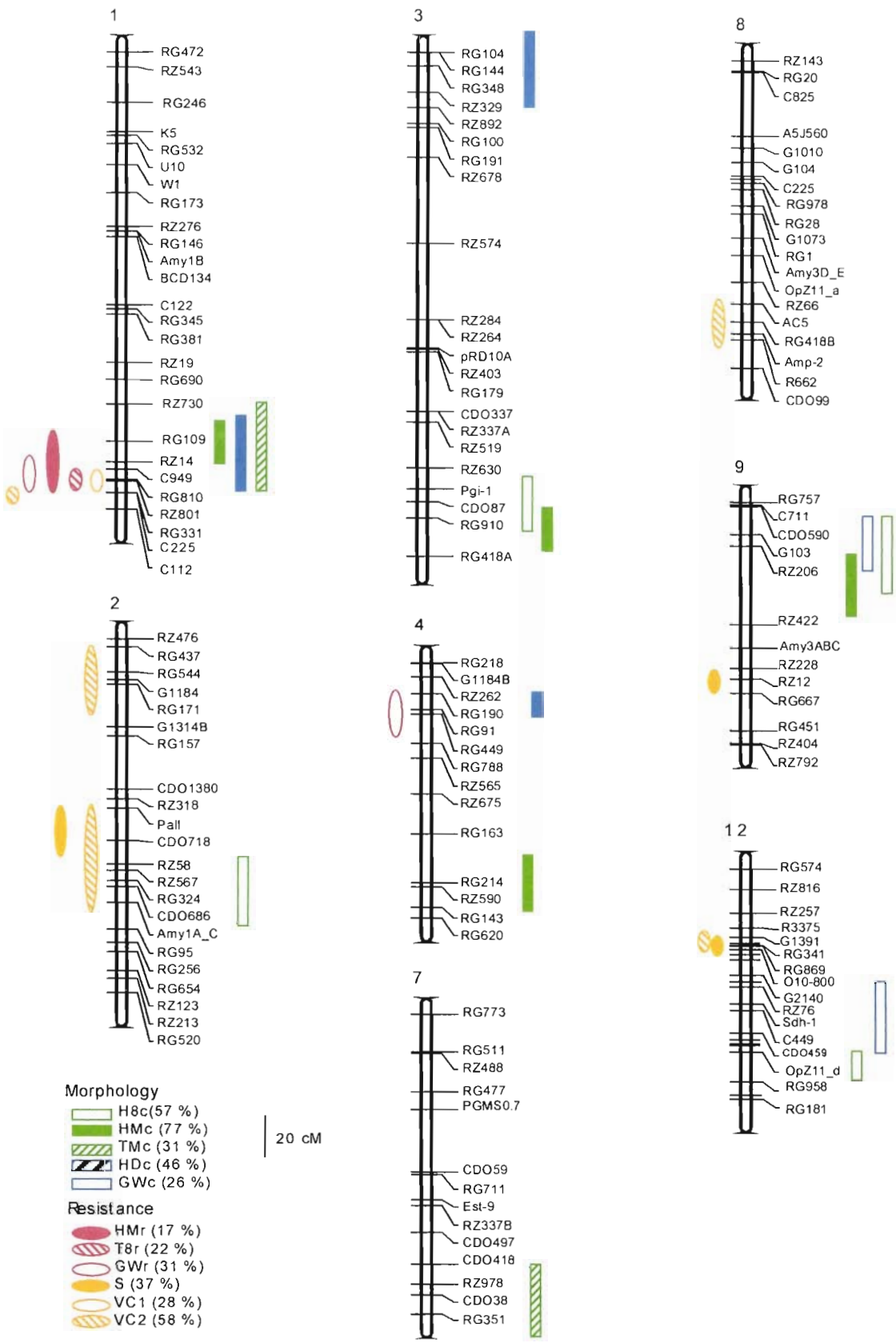


Figure 1. Distribution of heading date for control plants and plants mechanically inoculated with RYMV, in field conditions. Heading occurred, on average, ten days later on inoculated plants than on controls.

Figure 2 (right page). Map positions of QTLs involved in plant morphology and RYMV resistance in 'IR64/Azucena' DH population. QTLs were detected using Mapmaker/QTL, with threshold of LOD=2.6. QTLs are represented by boxes covering a 1-LOD confidence interval. Percentages of variance explained by the multi-QTL model are mentionned in the legend in front of each trait. H8c (plant height 8 weeks after sowing), HMc (plant height at maturity), TMc (number of fertile tillers), HDc (headind date) and GWc (grain weight) were measured on control healthy plants. S (symptoms), VC1 (virus content in the field, measured using an ELISA test) and VC2 (virus content in growth chamber, measured using an ELISA test) were estimated on plants mechanically inoculated with RYMV. HMr (impact of the disease on HMc), T8r (impact on tiller 8 weeks after sowing), GWr (impact on GWc) were estimated, for each line, using the ratio (score obtained on inoculated plants) / (score obtained on controls).


1
RG472
RZ543
RG246
K5
RG532
U10
W1
RG173
RZ276
RG146
Amy1B
BCD134
C122
RG345
RG381
RZ19
RG690
RZ730
RG109
RZ14
C949
RG810
RZ801
RG331
C225
C112
2
RZ476
RG437
RG544
G1184
RG171
G1314B
RG157
CDO1380
RZ318
Pall
CDO718
RZ58
RZ567
RG324
CDO686
Amy1A_C
RG95
RG256
RG654
RZ123
RZ213
RG520
3
RG104
RG144
RG348
RZ329
RZ892
RG100
RG191
RZ678
RZ574
RZ284
RZ264
pRD10A
RZ403
RG179
CDO337
RZ337A
RZ519
RZ630
Pgi-1
CDO87
RG910
RG418A
4
RG218
G1184B
RZ262
RG190
RG91
RG449
RG788
RZ565
RZ675
RG163
RG214
RZ590
RG143
RG620
7
RG773
RG511
RZ488
RG477
PGMS0.7
CDO59
RG711
Est-9
RZ337B
CDO497
CDO418
RZ978
CDO38
RG351
8
RZ143
RG20
C825
A5J560
G1010
G104
C225
RG978
RG28
G1073
RG1
Amy3D_E
OpZ11_a
RZ66
AC5
RG418B
Amp-2
R662
CDO99
9
RG757
C711
CDO590
G103
RZ206
RZ422
Amy3ABC
RZ228
RZ12
RG667
RG451
RZ404
RZ792
12
RG574
RZ816
RZ257
R3375
G1391
RG341
RG869
O10-800
G2140
RZ76
Sdh-1
C449
CDO459
OpZ11_d
RG958
RG181
Morphology
H8c(57 %)
HMc (77 %)
TMc (31 %)
HDc (46 %)
GWc (26 %)
Resistance
HMr (17 %)
T8r (22 %)
GWr (31 %)
S (37 %)
VC1 (28 %)
VC2 (58 %)
20 cM

Genetic basis and mapping of the resistance to the rice yellow mottle virus. II. Evidence of a complementary epistasis between two QTLs

Pressoir G., Albar L., Ahmadi N., Rimbault I., Lorieux M., Fargette D. and Ghesquière A.

Theoretical and Applied Genetics, 1998, 97 : 1155-1161.

The genetic basis of resistance to rice yellow mottle virus (RYMV) was studied in a doubled-haploid (DH) population derived from a cross between the very susceptible *indica* variety IR64 and the resistant upland *japonica* variety Azucena. As a quantitative trait locus (QTL) involved in virus content estimated with an ELISA test has been previously identified on chromosome 12, we performed a wide search for interactions between this QTL and the rest of the genome, and between this QTL and morphological traits segregating in the population. Multiple regression with all identified genetic factors was used to validate the interactions. Significant epistasis accounting for a major part of the total genetic variation were observed. A complementary epistasis between the QTL located on chromosome 12 and a QTL located on chromosome 7 could be the major genetic factor controlling the virus content. Resistance was also affected by a morphology-dependent mechanism since tillering was interfering with the resistance mechanism conditioned by the epistasis between the two QTLs. Marker-assisted backcross breeding was developed to introgress the QTLs of chromosome 7 and chromosome 12 in the susceptible IR64 genetic background. First results confirmed that if both QTLs do not segregate in a backcross derived F2 population, then QTL of chromosome 12 cannot explain differences in virus content. A near isogenic line (NIL) approach is currently developed to confirm the proposed genetic model of resistance to RYMV.

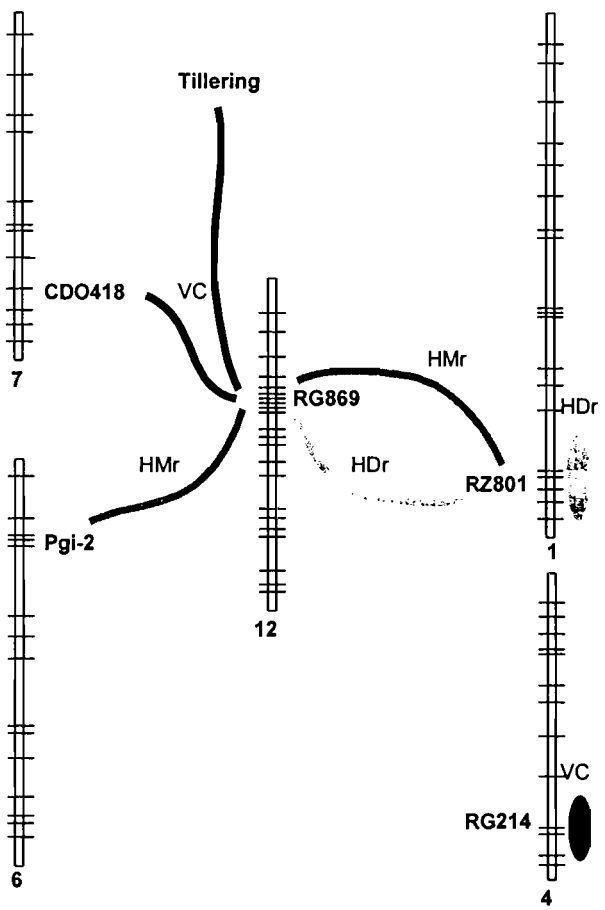


Figure. QTLs and epistasis between QTLs involved in virus content (VC), impact of RYMV inoculation on heading date (HDr) and on plant height at maturity (HMr). VC was measured using an ELISA test on plants grown in growth chamber and mechanically inoculated with RYMV. HDr and HMr were estimated in field conditions, using the ratio (days to heading -or plant height- on inoculated plants) / (days to heading -or plant height- on healthy controls). A major complementary interaction between RG869 (chromosome 12) and CDO418 (chromosome 7) explained 36.5% of VC. Models including all the QTLs and the interactions detected explained 61% of VC, 31% of HMr and 36% of HDr.

Expression and inheritance of multiple transgenes in rice plants

Chen L., Marmey Ph., Taylor N.J., Brizard J.P., Espinoza C., D'Cruz P., Huet H., Zhang S., de Kochko A., Beachy R.N. and Fauquet C.M.

Nature Biotechnology, 1998, 16 (11) : 1060-1064.

The ability to control integration, inheritance, and expression of multiple transgenes is a prerequisite for manipulating biosynthetic pathways and complex agronomic characteristics in plants. One hundred and twenty-five independent transgenic rice plants were regenerated after cobombarding embryogenic tissues with a mixture of 14 different pUC-based plasmids. Eighty-five percent of the R0 plants contained more than two, and 17% more than nine, of the target genes. Plants containing multiple transgenes dis-played normal morphologies and 63% set viable seed. Multigene cotransformation efficiency was corre-lated with the ratio in which the plasmids were mixed with respect to the selectable marker. All target genes had an equal chance of integration, indicating that the nature of the coding region had no effect on the efficiency of integration. Three plant lines containing 11, 10, and 9 transgenes, respectively, were ana-lyzed for patterns of integration and inheritance until the R3 generation. Integration of multiple trans-genes occurred at either one or two genetic loci, with inheritance conforming to a 3:1 Mendelian ratio. Coexpression of four marker genes was investigated until the R2 generation.

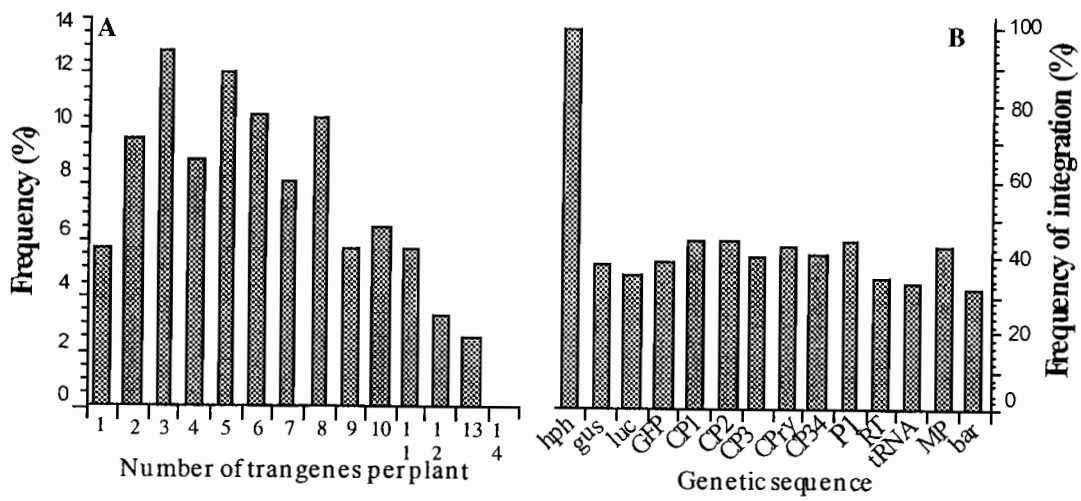


Figure 1. Integration of fourteen transgenes into *Japonica* rice plants by co-bombardment. **A**: Frequency of transgenic plants containing 1 to 14 genes, **B:** Integration frequency for each transgene in transgenic rice. A total of 125 R0 transgenic rice plants were analyzed by PCR for the integration of each of thirteen genes in addition to the *hph* selectable marker. Presence of the *bar* gene was assessed by resistance to the herbicide BASTA.

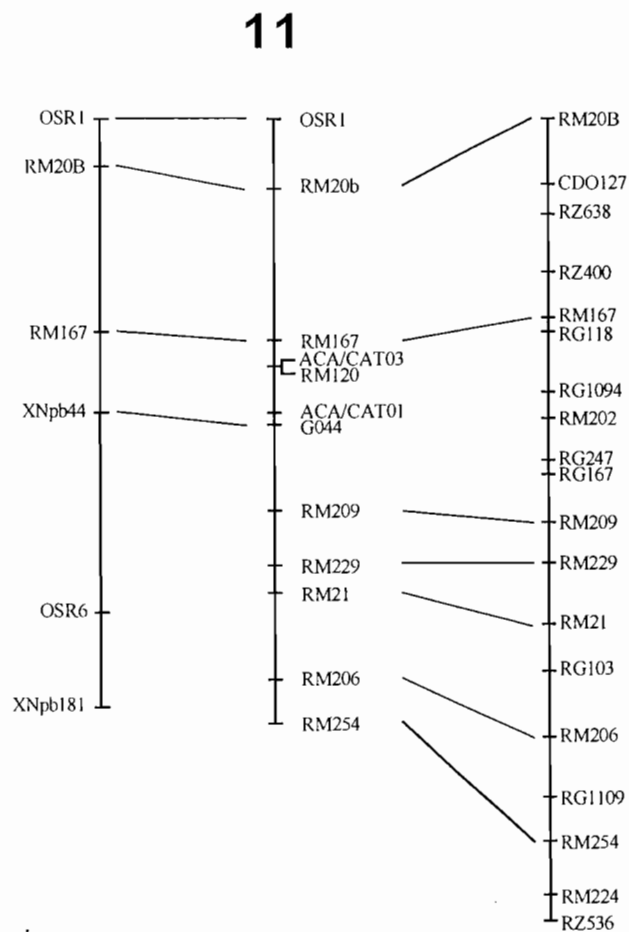
A first interspecific *Oryza sativa* x *O. glaberrima* microsatellite-based genetic linkage map.

Lorieux M., Ndjiondjop M.N. and Ghesquière A.

Theoretical and Applied Genetics, 2000, 100 : 593-601.

Oryza glaberrima is an endemic African cultivated rice species. To provide a tool for evaluation and utilisation of the potential of *O. glaberrima* in rice breeding, we developed an interspecific *O. glaberrima* x *O. sativa* genetic linkage map. It was based on PCR markers, essentially microsatellites and STSs. Segregation of markers was examined in a backcross (*O. sativa*/*O. glaberrima*//*O. sativa*) population. Several traits were measured on the BC_1 plants, and major genes and QTLs could be mapped for these traits. Several of these genes correspond well to previously identified loci. The overall map length was comparable to those observed in *indica* x *japonica* crosses, indicating that recombination between the two species occurs without limitation. However, three chromosomes show discrepancies with *indica* x *japonica* maps. The colinearity with intraspecific maps was very good, confirming previous cytological observations. A strong segregation distortion hot spot was observed on chromosome 6 near the *waxy* gene, indicating the presence of *s10*, a sporo-gametophytic sterility gene previously identified by Sano (1990). Main interests of such a PCR-based map for African rice breeding are discussed, including gene and QTL localisation, marker-assisted selection, and development of interspecific introgression lines.

Figure 1. PCR-based genetic map developed from a backcross population (*O. sativa*/*O. glaberrima*//*O. sativa*) and its comparison with two other rice genetic maps (example of chromosome 11). Left linkage groups: RFLP-based microsatellite map developed on RIL_1 population by Akagi et al. 1996. Center linkage groups: microsatellite-based map developed on BC_1 population (present study). Right linkage groups: RFLP-based microsatellite map developed on DH_1 population by Chen et al. 1997.



First intergeneric hybrids (*Psilanthus ebracteolatus* Hiern x *Coffea arabica* L.) in coffee trees.

Couturon E., Lashermes P. and Charrier A.

Canadian Journal of Botany, 1998, 76 : 542-546.

Intergeneric hybrids between *Psilanthus ebracteolatus* and *Coffea arabica* were successfully produced by crossing at the tetraploid level. Although a total of 41 plants was obtained, only nine plants survived after 5 months growth in the nursery. Hybrid status was confirmed by means of cytological and molecular methods. For most of morphological characteristics analyzed, hybrids appeared intermediate between the two parental species. The mean production of two surviving hybrids per 100 pollinated flowers as well as their fertility are comparable with those reported for intrageneric crosses between *Coffea* species, and do not support the present division of coffee-trees in two genera. Both the capacity of *C. arabica* to hybridize with *P. ebracteolatus*, and the fertility of hybrids produced appear high enough to envisage intergeneric gene transfers from *P. ebracteolatus* into *C. arabica*.

Cross	No. of pollinated flowers	No. of hybrids obtained	No. of surviving hybrids	Surviving hybrids per 100 flowers
PET1 x AR59	78	6	3	4
PET2 x AR59	304	25	5	2
PET1 x AR60	200	10	1	<1

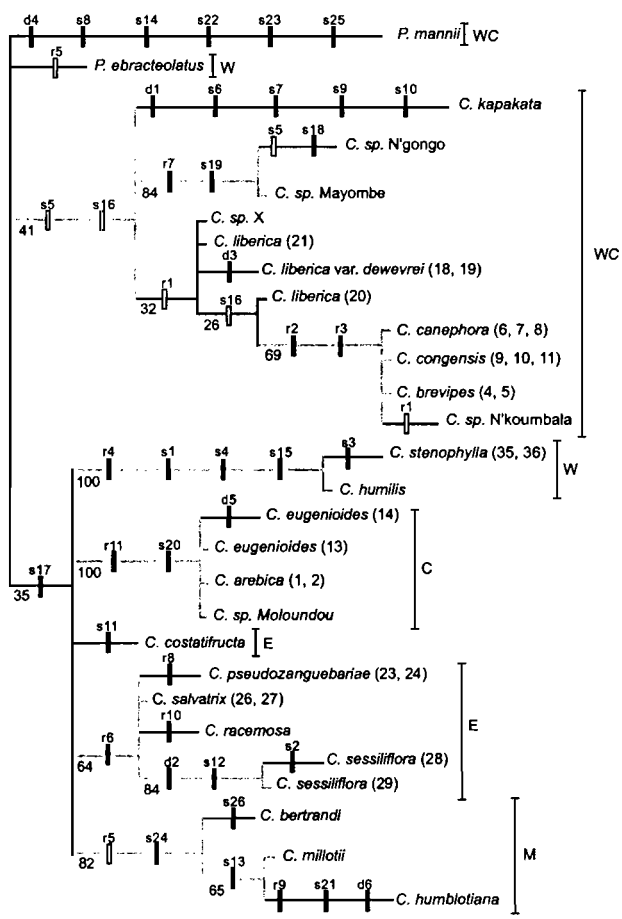
Production of intergeneric hybrids between *P. ebracteolatus* (accessions PET1 and PET2) and *C. arabica* (accessions AR59 and AR60)

Phylogenetic analysis of chloroplast DNA variation in *Coffea* L.

Cros J., Combes M.C., Trouslot P., Anthony F., Hamon S., Charrier A. and Lashermes Ph.

Molecular Phylogenetics and Evolution, 1998, 9 : 109-117.

The *trn*L-*trn*F intergenic spacer of the chloroplast DNA (cpDNA) has been sequenced from 38 tree samples representing 23 *Coffea* taxa and the related genus *Psilanthus*. By studying the cpDNA variation, we sought to gain insights into *Coffea* evolution. These sequences were used for phylogenetic reconstruction using parsimony analyses. An analysis was also conducted with a combined data set using the *trn*L-*trn*F sequences and 11 restriction site changes detected by RFLP analysis of the chloroplast genome in a previous study. The results suggest a radial mode of speciation and a recent origin in Africa for the genus *Coffea*. Phylogenetic relationships inferred from the cpDNA analysis suggest several major clades, which present a strong geographical correspondence (i.e. West Africa, Central Africa, East Africa and Madagascar). The overall results agree well with the phylogeny previously inferred from nuclear genome data. However, several inconsistencies are observed among taxa endemic to West Africa, suggesting the occurrence of introgressive hybridization.



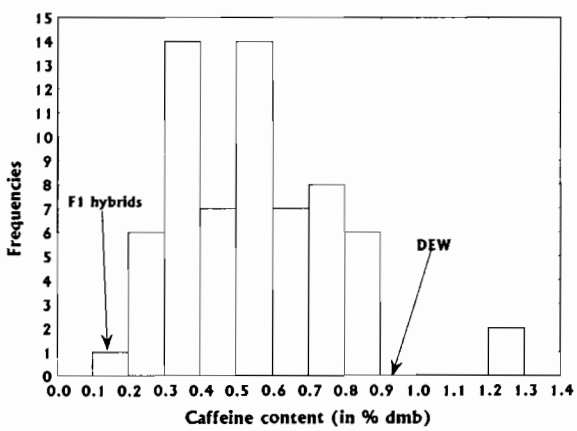
One of the most parsimonious trees inferred from cpDNA polymorphism analysis. Characters appearing only once on the tree are solid boxes; parallel and reversal changes are designated with open boxes.

Inheritance of caffeine and heteroside contents in an interspecific cross between a cultivated coffee species *Coffea liberica* var *dewevrei* and a wild species caffeine-free *C. pseudozanguebariae*.

Barre P., Akaffou S., Louarn J., Charrier A., Hamon S. and Noirot M.

Theoritical Applied Genetics, 1998, 96, 306-311.

Coffee species originating from Africa, in particular the two major cultivated species *C. arabica* and *C. canephora*, usually contain caffeine in their beans, whereas almost all Malagasy coffee species are caffeine-free. However, one wild coffee species *C. pseudozanguebariae,* collected near the coast in south Kenya, is caffeine-free. Beans of this species contain a specific heteroside diterpene (hereinafter referred to simply as heteroside) and give a bitter coffee beverage. We have investigated the inheritance of the caffeine and heteroside contents in the first and second generations of an interspecific cross between *C. pseudozanguebariae* and *C. liberica* var. *dewevrei,* for which the caffeine content is about 1 % dmb (dry matter basis). The caffeine content of F1 hybrids (0.2 % dmb) was lower than the parental average (0.47 % dmb). Caffeine and heteroside contents appeared to be under polygenic control with a strong genetic effect. Nevertheless, one major gene with two alleles seemed to be involved in the control of both compounds. Absence of caffeine was apparently con-trolled by one recessive gene. Heteroside content seemed to be controlled by one codominant gene, heterozygotes being intermediate between the two different groups of homozygotes.



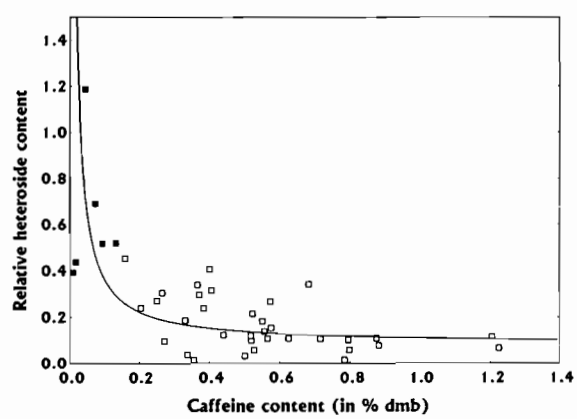


Figure 1 (Left) : Distribution of caffeine content in the backcross to DEW (BCDEW).

Figure 2 (Right) : Relationship between caffeine (CAF) and relative heteroside (RHET) contents in second-generation hybrids with caffeine higher than 0.004 and with RHET higher than 0.006. A hyperbolic function y = 0.03/x+0.08 was fitted to the curve. Filled squares indicate BCPSE hybrids and empty squares indicate BCDEW hybrids.

One aim of this study was to transfer the absence of caffeine from *C. pseudozanguebariae* into coffee species with caffeine, such as *C. canephora,* without transferring other undesirable traits, such as the presence of heteroside which could be involved in the bitterness of the coffee beverage. This transfer should be greatly facilitated if the simple genetic determinism of absence of caffeine is confirmed. Recessiveness of the absence of caffeine requires the help of linked molecular markers to follow the allele through generations of successive backcrosses. Indeed, this should allow the distinction in successive backcrosses to DEW or *C. canephora* between the dominant homozygotes and the heterozygotes. The selection of heterozygotes could be done at the seedling stage.

Relationship between parental chromosomic contribution and nuclear DNA content in the coffee interspecific hybrid : *Coffea pseudozanguebariae x C. liberica* var. *dewevrei.*

Barre P., Layssac M., D'Hont A., Louarn J., Charrier A., Hamon S. and Noirot M.

Theoritical Applied Genetics, 1998, 96 : 301-305.

F1 hybrids were obtained between two coffee species with the same chromosome number (2n = 22) but with different nuclear DNA contents (*C. pseudozanguebariae* (PSE) 2C = 1.13 pg and *C. liberica* var. *dewevrei* (DEW) 2C = 1.42 pg). G2 hybrids were obtained by open-pollination of the F1 hybrids. Genomic *in situ* hybridisation (GISH) and flow cytometry were used on 6 F1 hybrids and 7 G2 hybrids in order to determine their parental chromosomic contribution and their nuclear DNA content (qDNA), respectively. GISH efficiently identified chromosomes from both species. F1 hybrids had qDNA intermediate between the parental species and contained 11 chromosomes from each species as expected. There was a linear relationship between the number of PSE chromosomes and the nuclear DNA content. This result allows flow cytometry to give a rough estimate of the parental chromosomic contribution in G2 hybrids.

In introgression programmes of wild traits into the cultivated species, the aim is to produce plants with the introgressed trait and the background of the cultivated species. This is usually done by backcrossing the hybrids with the favourable trait on the cultivated species. This is time-consuming, especially for coffee trees which yield after four years. The use of molecular markers and flow cytometry for early detection of hybrids having the expected trait with the minimum number of chromosomes from the wild species could accelerate this process.

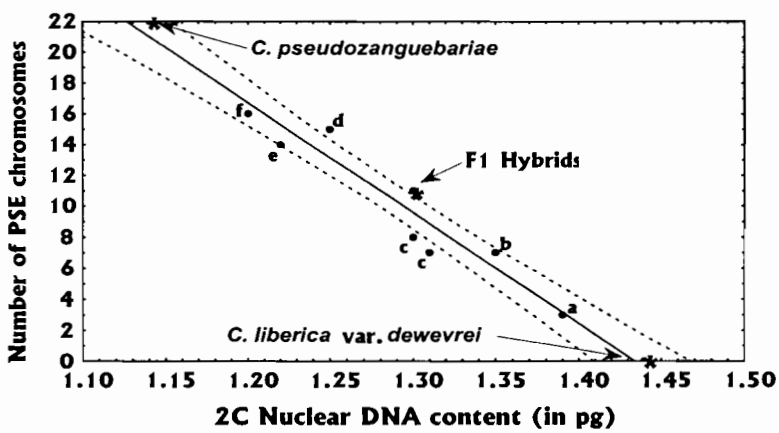
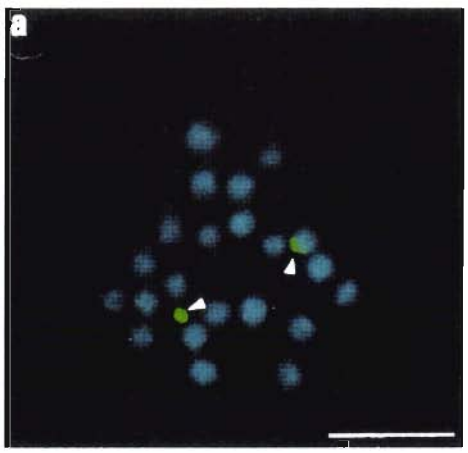
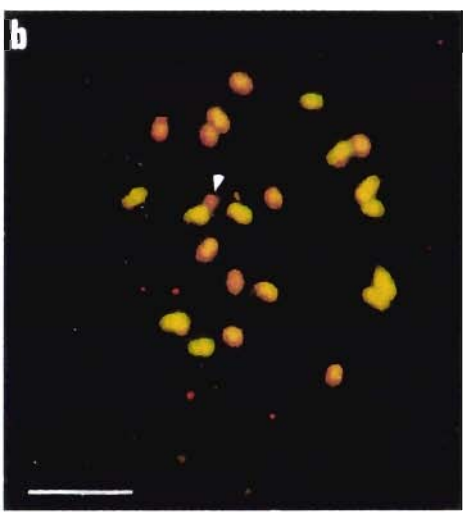


Figure 1 : Linear relationship (y = -71.41 x + 102.42 r = 0.98) between the nuclear DNA content and the number of chromosomes on DEW and PSE, F1 and G2 hybrids. Results of the Newmann & Keuls test on qDNA are indicated with letters.

Figure 2 (right page) : **a)** *In situ* hybridisation of the rDNA probe (pTA 71) detected with FITC to metaphase of *Coffea pseudozanguebariae* counterstained with DAPI. **b)** Genomic *in situ* hybridisation to metaphase of F1 hybrid between *C. pseudozanguebariae* (fluoresced in red) and *C. liberica* var. *dewevrei* (fluoresced in yellow). **c and d)** GISH to metaphase of G2 hybrids with 17 and 3 chromosomes of *C. pseudozanguebariae*, respectively. Arrows indicate rDNA sites. Bar represents 10 µm. **b', c' and d'**: Schematic representation of figures b, c and d, respectively, showing the specific origin of the chromosomes, after correction for staining of rDNA sites. The black and white chromosomes correspond to chromosomes of *C. pseudozanguebariae* and *C. liberica* var. *dewevrei*, respectively.





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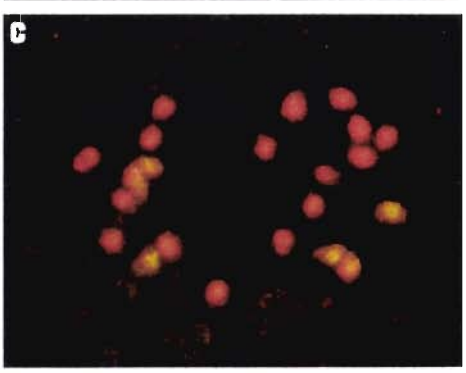

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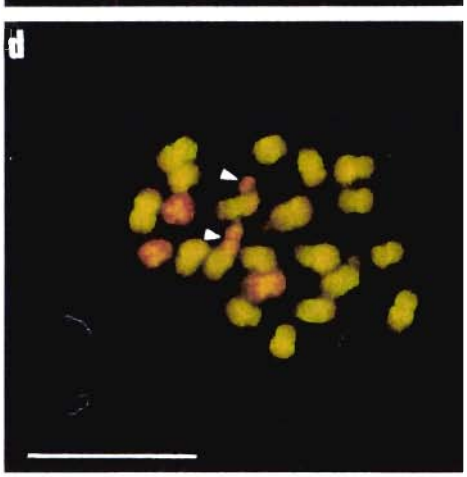

d'

Relations between and Inheritance of chlorogenic acid contents in an interspecific cross between *Coffea pseudozanguebariae* and *C. liberica* var *dewevrei*

Ky C.L., Louarn J., Guyot B., Charrier A., Hamon S. and Noirot M.

Theor. Appl. Genet, 1999, 98 : 628-637.

The esters of quinic acid are trivially named chlorogenic acids (CGA) and are particularly abundant in coffee green beans. They include six classes, but three classes [caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), and feruloylquinic acids (FQA)] represent about 98 % of the CGA content Each of these classes is commonly divided into three isomers on the basis of the number and position of the acylating residues.

The CGA content of coffee beans modifies cup taste through direct and indirect effects. As an example of direct effect, diCQA is known to increase astringency. The indirect effects are due to molecular changes during roasting which have mainly negative influences. To summarize, the quality of the beverage increases when the CGA content decreases. This largely explains taste differences between Robusta and Arabica coffees.

CGA inheritance was studied in a interspecific cross between *C. pseudozanguebariae* BRIDSON (PSE) (1.3 % dmb CGA) and *C. liberica* var *dewevrei* (DEW) (6.9 % dmb CGA).

Between-tree differences were recorded for all isomer contents. Nevertheless, most traits showed both year and interaction effects and the importance of environmental effects (years + interactions) varied between traits from 8.4% (3-FQA/ FQA) to 57% (CQA/CGA). In general, minor isomers presented greater environmental effects.

In F1 hybrids, CGA content was similar to that of the mid-parental species, and differed significantly from those of parental species. CGA content in BCPSE was intermediate between F1 and PSE averages, whereas CGA content in BCDEW was between F1 and DEW values. This suggests an additivity hypothesis for this trait, as can be verified in figure 1.

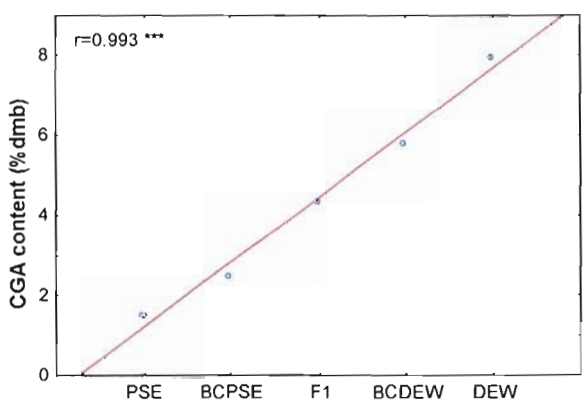

Fig. 1 : Regression analysis showing additivity of CGA content.

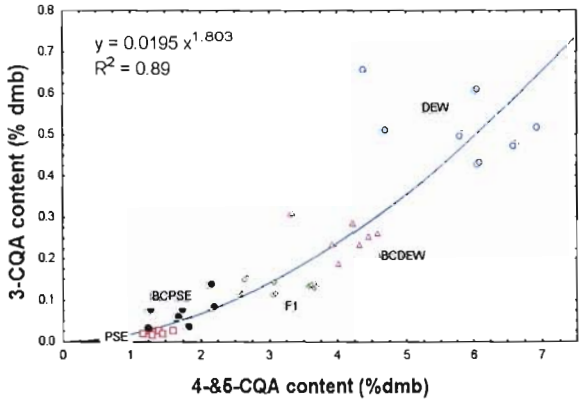

Fig. 2: Curvilinear relationship between 5-CQA and 3-CQA

CQA represented 79% and 93% of CGA in DEW and PSE, respectively, whereas diCQA represented 15% and 2.5% of CGA, respectively. FQA represented 7% of CGA on average in the two species.

Additivity was verified for CQA, diCQA contents. It was also verified for FQA content after transformation. A similar situation existed for isomers: the 5-CQA isomer content was additive, whereas only the square root of the 3-CQA content was additive. In fact, a curvilinear relationship was emphasized between 3-CQA and 4-&5-CQA contents (Fig. 2), explaining the inheritance behaviour of 3-CQA in regards with 5-CQA.

Inheritance of coffee bean sucrose content in the interspecific cross: *Coffea pseudozanguebariae x Coffea liberica dewevrei*

Ky C.L., Doulbeau S., Guyot B., Akaffou S., Charrier A., Hamon S., Louarn J. and Noirot M.

Plant breeding, 2000, 119 : 165-168.

Coffee flavour is developed during the roasting process from aroma precursors present in green beans. The aroma formation is very complex and includes Maillard and Stecker's reactions and flavour precurseur degradations. Among them, sucrose which is degraded in furans, is one of the most important. Sucrose inheritance was studied in a interspecific cross between *C. pseudozanguebariae* BRIDSON (PSE) (7.4 % dmb) and *C. liberica* var *dewevrei* (DEW) (5.6 % dmb).

Between trees, differences were clearly observed. In contrast, no difference between years was found. Presence of an interaction means that the year effect changed between trees.

The sucrose content of F1 hybrids observed was close to the theoretical mid-parental value (6.46 % dmb) suggesting additivity. The between-F1 hybrid variance (s^2 = 1.141) did not differ from the within-parental species one (s^2 = 1.335).

Sucrose contents in the backcross hybrids BCDEW and BCPSE-1 overlap. The within-BCPSE-1 variance was four times lower (s^2 = 0.252) than in the F1, whereas the within-BCDEW variance was twice (s^2 = 2.130) the F1. The lower BCPSE-1 variance is explained by its low maximal value (5.2 % dmb).

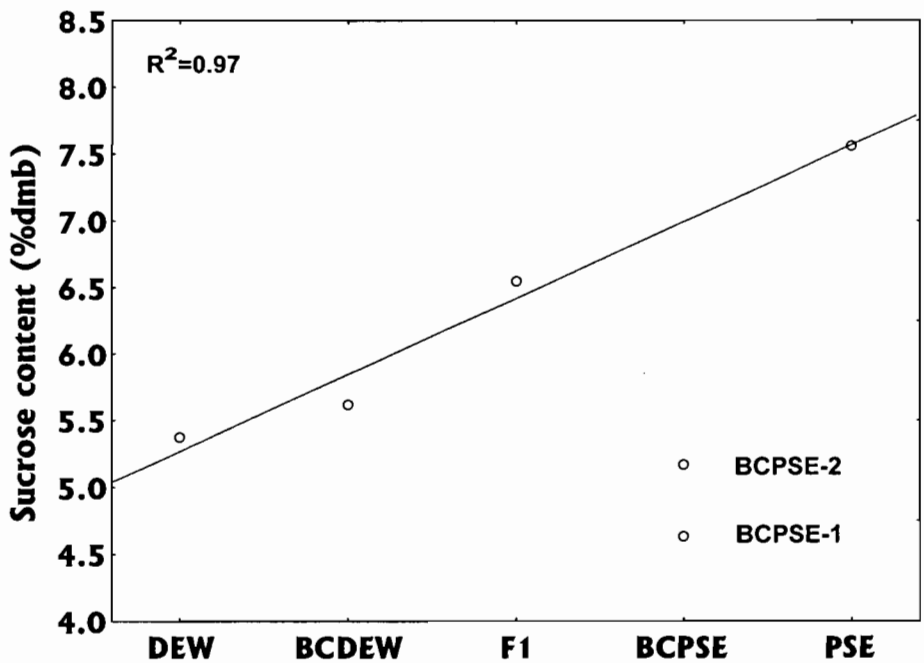

Figure 1 : Inheritance of bean sucrose content.

Sucrose content in BCDEW was close to the expected value in the additivity model [(F1+DEW)/2 = 5.15% dmb]. In contrast, sucrose content in BCPSE-1 was significantly different from the expected value [(F1+PSE)/ 2 = 7.06% dmb]. A second sample of seven BCPSE hybrids (BCPSE-2) showed the same discrepancy.

The result of the additivity test is summarized in Fig. 1. The linear regression involved the DEW, BCDEW, F1 and PSE group averages. The relation-ship is linear and significant representing 97 % of the variance.

Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.).

Ky C.L., Barre P., Lorieux M., Trouslot P., Akaffou S., Louarn J., Charrier A., Hamon S. and Noirot M.

Theor. Appl. Genet., 2000, 101 : 669-676.

Coffee trees belong to the genus *Coffea* sub-genus *Coffea*, family *Rubiaceae* and are mostly present in tropical and subtropical regions of the world. An interspecific cross between *C. pseudozanguebariae*, native from Kenya and Tanzania, and *C. liberica* var. *dewevrei*, native from Centrafrique have been realised. A genetic mapping was carried out using 192 PSE-specific AFLP markers.

Segregation distortion were observed for 30% of loci (53 AFLP and 1 RFLP markers). The distribution of segregation ratio is symetric with three modes centred on three different ratios: (1 : 3), (1 : 1) and (3 : 1) (Fig. 1). The distribution of the 54 distorted markers on the genetic map is illustrated on figure 2. Such distortions can explained by genetic conversion.

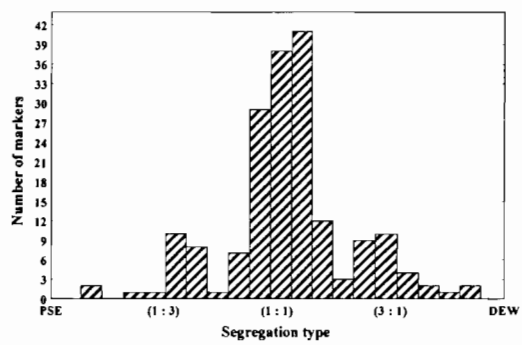

Figure 1: Distribution of segregation ratio

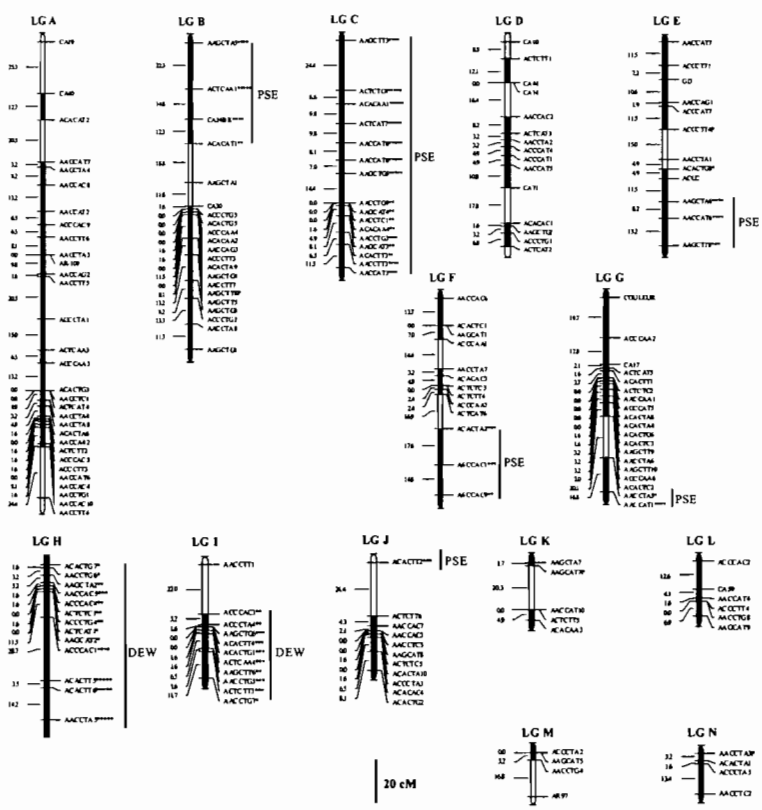

Figure 2: Genetic linkage map

TRIGONELLINE INHERITANCE IN THE INTERSPECIFIC COFFEA PSEUDOZANGUEBARIAE X C. LIBERICA VAR. DEWEVREI CROSS

Ky C.L., Guyot B., Louarn J., Hamon S. and Noirot M.

Theoretical and Applied Genetics, 2000, (sous presse).

Trigonelline alkaloid is present in coffee beans, and during roasting, it give rise to the major coffee aroma compounds (several alkyl-pyridines and pyrroles). In this study, we investigated the genetic inheritance of trigonelline accumulation in green beans in an interspecific cross between a wild east African species *Coffea pseudozanguebariae* (PSE) and the west African species *C. liberica* var. *dewevrei* (DEW). Trigonelline content was measured by HPLC in both parental species, F1 hybrids and the reciprocal back-cross hybrids (BCDEW and BCPSE). The results showed that, on average, PSE accumulated twice as much trigonelline as DEW. No year effect or inter-action (genotype x year) was recorded. Trigonelline showed high heritability (71%), which meant that the genotypic value could be easily estimated from the phenotypic value However, the fact that this trait was not additive suggested the possibility of nucleo-cytoplasmic inheritance. This hypothesis was confirmed by: i) similar levels of trigonelline content in the PSE, F1, BCPSE and BCDEW groups, all having the same maternal cytoplasm, and ii) the location of one nuclear QTL on the G linkage group.

Table 1 : Trigonelline contents (% dmb) in parental species (DEW and PSE), F1 hybrids and back-cross hybrids (BCDEW and BCPSE). Between-tree differences are given by F test results (***: Very highly significant, $p < 0.001$). Newmann & Keuls test results are indexed with a and b letters.

	DEW	BCDEW	F1	BCPSE	PSE
Average	0.57[a]	1.08[b]	1.13[b]	1.21[b]	1.02[b]
Min-Max	0.51-0.66	0.74-1.38	0.87-1.44	1.03-1.52	0.86-1.19
Range	0.15	0.64	0.57	0.49	0.33
$F_{6,21}$	17.8 ***	6.43 ***	22.3 ***	18.0 ***	17.3 ***

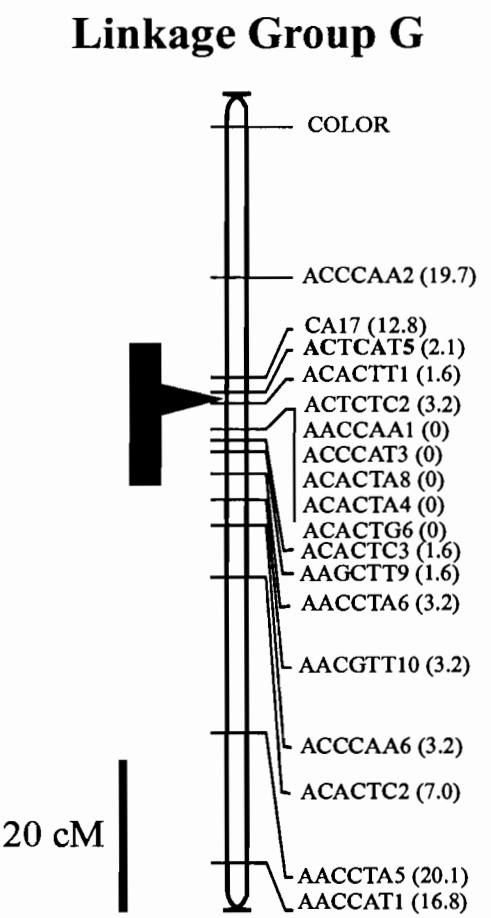

Figure 1 : Trigonelline QTL location on the linkage group G of the genetic map constructed in KY *et al.* (2000a). AFLP markers names are symbolised on the right side of the linkage group. Number in brakets correpond to genetic distance (cM) between markers. Bar to the left of linkage group correspond to the 2.0 *LOD* support intervals for the location of the QTL. Arrow indicates the most likely position (highest *LOD* peak : 3.56) estimated with MAPMAKER/ QTL.

Molecular characterisation and origin of the *Coffea arabica* L. genome

Lashermes P., Combes M.C., Robert J., Trouslot P., D'Hont A., Anthony F. and Charrier A.

Molecular and General Genetics, 1999, 261 : 259-266.

Restriction fragment length polymorphism (RFLP) loci-markers in combination with genomic *in situ* hybridisation (GISH) were used to investigate the origin of the allotetraploid species *Coffea arabica* (2n=4x=44). By comparing the RFLP patterns of potential diploid progenitor species with those of *C. arabica*, the source of the two sets of chromosomes, or genomes, combined in *C. arabica* was specified. The genome organisation of *C. arabica* was confirmed by GISH using simultaneously labelled total genomic DNA from the two putative genome donor species as probes. These results clearly suggested that *C. arabica* is an amphidiploid formed from the hybridisation between *C. eugenioides* and *C. canephora* or ecotypes related to those diploid species. Results also indicate low divergence between the two constitutive genomes of *C. arabica* and those of its progenitor species, suggesting that the speciation of *C. arabica* took place very recently. Precise localisation in central Africa of the speciation process of *C. arabica* based on the present distribution of the coffee species appeared difficult since the constitution and extent of tropical forest varied considerably during the late quaternary period.

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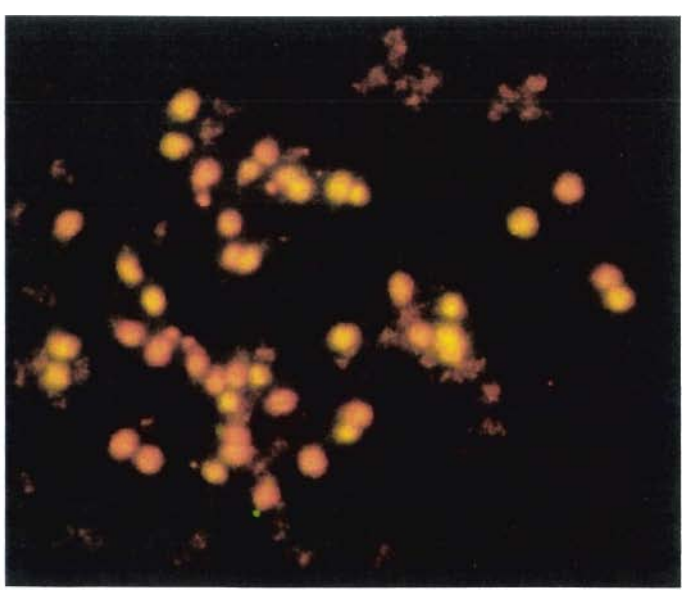
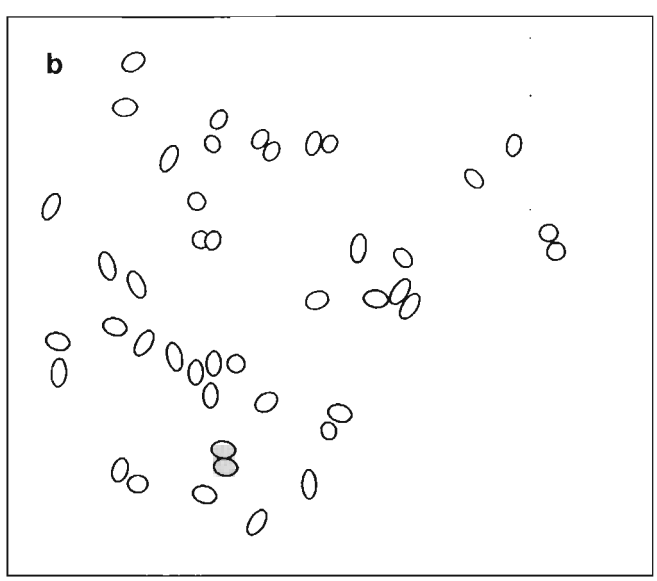




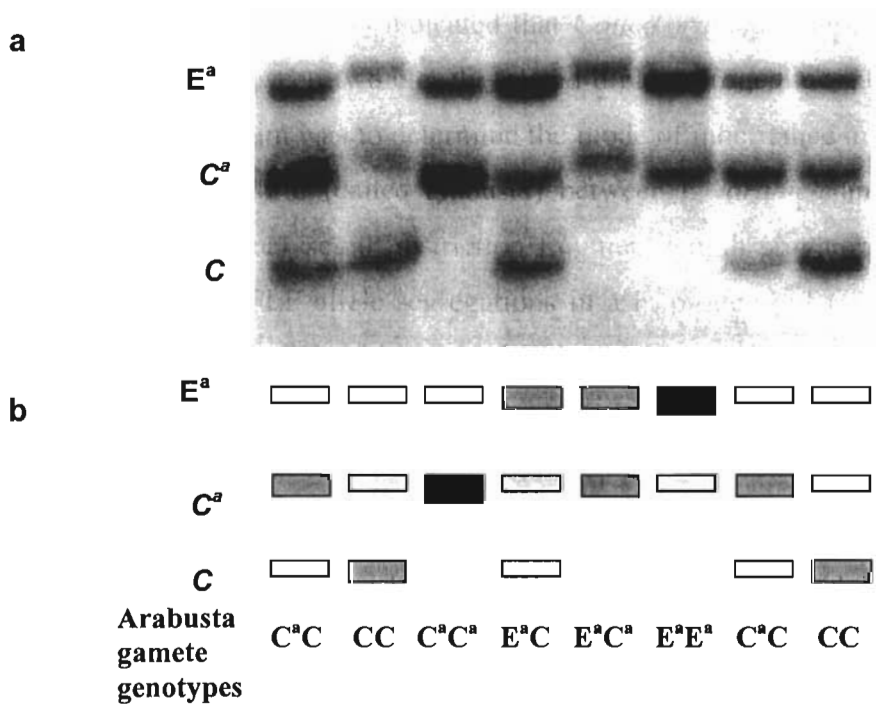
Root-tip mitotic chromosomes of *C. arabica*. **a-** Preparation following simultaneous *in situ* hybridisation with digoxigenin-labelled total DNA from *C. canephora* and biotin-labelled total DNA from *C. eugenioides* ; the two signals were superimposed by double exposure ; **b-** Schematic distribution of the chromosomes in two groups.

Single-locus Inheritance in the Allotetraploid *Coffea arabica* L. and Interspecific Hybrid *C. arabica* x *C. canephora*

Lashermes P., Paczek V., Trouslot P., Combes M.C., Couturon E., and Charrier A.

Journal of Heredity, 2000, 91 : 81-85.

Molecular cytogenetic analysis has indicated that *Coffea arabica* is an amphidiploid formed from the hybridization between two closely related diploid progenitor species, *C. canephora* and *C. eugenioides*. Our aim was to determine the mode of inheritance in *C. arabica* and in a tetraploid interspecific hybrid (called arabusta) between *C. arabica* and *C. canephora* as revealed by segregation analyses of restriction fragment length polymorphism (RFLP) loci-markers. The observed RFLP allele segregations in a F_2 progeny of *C. arabica* conform to disomic inheritance as expected, with regular bivalent pairing of homologous chromosomes in the F_1 hybrid. In contrast, RFLP loci followed tetrasomic inheritance in the arabusta interspecific hybrid, although bivalents have been reported to predominate greatly at meiosis in its hybrid. These results suggest that homoeologous chromosomes do not pair in *C. arabica*, not as a consequence of structural differentiation, but because of the functioning of pairing regulating factors. Moreover, the arabusta hybrid seems to offer the possibility of gene exchange between the homoeologous genomes.



Figures.. a- Example of RFLP allele segregations (locus gA1) observed among the tetraploid progeny resulting from the backcross of an arabusta hybrid (*C. arabica* x *C. canephora* 4x) to *C. arabica*. C^a, E^a, designate alleles attributed to the two constitutive genomes of *C. arabica* while C designates the allele of *C. canephora*. **b**- Dose interpretation diagram and the deduced arabusta gamete genotypes assuming one copy of both alleles C^a and E^a transmitted by the arabica parent.

Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.)

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Graziosi G., Trouslot P. and Anthony F.

Theoritical Applied Genetics, 2000, 100 : 139-146.

Nineteen arabica coffee introgression lines (BC_1F_4) and two accessions derived from a spontaneous interspecific cross (i.e. Timor Hybrid) between *Coffea arabica* (2n = 4x = 44) and *C. canephora* (2n = 2x = 22) were analysed for the introgression of *C. canephora* genetic material. The Timor Hybrid-derived genotypes were evaluated by AFLP, using 42 different primer combinations, and compared to 23 accessions of *C. arabica* and 8 accessions of *C. canephora*. 1062 polymorphic fragments were scored among the 52 accessions analysed. A total of 178 markers consisting of 109 additional bands (i.e. introgressed markers) and 69 missing bands distinguished the group constituted by the Timor Hybrid-derived genotypes from the accessions of *C. arabica*. The genetic diversity observed in the Timor Hybrid-derived genotypes appeared approximately double that in *C. arabica*. Although representing only a small proportion of the genetic diversity available in *C. canephora,* the Timor Hybrid obviously constitutes a considerable source of genetic diversity for arabica breeding. Analysis of genetic relationships among the Timor Hybrid-derived genotypes suggested that introgression was not restricted to chromosome substitution but also involved chromosome recombinations. Furthermore, the Timor Hybrid-derived genotypes varied considerably in the number of AFLP markers attributable to introgression. In this way, the introgressed markers identified in the analysed arabica coffee introgressed genotypes were estimated to represent from 8% to 27% of the *C. canephora* genome. Nevertheless, the amount of alien genetic material in the introgression arabica lines remains substantial and should justify the development of adapted breeding strategies.

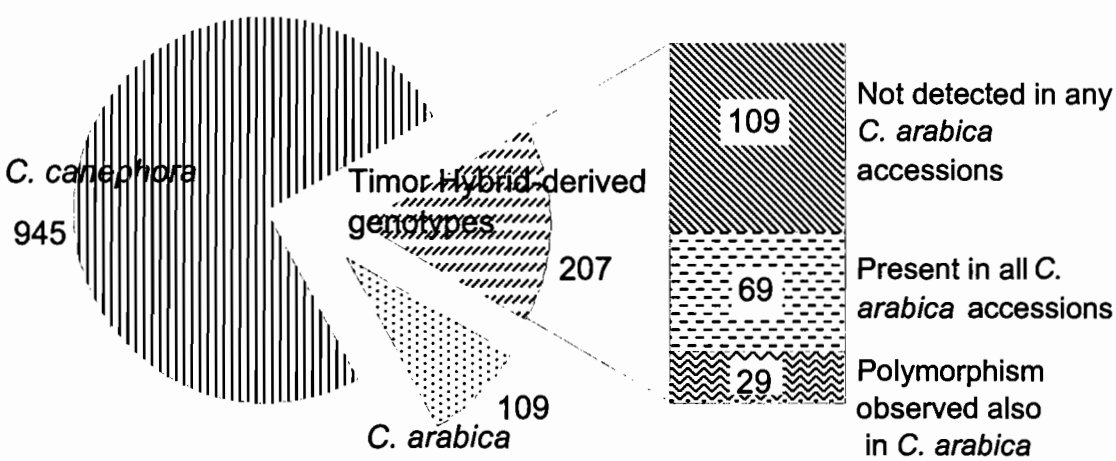


Figure. Pie charts depicting the numbers of polymorphic AFLP bands observed among individuals within each group constituted by the accessions of *C. arabica*, *C. canephora* and introgressed Timor Hybrid-derived genotypes, respectively. For the introgressed material, the polymorphic markers either attributable to the Arabica parent or associated with the introgression of *C. canephora* chromosome segments were distinguished.

Characterisation of microsatellite loci in *Coffea arabica* and related coffee species

Combes M.C., Andrzejewski S., Anthony F., Bertrand B., Rovelli P., Graziosi G. and Lashermes P.

Molecular Ecology, 2000, 9 : 1171- 1193.

The goal of the present study is to develop a set of molecular genetic markers, known as simple sequence repeats (SSRs) or microsatellite, suitable for genetic studies of coffee species. We assessed their potential as genetic markers in the discrimination of *C. arabica* and *C. canephora* genotypes, and examined cross-amplification in various coffee species. The species *C. arabica* were represented by 32 individuals sampled from different locations in Ethiopia and Yemen, while *C. canephora* were represented by 10 individuals collected in Central African Republic, Congo and Côte-d'Ivoire. A total of 13 *Coffea* taxa were surveyed. DNA clones from a partial genomic library (*C. arabica* var. Caturra) enriched for (TG)13 motifs were sequenced, and primers complementary to flanking regions of identified repeats were designed for 11 sequences. Only 5 of the 11 microsatellite loci appeared to be polymorphic in *C. arabica* while the microsatellite loci showed a broad range of genetic diversity across the accessions of *C. canephora*. The mean heterozygosity values were of 0.04 and 0.47 in the predominantly autogamous *C. arabica* and the self-incompatible species *C. canephora*, respectively. Although designed from sequences isolated in *C. arabica*, these primers worked well for most of the diploid coffee species tested. Altogether, the primers described here can provide useful markers to investigate levels of genetic variation in coffee species with respect to germplasm management and genetic study in natural plant populations.

Locus	EMBL Accession no.	Repeat motif*	Allele number/Heterozygosity**	
			C. arabica	*C. canephora*
M2a	AJ250250	$(GT)_8$/ $(GT)_6$/ $(GT)_7$	2 / 0.00	3 / 0.40
M3	AJ250251	$(CA)_6$/ $(CA)_3$/ $(CA)_3$/ $(CA)_3$/ $(CA)_4$/ $(CA)_3$/ $(CA)_3$/ $(CA)_3$	2 / 0.00	3 / 0.20
M11	AJ250252	$(GT)_4$/ $(GA)_4$/ $(GT)_4$/ $(GT)_6$	1 / 0.00	2 / 0.20
M20	AJ250253	$(GA)_5(GT)_8TT(GT)_4TT(GT)_7$ $(GA)_{11}(TC)_2(CT)_3GT$	5 / 0.00	6 / 0.70
M24	AJ250254	$(CA)_{15}(CG)_4CA$	6 / 0.13	10 / 0.80
M25	AJ250255	$(GT)_5CT(GT)_2$/ $(GT)_{12}$	3 / 0.03	3 / 0.30
M27	AJ250256	$(GT)_{11}$	2 / 0.00	4 / 0.60
M29	AJ250257	$(CTCACA)_4$/ $(CA)_9$	3 / 0.00	3 / 0.20
M32	AJ250258	$(CA)_3$/ $(CA)_3$/ $(CA)_{18}$	7 / 0.10	6 / 0.30
M42	AJ250259	$(GT)_3$/ $(GT)_7$	2 / 0.00	5 / 0.70
M47	AJ250260	$(CT)_9(CA)_8$/ $(CT)_4$/ $(CA)_5$	7 / 0.10	10 / 0.80

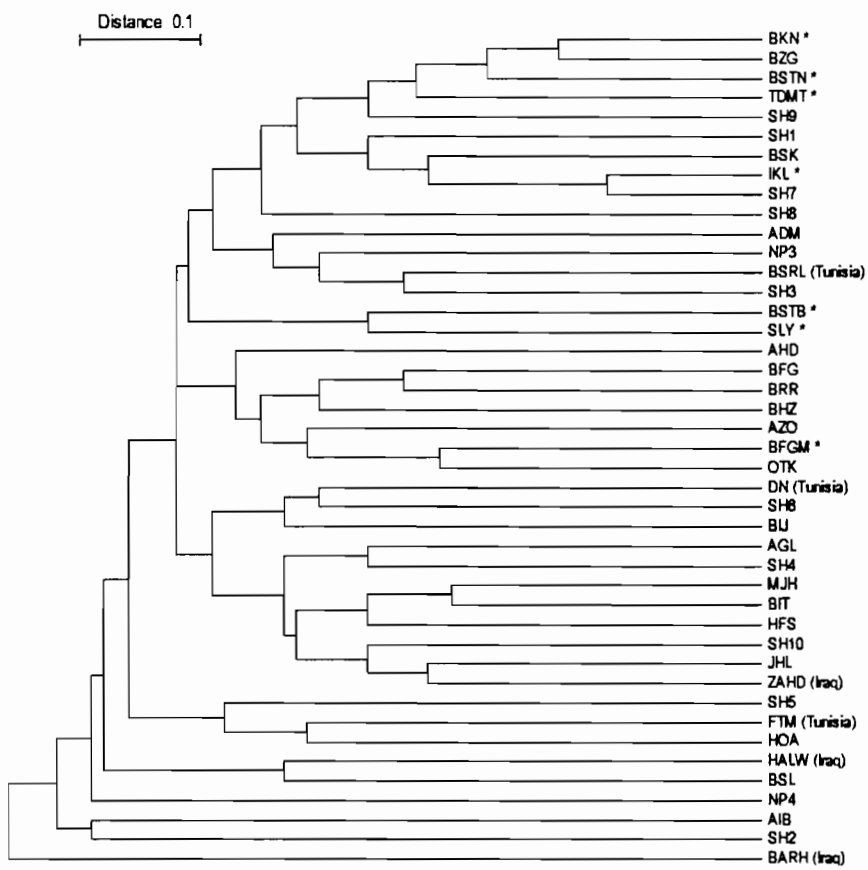
Table. Allele number, and heterozygosity level of 11 microsatellite loci isolated from *Coffea arabica* (*Microsatellite motifs are sequenced alleles of *C. arabica* var. Caturra)

Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers.

Sedra My H., Lashermes P., Trouslot P., Combes M.C. and Hamon S.

Euphytica, 1998, 103 : 75-82.

Genetic variation among 43 date palm (*Phoenix dactylifera* L.) accessions, including 37 accessions from Morocco and 6 cultivars from Iraq and Tunisia, was studied using Random Amplified Polymorphic DNA (RAPD) markers. The pre-screening of 123 primers on four genotypes allowed selection of 19 primers which revealed polymorphism and gave reproducible results. All 43 analysed genotypes were distinguishable by their band patterns. RAPD technology therefore appears very effective for identifying accessions of date palm. RAPD-based genetic distance was used to determine the relationships between the accessions. The grouping-association identified by cluster analysis was rather weak. However, morphologically similar varieties clustered together. A relatively low polymorphism and a lack of evident organisation are observed among the date palm varieties grown in Morocco. This could be related to the mode of introduction and maintenance of the Moroccan date palm germplasm involving limited foundation germplasm, exchange of cultivars between plantations, and periodic development of new recombinant cultivars following sexual reproduction.



Dendrogram of the accessions generated by group average clustering analysis (UPGMA) using RAPD-based genetic distance. The cultivars resistant to « Bayoud » are indicated by *.

Inheritance, distribution and biology of andromonoecy in the agamic complex of the *Maximae* (Panicoideae)

Assienan B. and Noirot M.

Theor. Appl. Genet., 1999, 98 : 622-627.

Panicum maximum belongs to the agamic complex of the *Maximae* (*Panicoideae*), native from Kenya and Tanzania. The complex includes two other species, *P. infestum* Anders., *P. trichocladum* K. Schum., and several morphologically intermediate types. Andromonoecy and hermaphroditism have been recorded in this complex.

In andromonoecious plants and within the spikelet, flowering of the male flower occurred 1 to 3 days after that of the hermaphrodite flower. This flowering discrepancy varied between clones in respect of both mean and variance. Neither the mode of reproduction, the level of ploidy nor the interspecific origin influences the difference of flowering date between male and hermaphrodite spikelets.

Andromonoecy showed a monogenic and recessive inheritance. Hermaphrodites were *Hhhh*, whereas andromonoecious plants were *hhhh* at the tetraploid level and *hh* at the diploid level.

Andromonoecy distribution depends on the species, the level of ploidy, the mode of reproduction and the geographical origin. All diploid sexuals were andromonoecious. Clones of *P. infestum, P. trichocladum,* and all their natural interspecific hybrids with *P. maximum,* were andromonoecious. By contrast, apomictic clones of *P. maximum* were polymorphic. In this case, the percentages of hermaphrodite and andromonoecious clones were 44.7% and 55.3%, respectively and did not differ from a 1:1 ratio.

The maritime region showed a higher andromonoecy rate than the continental region (Table 1); and ii) andromonoecy was more frequent in regions of Korogwe and Bagamoyo, where sexuals were present (Table 1). There was no difference between Kenya and Tanzania.

Table 1: Regional distribution of andromonoecy in apomictic *P. maximum*.

Contrast	% andromonoecy
Kenya vs Tanzania	50 vs 59
Continental vs Maritime	51 vs 66
Non sexuals vs sexuals	52 vs 69

Hermaphroditism seems to be absent in the diploid sexuals despite gene flow between the two pools. Several hypotheses can explain this result: i) diploids could be genetically hermaphrodite *Hh*, but hermaphroditism would not be expressed at the diploid level. Nevertheless, the genealogy of the tetraploid and sexual hybrids (1S17, 1S18, 2S24, 2S87) we used as parents in the study is consistent with their sexual colchiploidized parent being *hhhh;* ii) andromonoecy overran diploid populations; and iii) absence of hermaphroditism could be compared to the absence of apomixis in these plants. In apomixis, fertile diploids are *aa* (the rare *Aa* diploids are sterile, but show apomictic embryo-sacs), whereas apomicts are *Aaaa*. Absence of *Aaaa*, *AAAa*, and *AAAA* apomicts and sterility of *Aa* diploids lead us to suppose that an allelic dosage would trigger sterility when the proportion of the *A* allele is higher than 0.25. To-date, only *Hhhh* genotypes have been recorded among andromonoecious apo-micts, whereas all diploids were *hh*. The similarity is striking, although further studies are necessary to complete our genetic identification of andromonoecious apomicts. Screening of haploids in progenies from andromonoe-cious and sexual tetraploids may indicate whether Hh plants are sterile. Similarity does not imply linkage. Indeed, at the tetraploid level, andromonoecy concerns both sexuals and apomicts.

MOLECULAR DIVERSITY IN PINEAPPLE ASSESSED BY RFLP MARKERS

Duval M. F., Noyer J. L., Perrier X., Coppens d'Eeckenbrugge G. and Hamon P.

Theoretical and Applied Genetics, 2000, (sous presse).

Pineapple, *Ananas comosus* (L.) Merr, is the third most important tropical fruit cultivated in all tropical and subtropical countries. Pineapple germplasm includes all seven species of the genus *Ananas* and the unique species of the relative genus *Pseudananas*. The knowledge of the diversity structure is needed to develop new breeding programs. Restriction Fragment Length Polymorphism (RFLP) was used to study molecular diversity in a set of 301 accessions, most of these recently collected. This sample was analysed using 18 homologous genomic probes. Dissimilarities were calculated with Dice index and submitted to Factorial Analysis. The same data were represented as a diversity tree constructed with the score method. *Pseudananas sagenarius* displayed a high polymorphism and shares 58.7 % of bands with *Ananas*. Within *Ananas*, variation appears continuous and was found mostly at the intraspecific level, particularly in the wild species *A. ananassoides* and *A . parguazensis*. As for cultivated species, *A.comosus* appears relatively homogenous despite the wide morphological variation and *A. bracteatus* which is grown as a fence and for fruit appears still much less variable. On the contrary *A. lucidus*, cultivated by the Amerindians for fiber, displays a high polymorphism. The diversity tree displayed a loose assemblage of numerous clusters separated by short distances. Most species scattered in various clusters, few of these been monospecific. Some accessions which had not been classified as they shared morphological traits typical of different species regroup with one or the other, sometimes with both species in mixed clusters. No reproductive barrier exists in this germplasm and these data indicate the existence of a gene flow, enhancing the role of an effective sexual reproduction in a species of largely predominant vegetative mutiplication.

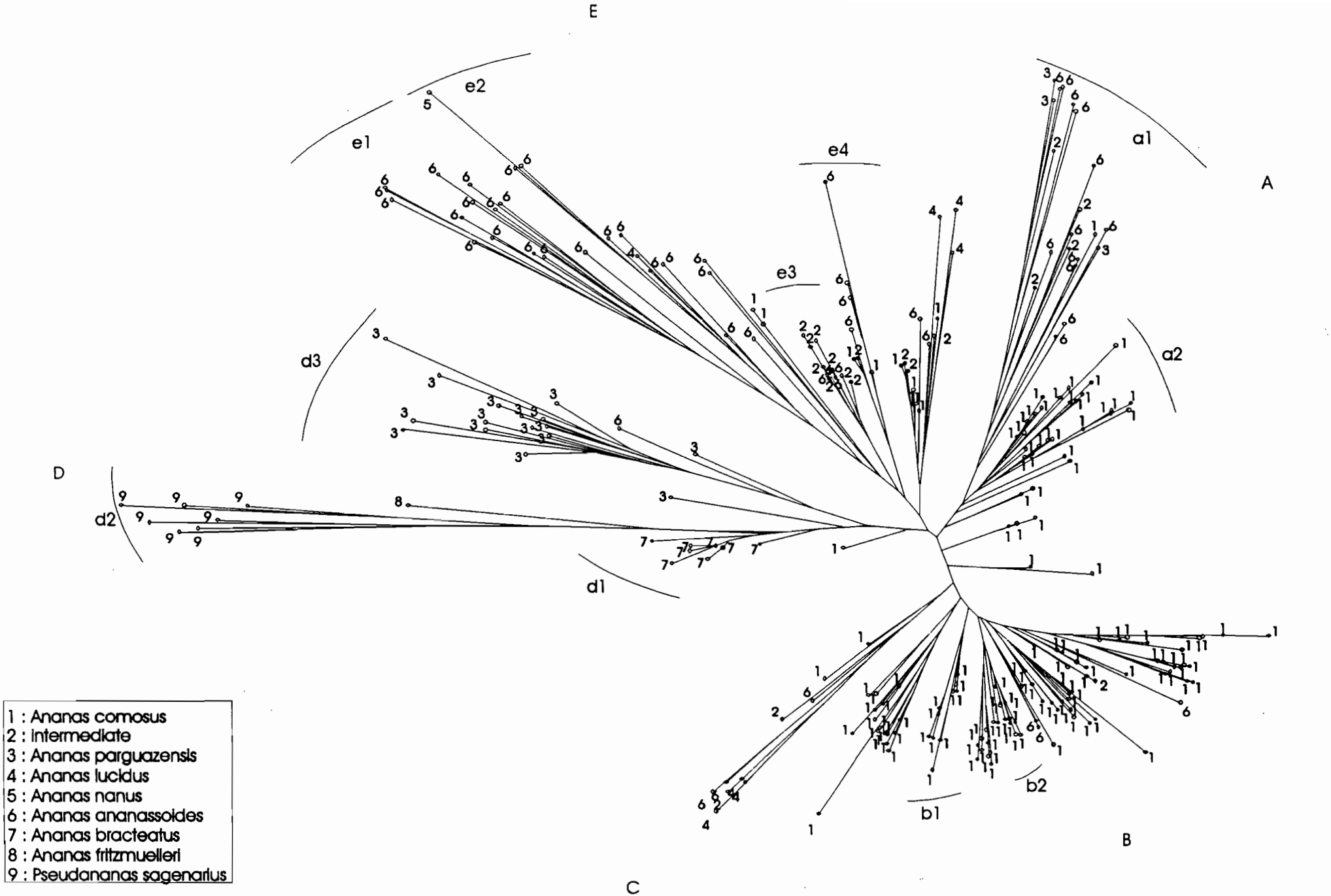


Figure 2 : Diversity Tree constructed by Add-Tree method with Dice dissimilarity indices.

Prediction of oil palm (*Elaeis guineensis* Jacq.) agronomic performances using the best linear unbiased predictor (BLUP)

Purba A.R., Flori A., Baudouin L. and Hamon S.

Theoritical and Applied Genetics, 2000, (sous presse).

Un schéma de Sélection Récurrente Réciproque (SRR) a été adopté en Indonésie. Les essais génétiques du palmier à huile sont difficiles à conduire compte tenu de la longueur du cycle de sélection de la plante et de la grande surface nécessaire. La connaissance des paramètres génétiques parentaux est très importante pour atteindre le progrès génétique escompté, mais l'évaluation de ces paramètres est difficile du fait de séries de données très déséquilibrées. Dans cette étude, les séries de données agronomiques déséquilibrées et l'information généalogique d'un programme de sélection en Indonésie ont été analysé selon les méthodes du Maximum de Vraisemblance Restreinte (REML = REstricted Maximum Likelihood) et de la Meilleure Prédiction Linéaire non Biaisée (BLUP = Best Linear Unbiased Predictor). Les caractères analysés ont été la production de régimes et la production d'huile à l'âge adulte (de 7 à 9 ans après plantation).

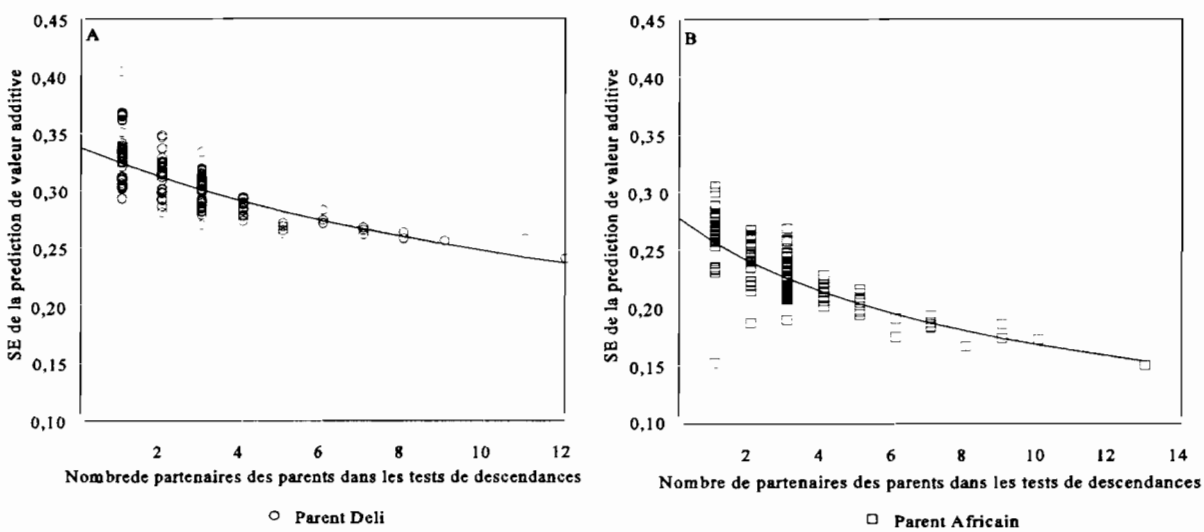
Les coefficients de parenté ont varié respectivement de 0,125 à 0,891 et de zéro à 0,750 chez les parents des groupes Deli et Africain. Les coefficients moyens de consanguinité des parents étaient de 0,269 et 0,166 respectivement à l'intérieur des groupes Deli et Africain. Les variances additives des caractères tels que le nombre de régimes, le taux d'extraction en huile et la production d'huile ont été supérieures chez les parents du groupe Deli par rapport à ceux du groupe Africain. Les coefficients de corrélation entre les valeurs prédites et les valeurs observées des performances hybrides ont varié de 0,55 à 0,64 pour la production d'huile, de 0,49 à 0,71 pour le nombre de régimes, de 0,47 à 0,58 pour la production totale de régimes, de 0,48 à 0,64 pour le taux d'extraction en huile et de 0,42 à 0,56 pour la croissance en hauteur (tableau 3.2). La précision de cette prédiction sera d'autant plus efficace que le nombre partenaires des parents dans les essais de descendances augment (figure 3.2). La capacité de prédiction de la performance d'un hybride par la méthode du BLUP devrait être suffisante pour sélectionner le caractère de la production d'huile, et représentera une contribution significative aux productions de semences et de clones du palmier à huile.

Coefficients de corrélations entre

les valeurs prédites et les valeurs observées.

	Nombre d'hybrides prédicteurs (p) (*)						
Caractère	60	90	120	150	180	210	240
OYD	0,55	0,58	0,60	0,61	0,62	0,63	0,64
BNO	0,49	0,55	0,60	0,64	0,66	0,69	0,71
ABW	0,37	0,45	0,53	0,58	0,63	0,67	0,70
FFB	0,47	0,51	0,53	0,55	0,56	0,57	0,58
IER	0,48	0,53	0,57	0,58	0,60	0,62	0,64
HIT	0,42	0,45	0,48	0,51	0,52	0,54	0,56

OYD = production d'huile ; BNO = nombre de régimes ; ABW = poids moyen de régimes ; FFB = production totale de régimes ; IER = rendement industriel en huile ; HIT = vitesse de croissance en hauteur



Relation entre l'erreur standard de la prédiction de la valeur additive de la production d'huile et le nombre de partenaires des parents dans les tests de descendances du groupe A (Deli) et du groupe B (Africain).

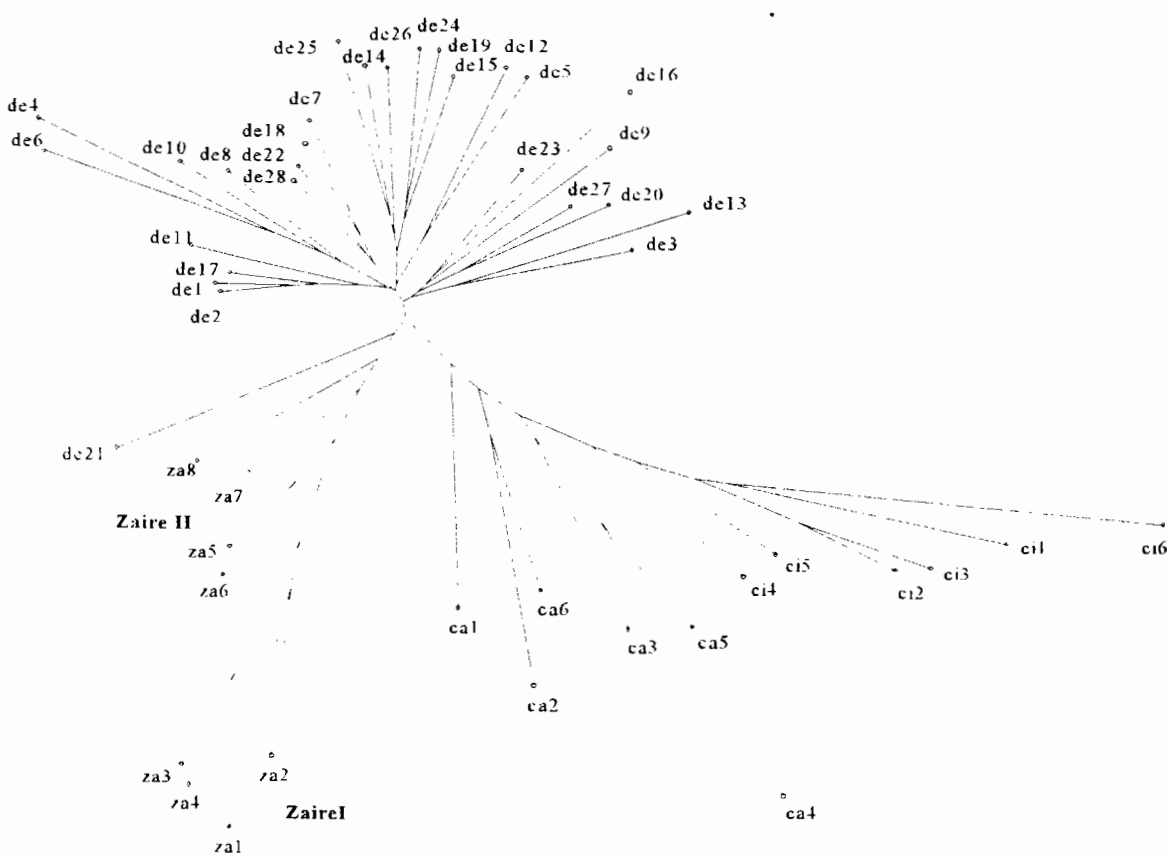
A new aspect of genetic diversity of Indonesian oil palm (*Elaeis guineensis* Jacq.) revealed by isoenzyme and AFLP markers and its consequences to breeding

Purba A.R., Noyer J.L., Baudouin L., Perrier X., Hamon S. and Lagoda P.J.L.

Theoritical and Applied Genetics, 2000, 101 : 956-961.

Le palmier à huile joue un rôle économique important dans divers pays d'Asie du Sud-Est parmi lesquels on compte l'Indonésie, second producteur mondial d'huile de palme et d'huile de palmiste. L'amélioration de la qualité du matériel de plantation passe par une meilleure connaissance des relations génétiques entre les génotypes des différentes populations utilisées dans les programmes de sélection. Dans cette étude, 48 géniteurs, représentatifs de quatre populations utilisées dans les programmes d'amélioration de l'IOPRI, ont été étudiés à l'aide de cinq paires d'amorces AFLP et de quatre systèmes isoenzymatiques.

L'analyse des isoenzymes a montré que toutes les populations ne sont pas à l'équilibre de Hardy-Weinberg. Cent cinquante-huit niveaux de bande utilisables ont été identifiés par l'analyse AFLP, parmi lesquels 96 (61%) ont révélé du polymorphisme. Les AFLP nous ont permis d'identifier des hors-type dans les descendances et qui ont été exclus de l'analyse. La distance non- biaisée de Rogers sépare clairement les quatre populations. La méthode du Neighbor Joining regroupe deux populations Africaines connues comme provenant de régions différentes. La variabilité révélée est en accord avec les connaissances acquises par les sélectionneurs. Les résultats obtenus avec les AFLP montrent que les croisements entre les sous-populations Africaines, actuellement exclues de la schéma de Sélection Récurrente Réciproque des programmes d'amélioration du palmier à huile, pourraient être plus intéressants que les croisements entre les populations Africaines et Deli.



Arbre obtenu par la méthode NJ pour les 48 parents analysés avec 158 marqueurs AFLP (ca = Cameroun ; de = Deli ; ci = Côte d'Ivoire ; za = Zaïre).

NUCLEUS-CYTOSOL INTERACTIONS – A SOURCE OF STOICHIOMETRIC ERROR IN FLOW CYTOMETRIC ESTIMATION OF NUCLEAR DNA CONTENT IN PLANTS

Noirot M., Barre P., Louarn J., Duperray C. and Hamon S.

Annals of Botany, 2000, 86 : 309-316.

Discrepancies of 2-20 % were noted in nuclear DNA content estimates using mixed and unmixed extracts of several plant species combi-nations (target-standard). This may be because cytosolic components affect dye accessibility to DNA. Several dilution experiments showed clearly how cytosol concentration modified dye accessi-bility. Heat treatments suggested that dye accessi-bility could be related to chromatin sensitivity to decondensation. Some methods (nucleus isolation, dilution) can decrease, but not eliminate, stoichio-metric error. The high sensitivity of the nucleus to the medium highlights the problem of reliability limits in estimating genome size. The choice bet-ween internal and external standardization and the interpretation of intraspecific variation in "nuclear DNA content" are discussed.

Table 1: Effects of various target/standard combinations on nuclear fluorescence and the fluorescence ratio.

Combinations	Variables	Unmixed	Mixed	$F_{1,8}$	P
	Oil palm peak	890	883	2.00	NS
Oil palm/Petunia	Petunia peak	632	635	0.25	NS
	Ratio	1.409	1.389	3.61	NS
	Coffee D peak	356	354	2.15	NS
Coffee D/Petunia	Petunia peak	742	726	29.9	***
	Ratio	0.480	0.488	52.4	***
	Cacao peak	232	239	0.88	NS
Cacao/Petunia	Petunia peak	864	815	20.2	**
	Ratio	0.269	0.293	11.9	**
	Coffee P peak	484	487	4.18	NS
Coffee P/Coffee H	Coffee H peak	739	761	38.8	***
	Ratio	0.654	0.640	84.3	***
	Yam peak	274	281	1.77	NS
Yam/Petunia	Petunia peak	904	737	1677	***
	Ratio	0.304	0.382	138	***
	Rice peak	259	247	16.4	**
Rice-Petunia	Petunia peak	736	745	12.6	**
	Ratio	0.352	0.332	19.6	**
	Rice peak	255	243	11.6	**
Rice/Coffee D	Coffee D peak	354	368	57.0	***
	Ratio	0.720	0.662	55.2	***
	Yam peak	647	593	25.8	**
Yam/Cacao	Cacao peak	518	509	99.8	***
	Ratio	1.25	1.18	15.9	*

Peak values are expressed in channel units. Coffee D: *Coffea liberica dewevrei*; Coffee P: *C. pseudozanguebariae*; Coffee H: *C. humilis*. NS: non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Effects of quantitative and qualitative Principal Component Score Strategies on the structure of coffee, rubber tree, rice and sorghum core collections

Hamon S., Dussert S., Deu M., Hamon P., Seguin M., Glaszmann J.C., Grivet L., Chantereau J., Chevallier M.H., Flori A., Lashermes Ph., Legnate H. and Noirot M.

Genetics Selection Evolution, 1998, 30 : 237-258.

The Principal Component Score Strategy (PCSS) is a multivariate method which allows the identification of a core subset from a germplasm collection. Previously described for quantitative data, the method is extended here to qualitative data provided by molecular markers. Quantitative and qualitative PCSS were then applied to real data on four tropical crops : coffee, rice, rubber tree and sorghum. The results show, in all cases, that the increase in the Cumulated Relative Contribution (CRC) is very rapid but may depend on the species. Ten percent of the entire collection yielded between 22 and 58 % of the CRC. As expected, the variability of the quantitative characters in the subsets was little or not modified by a qualitative selection but largely increased by a quantitative one, whereas qualitative PCSS was more efficient in preserving rare alleles and increased the global diversity with limited quantitative changes. The range of crop plants tested allowed comparison of the respective impacts of the two methods and highlighted the advantage of combining both types of characters.

Alleles	**Coffee**	I50 = 15	**Rice**	I50 = 29	**Rubber**	I50 = 30	**Sorghum**	I50 = 70
Frequency	Total	Selected	Total	Selected	Total	Selected	Total	Selected
$f < 5\%$	16	14	17	16	13	12	18	17
$5\% < f < 10\%$	2	2	4	4	8	8	3	3
$10\% < f < 20\%$	5	5	5	5	11	11	4	4
$20\% < f < 40\%$	8	8	5	5	6	6	8	8
$f > 40\%$	2	2	18	18	13	13	15	15
H	/	/	0.38	0.46	0.45	0.53	0.38	0.46

Table 1 : Selection of core subset accessions for four different crops using the PCSS strategy on qualitative data. The selected number of alleles are reported according to their frequency in the initial collection and to their presence in the subset. H is the heterozygosity of Nei.

Variable	Branc87	Circ87	Circ91	Circ94	Epec89	Epec96	Grum94	Pro87	Pro91	Pro95
BC	1.79	120.5	434.4	480.8	50.1	4.69	2.99	937	2935	7784
Core Qual	1.73	128.1	389.5	430.9	47.0	4.13	2.80	1052	3138	6075
VarDiff	*	*	NS	NS	NS	NS	NS	NS	NS	NS
MeanDiff	/	/	NS	NS	NS	*	NS	*	NS	NS
Core Quant	1.30	148.2	359.6	439.7	42.3	4.33	2.40	1164	6738	14556
VarDiff	***	***	***	***	***	***	***	***	***	***
MeanDiff	/	/	/	/	/	/	/	/	/	/

Table 2. Comparison, for the rubber tree case, of 8 traits distribution (Vardiff), means (MeanDiff) between the BC and the qualitative subset means (Core Qual); between the BC (Core Tot) and the quantitative subset means (Core Quant).

Coffee Genetic Resources and Biotechnologies : their putative uses for breeding

Hamon S., Anthony F., Barre Ph. , Berthaud J., Boursot M., Chabrillange N., Ky C.L., Combes M.C., Couturon E., Cros J., Dussert S., Engelmann F., Lashermes Ph., Le Pierrès D., Louarn J., Noirot M., Récalt C., Trouslot P. and Charrier A.

Agricultures, 1998, 7 : 480-487.

Arabica and Robusta coffee types are produced by recently domesticated species. The *Coffea* genepool, of African origin, is characterised by a high genetic diversity which was collected in different countries and is conserved in several field collections. The purpose of this paper is to give an overview of the current knowledge obtained using biotechnology on : the genetic diversity of the *Coffea* genepool, the breeding possibilities and the germplasm preservation.

Genetic diversity. Coffee phylogenetic relationships were estimated from different approaches. The nuclear DNA polymorphism of the sequences of the internal transcripted spacer ITS 2 region allowed to divided the pool into 4 major groups. Chloroplastic DNA, exclusively maternally inherited, has been sequenced from 38 *Coffea* taxa for the *trn*L-*trn*F intergenic spacer. Phylogenic relationships inferred from this analysis suggest an organisation into 6 clades. The genome size (DNA content - 2C values), estimated by flow cytometry, ranged from 0.95 to 1.78 pg and species could be classified into 3 groups suggesting that the coffee genome size increases from East to West Africa. The overall results agree well with both a geographical organisation of the diversity correspondence (Madagascar, East Africa, Central and West Africa) and a fast speciation process.

Possibilities for molecular breeding. A first linkage map for coffee *C. canephora* totalling 1402 cM was developed on the basis of a population of doubled haploids. Both RFLP and PCR-based markers were used to construct the linkage groups and to locate useful genes. As examples : the S-locus involved into the self-incompatibility of *C. canephora* is associated with an RFLP marker on linkage group 9 ; the resistance to Coffee Berry Disease (CBD) in Arabica coffee, is closely linked to the T gene. For CBD, the resistance is controlled by at least 3 genes which are present in the varieties Hibrido de Timor (T gene), Catimor (T gene), Rume Sudan (R and k genes) and K7 (k gene). Concerning the cultivated Arabica coffee, Random amplified polymorphic DNA (RAPD) markers have been successfully employed to analyse the genetic diversity among cultivated and subspontaneous accessions. The narrow genetic base of commercial cultivars was confirmed but a relatively large genetic diversity was observed within the germplasm collection demonstrating the importance of collecting missions. The large heterosis effect, reported in intergroup, hybrids could be related to this genetic differentiation. At the interspecific level, crossing behaviour show that, in most cases, it is possible to obtain hybrids between species. F1 and G2 hybrids, obtained between 2 species with different nuclear DNA content *C. pseudozanguebariae* (1.13 pg) and *C. devewrei* (1.42 pg), were selected as a model. Genomic *in situ* hybridisation (GISH) and flow cytometry were used on 6 F2 and 7 G2. In the G2, there was a linear relationships between the number of chromosomes and the nuclear DNA content, which indicates that flow cytometry could give an estimation of the parental chromosomic contribution. In addition, cafeine and heteroside contents were analysed in those hybrids. They appeared to be under polygenic control with a strong genetic effect. Nevertheless, one major gene with 2 alleles seemed to be involved in the control of both compounds. The use of gene from wild species into cultivated one is now obvious.

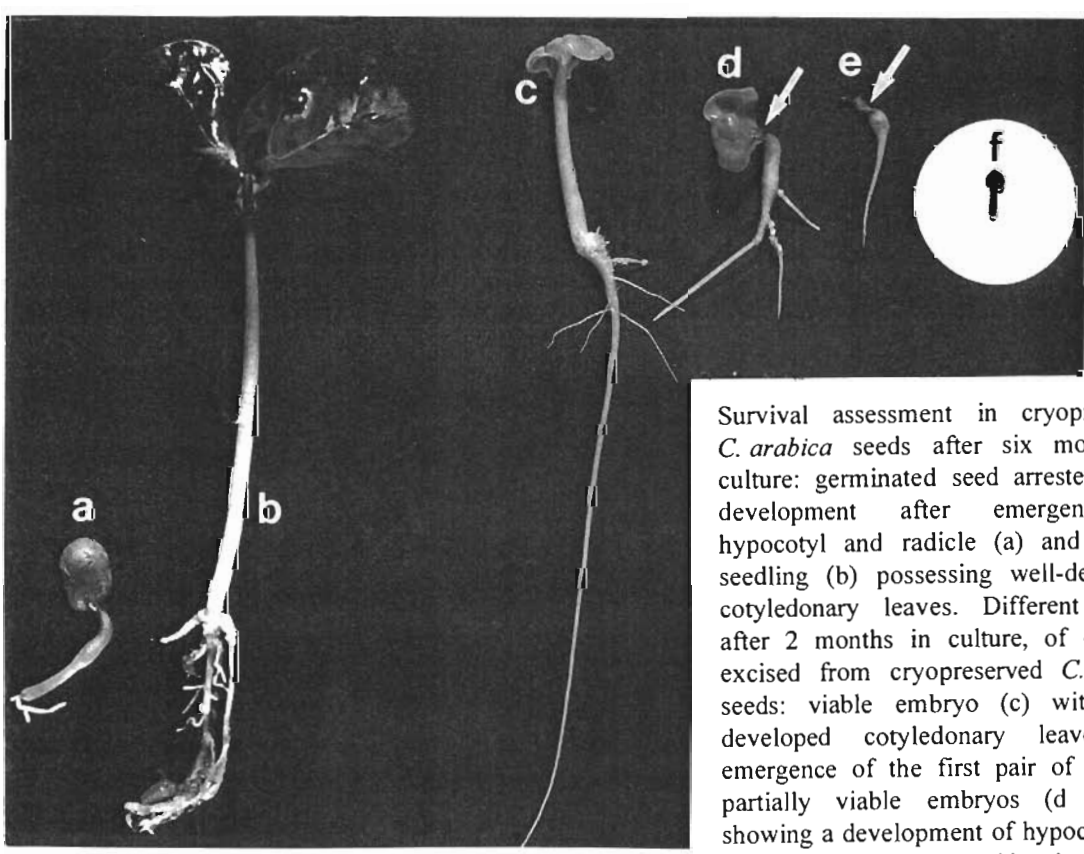
Conservation of genetic resources. At the moment, most coffee accessions are conserved in field collections. An *in vitro* core collection of African coffee germplasm, structured in 32 diploid diversity groups, was established and conserved *in vitro* for 3 years. Survival analysis indicated a broad variability of the accessions in their response to the storage conditions and confirmed the importance of structuring the coffee complex down to intraspecific level. Different approaches and medium have been tested for the long term preservation of coffee resources but the cryopreservation seems to be the most interesting one. The effect of the precooling temperature on the survival rate of cryopreserved *C. arabica* seeds and excised zygotic embryos were investigated. The optimal germination rate (70%) of cryopreserved seeds was achieved after precooling to -50°C. For embryos extracted after thawing from cryopreserved seeds, maximal survival rate (97%) was observed when seeds were immersed directly into liquid nitrogen directly after dehydration without precooling.

Cryopreservation of seeds of four coffee species (*Coffea arabica*, *C. costatifructa*, *C. racemosa* and *C. sessiliflora*) : importance of water content and cooling rate.

Dussert S., Chabrillange N., Engelmann F., Anthony F., Louarn J. and Hamon S.

Seed Science Research, 1998, 8 : 9-15.

In the range of water contents studied (0.1 to 0.4 g H2O g^{-1} dw), *Coffea arabica* seeds were less sensitive to desiccation than *C. costatifructa*, *C. racemosa* and *C. sessiliflora* seeds. At 0.20 g H2O g^{-1} dw, 53 % of *C. arabica* seeds germinated after direct immersion in LN (rapid cooling, 200°C min^{-1}), but none of them developed into normal seedlings. By contrast, in *C. costatifructa*, *C. racemosa* and *C. sessiliflora*, when seeds were dehydrated to the optimal water content (0.19, 0.28 and 0.31 g H2O g^{-1} dw, respectively), the seeds which developed into normal seedlings after LN exposure was 26, 78 and 31% of the desiccation control, respectively. Normal seedlings could be recovered from cryopreserved *C. arabica* seeds only if they were desiccated to 0.20 g H2O g^{-1} dw and precooled slowly to -50°C prior to immersion in LN. Precooling seeds at 2°C min^{-1} allowed 25% of seeds to develop into normal seedlings. The thawing rate had no effect on the survival of cryopreserved *C. arabica* seeds. In all cryopreservation experiments, the total germination did not reflect the percentage of seeds which developed into normal seedlings. Examination of excised embryos indicated a partial explanation of this difference since only the shoot apex was destroyed in non-viable embryos, whereas the hypocotyl and radicle were normal.



Survival assessment in cryopreserved *C. arabica* seeds after six months in culture: germinated seed arrested in its development after emergence of hypocotyl and radicle (a) and normal seedling (b) possessing well-developed cotyledonary leaves. Different stages, after 2 months in culture, of embryos excised from cryopreserved *C. arabica* seeds: viable embryo (c) with well-developed cotyledonary leaves and emergence of the first pair of leaves ; partially viable embryos (d and e) showing a development of hypocotyl and radicle while shoot apex (d and e, arrows) and cotyledons (e) were necrosed ; completely non-viable embryo (f).

Cryopreservation of coffee (*Coffea* spp.) seeds : interspecific variability in desiccation tolerance, amount of non-freezable water and optimal cooling rate.

Dussert S., Chabrillange N., Engelmann F. and Hamon S.

Cryobiology, 1999, 39 : 307-308.

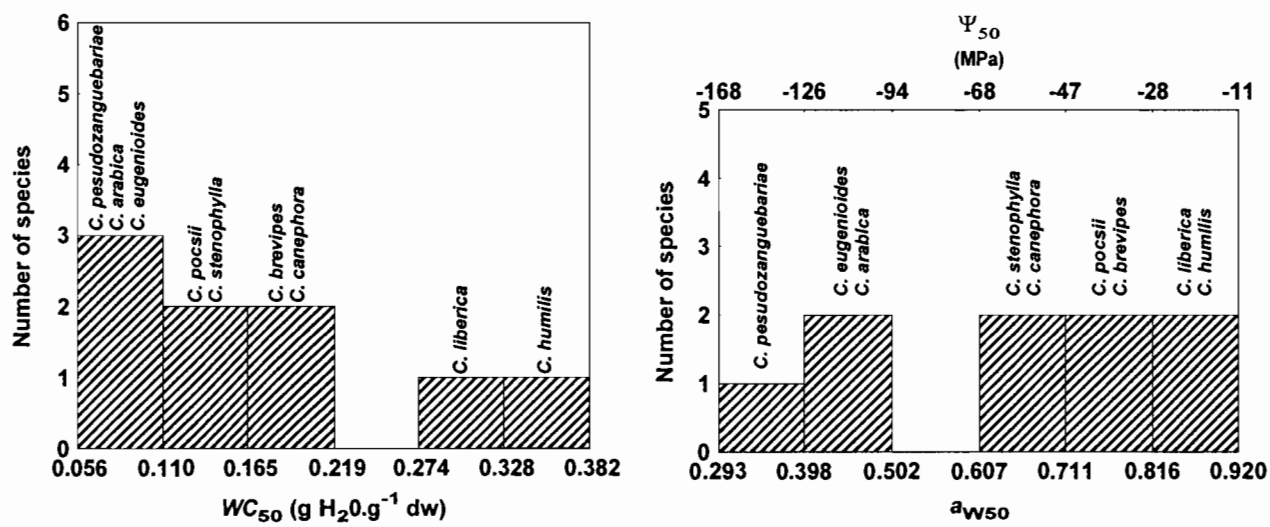
The effects of desiccation and of two cooling processes (direct immersion in LN or precooling at 1°C/min to -50°C prior to immersion in LN) on seed viability (recovery of normal seedlings) were investigated with eight coffee (*Coffea* spp.) species. A continuum of desiccation sensitivity was revealed within the species studied: the water content at which 50% of initial viability was reached, WC_{50}, ranged from 0.05 to 0.29 g H_2O/g dw. The inter-specific variability in response to cryopreservation parameters was observed at different levels. Firstly, three cases could be distinguished as regards the possibility of recovering normal seedlings after cryopreservation: i) with *C. liberica, C. canephora* and *C. sessiliflora,* no seed survived after cryopreservation, whatever the cooling process; ii) with *C. arabica* and *C. pocsii* (previously *C. sessiliflora* Kitulangalo), some seeds withstood LN exposure only if they were precooled slowly prior to immersion in LN; iii) with *C. pseudozanguebariae*, *C racemosa* and *C. eugenioides*, normal seedlings could be recovered from seeds cooled using both processes, but with the latter species, survival was higher after slow cooling. At a second level, with species for which survival was obtained, a large variability was observed as regards the water content at which survival after cryopreservation was first detected: it varied from 0.15 g H_2O/g dw for *C. pseudozanguebariae* to 0.26 g H_2O/g dw for *C. eugenioides*. In order to investigate if the differences observed at these two levels were caused by water crystallisation, the non-freezable water content of seeds of 5 species was determined using DSC. With *C. sessiliflora*, for which no survival was obtained after cryopreservation, seed viability was almost completely lost after desiccation to the non-freezable water content. Desiccation was thus considered the limiting factor for this species. With the four other species, the non-freezable water content coincided with the water content at which survival was first detected after cryopreservation. Inter-specific variability in response to LN exposure could also be observed at a third level, i.e. the maximal survival obtained after freezing seeds at the non-freezable water content: e.g. after rapid cooling, survival was 90% with *C. pseudozanguebariae* and only 8% with *C. eugenioides*.

Quantitative estimation of seed desiccation sensitivity using a quantal response model : application to nine species of the genus *Coffea* L.

Dussert S., Chabrillange N., Engelmann F. and Hamon S.

Seed Science Research, 1999, 9 : 135-134.

Seed desiccation sensitivity was studied in nine species of the genus *Coffea* by measuring seed viability after equilibration over various saturated salt solutions. A quantal response model based on the logistic distribution was developed in order to describe the typical S-shaped patterns observed. The goodness of fit of the desiccation sensitivity model was shown and the assumption that seed desiccation sensitivity follows a continuous distribution within species was verified. For each species, the water content at which 50% of initial viability was reached, WC_{50}, and a specific parameter describing the intra-specific variability, β, were calculated using a non-linear regression. A simplified water sorption model was developed which allowed easy calculation of water activity and water potential corresponding to WC_{50} (a_{w50} and Ψ_{50}) for relative humidities ranging between 10 and 100%. Distribution of WC_{50} and Ψ_{50} (or a_{w50}) in the genus *Coffea* was homogeneous within the following intervals: from 0.05 to 0.38 g $H_2O.g^{-1}$ dw for WC_{50} and from -168 to -11 MPa for Ψ_{50}. Different classifications of the coffee species studied as regards their desiccation sensitivity were obtained depending on whether WC_{50} or Ψ_{50} was used for classification. The continuum for desiccation sensitivity observed within the 9 species studied confirmed that coffee is an appropriate material for studying desiccation sensitivity.



Evidence for a continuum of desiccation tolerance: distributions for the nine *Coffea* species of the water content at which 50% of initial viability was reached, WC_{50}, and the corresponding water activity, a_{w50}, and water potential, Ψ_{50}.

Relationship between seed desiccation sensitivity, seed water content at maturity and climatic characteristics of native environments of nine *Coffea* L. species.

Dussert S., Chabrillange N., Engelmann F., Anthony F., Louarn J. and Hamon S.

Seed Science Research, 2000, 10 (3) : 293-300.

A broad variability for seed desiccation sensitivity, as quantified by the water content and the water activity at which half of the initial viability is lost, has been previously observed within nine African coffee species. In order to investigate if these different degrees of desiccation sensitivity correspond to an adaptive trait, additional data such as the duration of seed development and seed water content at maturity were measured for these species, and the relationship between these parameters and some climatic characteristics of their specific native environments was investigated. Since flowering in all coffee species occurs only a few days after the main rainfall marking the end of the dry season, simulations could be made, based on the continuous sequences of rainfall data compiled in databases of nine climatic stations chosen for their appropriate location in the collecting areas. The simulations revealed a highly significant correlation between the duration of seed development and that of the wet season. Consequently, mature seeds are shed at the beginning of the following dry season. Moreover, the mean number of dry months that seeds have to withstand after shedding was significantly correlated with the parameters used to quantify seed desiccation sensitivity. By contrast, seed moisture content at maturity was not correlated with the level of seed desiccation tolerance. All these results are discussed on the basis of more detailed descriptions of the natural habitats of the coffee species studied.

Desiccation tolerance in relation to soluble sugar contents in seeds of ten coffee (*Coffea* L.) species

Chabrillange N., Dussert S., Engelmann F., Doulbeau S. and Hamon S.

Seed Science Research, 2000, 10 (3) : 393-396.

Large differences in seed desiccation sensitivity have been observed previously among ten coffee species (*Coffea arabica, C. brevipes*, *C. canephora*, *C. eugenioides*, *C. humilis*, *C. liberica*, *C. pocsii*, *C. pseudozanguebariae*, *C. sessiliflora* and *C. stenophylla*). Of these species, *C. liberica* and *C. humilis* were the most sensitive to desiccation and *C. pseudozanguebariae* the most tolerant. A study was carried out using the same seed lots to investigate if these differences in desiccation tolerance could be correlated with differences in soluble sugar content. Soluble sugars were extracted from dry seeds and analysed using high performance liquid chromatography. The seed monosaccharide (glucose and fructose) content was very low (1.5 to 2 $mg.g^{-1}dw$) in all species studied. The sucrose content ranged from 33 $mg.g^{-1}dw$ in *C. liberica* seeds to 89 $mg.g^{-1}dw$ in seeds of *C. pocsii*. Raffinose was detected only in seeds of five species (*C. arabica*, *C. brevipes*, *C. humilis*, *C. sessiliflora*, *C. stenophylla*), among which only three species (*C. arabica*, *C. sessiliflora* and *C. brevipes*) also contained stachyose. Both raffinose and stachyose were present in very low quantities (0.3-1.4 $mg.g^{-1}dw$ and 0.1-0.7 $mg.g^{-1}dw$, respectively). Verbascose was never detected. No significant relationship was found between seed desiccation sensitivity and: i) the sugar content; ii) the presence/absence of oligosaccharides; and iii) the oligosaccharide: sucrose ratio.

Beneficial effect of post-thawing osmoconditioning on the recovery of cryopreserved coffee (*Coffea arabica* L.) seeds

Dussert S., Chabrillange N., Vasquez N., Engelmann F., Anthony F., Guyot A. and Hamon S.

Cryo-Letters, 2000, 21 : 47-52.

Osmoconditioning - controlled rehydration of seeds in a solution with low osmotic potential - has been shown to reinvigorate aged seeds. The present work aimed at investigating the effect of osmoconditioning on the germination of cryopreserved seeds of *Coffea arabica*, whose viability and vigour are drastically affected by cryopreservation. For cryopreservation, seeds were desiccated to 0.21 g H_2O/g dw, cooled at 1°C/min to -50°C, then immersed rapidly in liquid nitrogen. After rapid rewarming, seeds were osmoconditioned for 1 to 6 weeks using solutions with osmotic potentials between -1 and -4 MPa. The time to produce half of the final percentage of normal seedlings, T_{50}, was about three fold lower with osmoconditioned seeds than with non-osmoconditioned seeds (12-14 d *vs* 36 d). Moreover, after a 6-week osmoconditioning treatment with solutions with osmotic potential of -1 and -1.25 MPa, the percentage of seedlings recovered from cryopreserved seeds was 64-74%, against 13-16% only for cryopreserved seeds which were not osmoconditioned.

Percentage of normal seedlings recovered from cryopreserved seeds of *C. arabica* variety Typica after a 2-, 4- and 6- week osmoconditioning treatment with PEG solutions with osmotic potentials of -1, -1.25 and -1.5 MPa or without osmoconditioning treatment. Percentages of normal seedlings followed by the same letter were not significantly different at the P=0.05 level as tested by the Ryan's test of multiple comparison of proportions.

	Osmotic potential (MPa)	Normal Seedlings (%)
Control	0	16 [a]
2-week osmoconditioning	-1	52 [b]
	-1.25	50 [b]
	-1.5	2 [a]
4-week osmoconditioning	-1	54 [b]
	-1.25	62 [b]
	-1.5	22 [a]
6-week osmoconditioning	-1	64 [b]
	-1.25	74 [b]
	-1.5	10 [a]

Assessment of Genetic Diversity in Three Subsets Constituted from the ICRISAT Sorghum Collection Using Random vs. Non-Random Sampling Procedures.
A - Using morpho-agronomical and passport data

Grenier C., Bramel-Cox, P.J., Noirot M., Prasada Rao K.E. and Hamon P.

Theoretical and Applied Genetics, 2000, 101 : 190-196.

A large collection, such as the sorghum (*Sorghum bicolor* [L.] Moench) landrace collection held at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), represents a challenge for maintenance of both the accessions and the information documented on the germplasm collection. The accessibility and knowledge of the landrace collection are the essential factors for an efficient utilization of the genetic resources by both breeders and farmers. Different sampling strategies, either random or non-random were proposed to obtain subsets of reduced size (core collection). Three subsets were established; a random sampling within stratified collection (Logarithmic strategy: L); a sample based upon morpho-agronomic diversity (Principal Component Score strategy: PCS); and a sample based upon an empirical knowledge of sorghum (Taxonomic strategy: T). Comparisons of these three samples for morpho-agronomic characterization and passport information were assessed to determine the impacts on the phenotypic diversity. For their overall diversity, the three subsets did not differ as shown with the two-dimensional representation of the morpho-agronomic diversity and the Shannon-Weaver diversity indices. When comparison for morpho-agronomic and passport data were considered, the PCS subset looked similar to the entire landrace collection. The L subset showed differences for characters associated with the photoperiod reaction that was considered in the stratification of the collection. The T subset was the most distinct from the entire landrace collection as it over represented the landraces selected by farmers for specific uses and it covered the widest range of geographical adaptation and morpho-agronomic characteristics.

Assessment of Genetic Diversity in Three Subsets Constituted from the ICRISAT Sorghum Collection Using Random vs. Non-Random Sampling Procedures.
B - Using molecular markers

Grenier C., Deu M., Kresovich S., Bramel-Cox P.J. and Hamon P.

Theoretical and Applied Genetics, 2000, 101 : 197-202.

The large size of the sorghum (*Sorghum bicolor* (L.) Moench) landrace collection maintained by ICRISAT lead to the establishment of a core collection. Thus, three subsets of around 200 accessions were established from i) a random sampling after stratification of the entire landrace collection (L), ii) a selective sampling based on quantitative characters (PCS) and iii) a selection based on the geographical origin of landraces and the traits under farmers' selection (T). The assessment was done of the genetic diversity retained by each sampling strategy using the polymorphism at 15 microsatellite loci. The landraces of each subset were genotyped with three multiplex polymerase chain reactions (PCRs) of five fluorescent primer-pairs each with semi-automated allele sizing. The average allelic richness for each subset was equivalent (16.1, 16.3 and 15.4 alleles per locus for the subset PCS, L, and T, respectively). The average genetic diversity was also comparable for the three subsets (0.81, 0.77 and 0.80 for the subset PCS, L, and T, respectively). Allelic frequency distribution for each subset was compared with a chi-square test but few significant differences were observed. A high percentage of rare alleles (71 to 76% of 206 total rare alleles) was maintained in the three subsets. The global molecular diversity retained in each subset was not affected by a sampling procedure based upon phenotypic characters.

CORE COLLECTION OF SORGHUM: I . STRATIFICATION BASED ON ECO-GEOGRAPHICAL DATA.

Grenier C., Bramel-Cox P.J. and Hamon P.

Crop Science, 2000, (sous presse).

ICRISAT conserves a large (36 719 accessions) collection of sorghum, *Sorghum bicolor* (L.) Moench, in India. This collection of cultivated and wild sorghums was acquired over 25 years of collection and acquisition from 90 countries. A study of the morpho-agronomic diversity is used to describe the genetic structure of the collection. Morphological traits like date of flowering and plant height can be affected by day length variation. These two characters were highly correlated to the latitudinal and racial distributions of landraces. Thus, stratifying the entire collection for response to photoperiod, estimated by flowering date and plant height, was indicative of a major source of specific adaptation within the collection. This clustering resulted in four clusters, which described the sensitivity of the genotypes to the photoperiod in the latitudinal range where farmers selected them. These four clusters will be the basis for a random stratified sampling to establish core collections following different strategies.

CORE COLLECTION OF SORGHUM : II. COMPARISON OF THREE RANDOM SAMPLING STRATEGIES.

Grenier C., Hamon P. and Bramel-Cox P.J.

Crop Science, 2000, (sous presse).

Since 1972, ICRISAT has maintained a large collection of sorghum, *Sorghum bicolor* (L.) Moench subs. *bicolor*, in India. The collection size has continuously increased and the total number of accessions at present conserved in the gene bank has reached about 36,000 accessions. The need to help management was considered and this study was conducted to establish core collections. This sorghum collection was earlier stratified into four clusters according to the photoperiod sensitivity. Then, considering the core collection strategy, we used three random sampling procedures to determine the specific accessions to be included in the core; i.e. a constant portion (C), a proportional (P), and a proportional to the logarithm (L) of the photoperiod group's size sampling strategy. Both the core C and L strategies were significantly different from the landrace collection with better representation of the smallest groups, such as landraces insensitive to photoperiod. Despite differences between the three core collections, estimates of global diversity through the Shannon-Weaver Diversity Indices were of the same magnitude as the landrace collection. When compared, the core C and L were significantly different. Core L sampled better for the characters, the race, and the latitudinal classes that were related to the photoperiod sensitive landraces. Thus for establishing a core collection with the widest range of adaptation to photoperiod, we propose to use a logarithmic sampling strategy, which identifies a broadly adapted set of genotypes.

Les arbres tropicaux fixateurs d'azote

Dommergues Y., Duhoux E. et Diem H.G.

Alors que nos connaissances sur la génétique, la physiologie et l'écologie de la fixation de l'azote ont fait des progrès considérables au cours des dernières années notamment grâce au développement spectaculaire des moyens modernes d'investigations aussi bien à l'échelle de l'écosystème qu'à l'échelle moléculaire, on est surpris de constater que les retombées de ces découvertes dans le domaine appliqué sont encore insignifiantes. Ce livre présente pour la première fois, sous un même titre, d'une part, les derniers résultats de la recherche fondamentale relatifs à l'ensemble des arbres fixateurs d'azote (AFN), Légumineuses et non Légumineuses (plantes actinorhiziennes) (chapitres 1 à 6), d'autre part, les observations et les investigations de terrain concernant les principales espèces d'AFN ainsi que les problèmes majeurs rencontrés au champ (chapitres 7 à 9). Pour chaque problème étudié, on a rassemblé dans un ensemble logique des données expérimentales et des faits jusqu'alors ponctuels ou disparates. Ainsi ressortent nettement les grandes lignes de force des recherches contemporaines.

Cet ouvrage de synthèse, rédigé pour un large public, est destiné à informer tous ceux qui s'intéressent aux possibilités et aux promesses offertes par les AFN en zones méditerranéenne et tropicale. Pour ces lecteurs, il constitue un livre de référence.

In vitro culture of sahalian acacias : aspects of microcutting and somatic embryogenesis.

Borgel A., Sané D., Kparé Y., Diouf M. and Chevallier M.H.

in: «L'acacia au Sénégal», Campa, Grignon, Gueye, Hamon Eds., Orstom-Isra Publishers, ISBN 2-7099-1423, Paris 1998 : 257-272

In vitro multiplication of several subspecies of Sahalian acacias has been studied using different approaches:

- Microcutting production was studied with special reference to sustained root growth after several subcultures :

Results show that the auxin used for rooting the microcuttings (either indol-butyric acid IBA or naphtalen-acetic acid NAA) plays a specific role on root production rate and shape. IBA induces a higher rooting rate than NAA but only roots induced by NAA show a normal, thick tap-root shape that can survive when transplanted into soil.

- Somatic embryogenesis from different explants and detection of qDNA variants among regenerating somatic embryos :

Flow cytometric analyses were performed on regenerating somatic embryos of *A. nilotica* and *A. tortilis* and zygotic embryos excised from germinating seeds of the same two species. Most somatic embryos were found to have the same qDNA as the zygotic embryos of the same species. In some cases, significant differences were observed between somatic and zygotic embryos. Some somatic embryos show a doubled qDNA, others are chimeras of cell types containing normal and doubled qDNA (Figure 1).

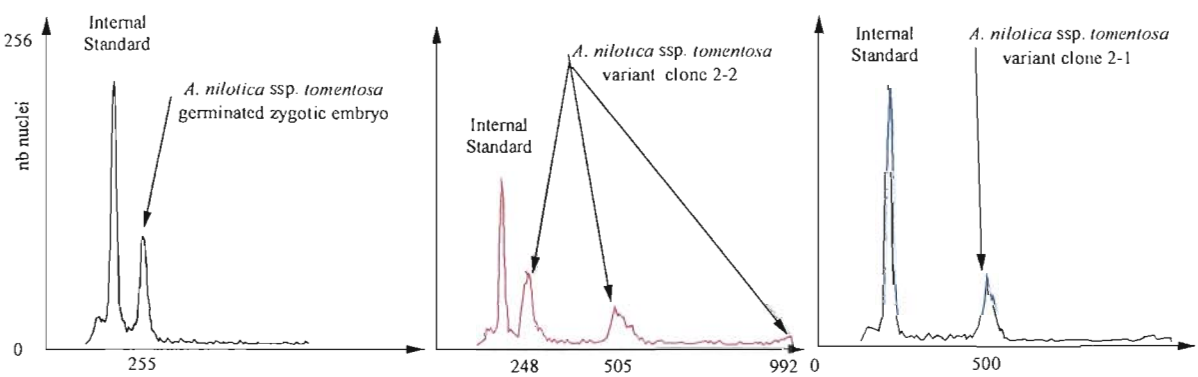


Figure 1: Flow cytometry permits to detect somatic embryos with abnormal qDNA (clones 2-1 and 2-2) with reference germinated zygotic embroys (left). The internal standard (latex fluorescent balls) is common to all individuals analysed. The fluorescent intensity shows the nuclear qDNA of each individual analysed (>2000 nuclei counted).

- Two new protocols have been developed :
  - With the first protocol it is now possible to obtain a normal rooting of *in vitro* microcuttings of *A. tortilis* even after several subcultures
  - The second protocol makes it possible to produce somatic embryos from different genotypes of *A. tortilis* and *A. nilotica* (Table 1)

Species	Callus		Embryos		
	Number	% with embryos	Number	% mature embryos	% plantlets produced
A. nilotica tomentosa	46	83	547	100	60
A. nilotica adstringens	41	85	629	83	54
A. tortilis raddiana	40	65	230	59	23
Total			1 406	85	51

Genetic diversity in the genus *Acacia*

Chevallier M.H. and Alain Borgel A.

in: «L'acacia au Sénégal», Campa, Grignon, Gueye, Hamon Eds., Orstom-Isra Publishers, ISBN 2-7099-1423, Paris 1998 : 287-308.

The genus *Acacia* is composed of ca. 1250 species, mostly originating from Australia, only 134 originating from Africa. Some of these species are essential to the local economy in the sahelian area. The study of the genetic variability of natural populations is a necessary prerequisite for defining strategies for genetic resources management.

This review compares the organisation of the genetic diversity of firstly long-lived forest trees, secondly of acacia trees, and thirdly of several African acacia species. The different types of genetic markers and the corresponding analytical methods are described. Despite a great disparity among the results analysed, it has been possible to compare the levels of diversity of the species, in particular in terms of the extent of their geographic distribution and their biologic characteristics.

Trees are a group of plants which show the highest genetic diversity in the plant kingdom. The dispersion area and the reproductive system are the main factors which help to explain this diversity. On the other hand, differentiation between populations is generally low.

The genus *Acacia* shows a heterogeneous structure in its genetic diversity. When compared to African species, Australian acacias show a lower intra-population diversity but a higher differentiation between populations originating from well separated geographic areas. Allogamy is the general rule but there are some exceptions, especially among some African species that show a high level of autogamy. Several ploidy levels and nuclear DNA amounts are observed: this may explain the high level of genetic diversity. These cytogenetic traits can be helpful for taxonomic purposes.

Experimental results are presented using isozyme markers of several African *Acacia* species and populations. These markers clearly explain the taxonomic relationship among two complexes of species grouped together on the basis of common isozyme markers and ploidy levels. The *A. senegal* group includes *A. senegal*, *A. dugeoni*, *A. laeta*, *A. mellifera* and *A. gourmaensis*, the *A. nilotica* group includes seven subspecies (*subalata*, *cupressiformis*, *jacquemonti*, *tomentosa*, *adstringens*, *nilotica*, and *indica*) distributed among two ploidy level groups (Table infra). The classification thus obtained improves the previous classification based on morphological and cytological traits.

Nuclear 2C DNA amount (picogram) and ploidy level in the complex *«A. nilotica»*

Species	Origin	2C (pg)
DIPLOIDS 2C=0.88 to 1.02 pg, 26 chromosomes		
A. nilotica subalata	India	0.96
A. nilotica cupressiformis	India	0.88
A. nilotica cupressiformis	India	0.89
A. nilotica jacquemontii	India	0.94
A. nilotica tomentosa	Senegal	1.02
A. nilotica tomentosa	Senegal	0.95
TETRAPLOIDS 2C=1.83 to 2.11 pg, 52 chromosomes		
A. nilotica jacquemontii	India	1.91
A. nilotica adstringens	Senegal	1.83
A. nilotica adstringens	Senegal	2.01
A. nilotica adstringens	Senegal	2.11

GENETROP PUBLICATIONS

(ALPHABETIC LIST)

PUBLICATIONS

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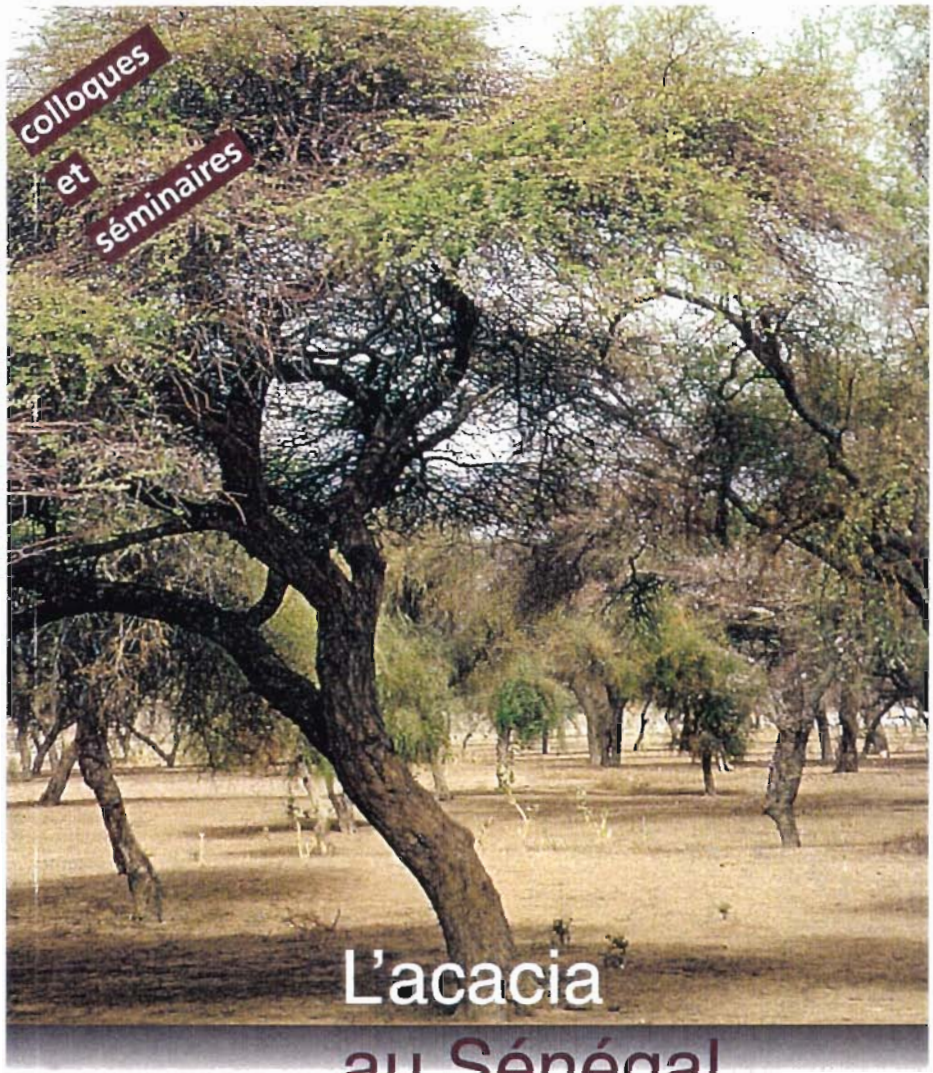

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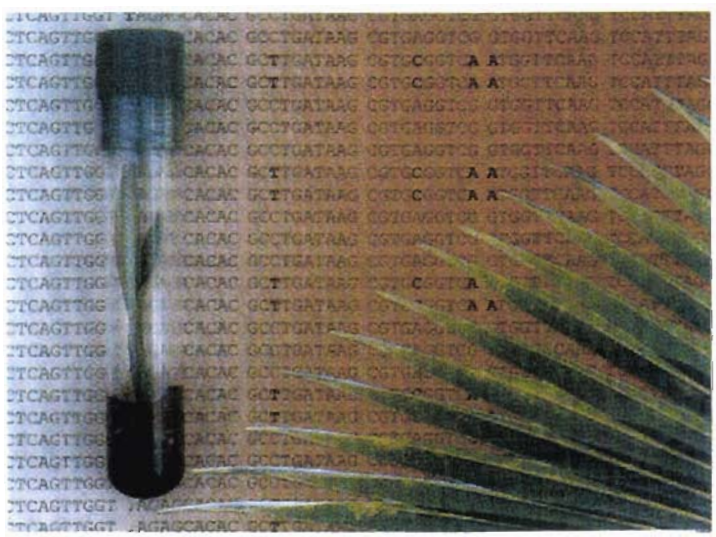

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CFR, Centre Français du Riz, France
CIAT, Centro Internacional de Agricultura Tropical, Colombie
CICY, Centro de Investigacion Cientifica de Yucatan, Mexique
CIFC, centro de investigacao das Ferrugens do Cafeeiro, Portugal
CIMMYT, Centro International de Mejoramiento de Maiz y Trogo, Mexique
CIRAD, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France
COGENT, Coconut Genetic Ressources Network, Malaysie
CRF, Coffee Research Fondation, Kenya
ENSA-M, Ecole Nationale de Sciences Agronomiques de Montpellier, France
FAST, Faculté des Sciences, Côte d'Ivoire
FELDA, Federal Land Development Authority of Malaysie
IACR, Long Ashton Research Station, U.K.
ICRISAT, International Crop Research Institute for the Semi-Arid Tropics, Inde
IDEFOR, Institut des Forêts, Côte d'Ivoire
IDESSA, Institut des Savanes, Côte d'Ivoire
ILTAB, International Laboratory for Tropical Agronomy Biotechnology, CA USA
INIFAP, Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, Mexique
INRA-F, Institut National de Recherche Agronomique, France
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