Use of fluorescence in situ hybridization as a tool for introgression analysis and chromosome identification in coffee (Coffea arabica L.)

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Abstract: Fluorescence in situ hybridization (FISH) was used to study the presence of alien chromatin in interspecific hybrids and one introgressed line (S.288) derived from crosses between the cultivated species *Coffea arabica* and the diploid relatives *C. canephora* and *C. liberica*. In situ hybridization using genomic DNA from *C. canephora* and *C. arabica* as probes showed elevated cross hybridization along the hybrid genome, confirming the weak differentiation between parental genomes. According to our genomic in situ hybridization (GISH) data, the observed genomic resemblance between the modern *C. canephora* genome (C) and the *C. canephora*–derived subgenome of *C. arabica* (C^a) appears rather considerable. Poor discrimination between C and C^a chromosomes supports the idea of low structural modifications of both genomes since the *C. arabica* speciation, at least in the frequency and distribution of repetitive sequences. GISH was also used to identify alien chromatin segments on chromosome spreads of a *C. liberica*–introgressed line of *C. arabica*. Further, use of GISH together with BAC-FISH analysis gave us additional valuable information about the physical localization of the *C. liberica* fragments carrying the S$_H$3 factor involved in resistance to the coffee leaf rust. Overall, our results illustrate that FISH analysis is a complementary tool for molecular cytogenetic studies in coffee, providing rapid localization of either specific chromosomes or alien chromatin in introgressed genotypes derived from diploid species displaying substantial genomic differentiation from *C. arabica*.

Key words: coffee, BAC-FISH, interspecific hybridization, GISH, introgression, genome evolution.

Résumé : L'hybridation in situ fluorescente (« FISH ») a été utilisée pour étudier la présence de chromatine introgressée chez des hybrides interspécifiques mais aussi chez une lignée cultivée, issues du croissement entre l'espèce cultivée *Coffea arabica* et ses proches diploïdes *C. canephora* et *C. liberica*, respectivement. L'hybridation in situ en utilisant l'ADN génomique des espèces *C. canephora* et *C. arabica* comme sondes, a montré une hybridation croisée importante chez les génomes hybrides, ce qui confirme une étroite différentiation génomique entre les parents. Cette ressemblance entre le génome actuel de *C. canephora* et le sous-génome de *C. arabica* dérivant de l'espèce *C. canephora* (C^a), semble très élevée. De plus, la faible discrimination entre les chromosomes du type C et ceux du type C^a, suggère des modifications structurales mineures dès l'origine même de l'espèce *C. arabica*, au moins dans ce qui concerne la fréquence et distribution des séquences répétées. L'hybridation génomique in situ (« GISH ») a permis aussi l'identification de différents fragments d'introgression chez une lignée de *C. arabica* introgressée par *C. liberica*, ce qui a conduit à l'identification de quatre fragments d'introgression. De plus, l'analyse combiné GISH plus BAC-FISH a donné des informations importantes sur la localisation physique du fragment issu de *C. liberica* porteur du facteur S$_H$3 de résistance à la rouille des caféiers. L'ensemble des résultats montre que la technique FISH représente un outil puissant pour l'étude cytogénétique des caféiers permettant l'identification rapide de chromosomes mais aussi de l'ADN introgressé chez les génotypes issus de croissements concernant des espèces diploïdes caractérisées par une différentiation génomique importante par rapport à *C. arabica*.

Mots-clés : caféier, BAC-FISH, GISH, hybridation interspécifique, introgression, évolution du génome.

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Introduction

Introgression represents one of the most important genetic strategies for plant breeding. However, introgression of desirable traits from exotic genetic resources into elite lines is often a time-consuming and laborious process (Simmonds 1993). Several methods including biochemical, genetic, or molecular markers have been successfully applied to detect and study alien introgression in plant genomes. During the last decade, cytogenetic methods based on DNA–DNA in situ hybridization have been proposed as complementary tools for the direct characterization of genomic constitution and alien introgression in plants (Rieseberg et al. 2000; Anamthawat-Jónsson 2001). In particular, fluorescence in situ hybridization (FISH) has been successfully applied in

diverse breeding strategies with various plant species (Jiang and Gill 1994; Puertas and Naranjo 2005). The main advantage of FISH is that it allows detection of the extent of introgression across the entire genome in a single hybridization experiment, thanks to the in situ labeling of homologous chromosomes or chromosome regions on the basis of divergent dispersed repeats. In situ hybridization could be carried out using total genomic DNA (genomic in situ hybridization, GISH), chromosome-derived DNA probes, or large genomic insert clones such as bacterial artificial chromosomes (BAC-FISH). Until now, GISH has provided valuable information for characterization of genomes and chromosomes in hybrid polyploids, hybrid plants, and recombinant breeding lines (Anamthawat-Jónsson 2001; Schubert et al. 2001; Raina and Rani 2001). Coffee is now one of the most important export commodities in the world. Commercial production is supported by 2 species: *Coffea arabica* and *C. canephora*. Within the genus *Coffea* (which includes more than 100 different species), *C. arabica* is the only tetraploid species ($2n = 4x = 44$) and it is characterized by self-fertilization. In contrast, *C. canephora* and all other species are diploid and predominantly self-incompatible (Charrier and Berthaud 1985). Furthermore, molecular analyses support the hypothesis that *C. arabica* is an amphidiploid resulting from the association of 2 genomes, namely E^a and C^a, related to *C. eugenioides* (E genome) and *C. canephora* (C genome), respectively (Lashermes et al. 1999). *Coffea arabica* is characterized by low genetic diversity, and the transfer of desired characters from related diploid species into cultivars of *C. arabica* has therefore been a continuous priority in coffee breeding (Van der Vossen 2001). Occurrence of spontaneous interspecific hybrids between *C. arabica* and related diploid species such as *C. canephora* or *C. liberica* is common, especially when these species grow in direct proximity (Cramer 1957). In artificial hybridization conditions, viable triploid, tetraploid (i.e., resulting from hybridization between *C. arabica* and autotetraploidized diploid parents), or hexaploid (i.e., obtained by duplication of triploid hybrids) hybrids could also be obtained. In recent decades, those hybrids have been used intensively in coffee breeding programmes as the main source of resistance to pests and diseases. However, conventional breeding strategies face considerable difficulties. In particular, strong limitations are due to the long generation time of the coffee tree (5 years), the high cost of field trials, and the lack of accuracy of current strategies. In the present work, we investigated whether total genomic DNA from the 2 diploid relatives *C. canephora* and *C. liberica* could be used as a probe to identify the presence of alien chromatin in introgressed genotypes of *C. arabica* by GISH. Therefore, the chromosome origin of triploid interspecific hybrids derived from crosses between *C. arabica* and *C. canephora* was examined by GISH. Further, to detect an introgression mediating disease resistance in chromosome 1 (linkage group 1) of a recombinant line of *C. arabica* (S.288), we used GISH with labeled *C. liberica* genomic DNA and subsequently performed FISH with a chromosome-specific BAC clone to identify the particular chromosomes that carry the resistance factor S_H3 to leaf rust (*Hemileia vastatrix*). The overall results are discussed in relation to genome closeness between *C. arabica* and

its diploid relatives as well as the future implications of FISH analysis in physical mapping of coffee introgression.

Materials and methods

Plant material

Root tip meristems were obtained from both clonal propagated plants of triploid ($3x = 33$) interspecific hybrids between *C. arabica* and *C. canephora* and seedling plants of a *C. liberica*–introgressed *C. arabica* genotype (i.e., line S.288). Both cloned and seedling plants were grown in pots under appropriate greenhouse conditions. Triploid F_1 plants (E^aC^aC) were generated by crossing the tetraploid *C. arabica* (accession ET 30) as female parent with the diploid *C. canephora* (accession IF 181). (Accessions ET 30 and IF 181 correspond to representative genotypes available at IRD, Montpellier, France.) The S.288 line is derived from a selfed offspring of S.26, a natural hybrid between *C. arabica* and *C. liberica* (Vishveshwara 1974). This line has been reported to carry the S_H3 resistance factor against the coffee leaf rust, introgressed from *C. liberica* (Rodrigues et al. 1975; Prakash et al. 2002, 2004). Therefore, it has been used as one of the main sources for rust resistance in India (Vishveshwara 1974). Recently, several markers linked to the S_H3 factor from *C. liberica* have been successfully mapped in a homoeologous region on chromosome 1 of *C. canephora* (Mahé 2006).

Chromosome preparation

For preparation of mitotic metaphase chromosome spreads, long (approximately 0.5 cm) actively growing root tips were collected. To arrest mitotic division at the metaphase stage, root tips were treated in the dark with a saturated α-bromonaphthalene solution for 2 h at 4 °C followed by 2 h at room temperature. Treated root tissues were rinsed 2 times with a fixative solution (ethanol : glacial acetic acid, 3:1 v/v) and stored at 4 °C until use. After being washed in distilled water for 30 min, fixed root tips were hydrolyzed in 0.25% HCl for 10 min and then washed thoroughly with 0.01 mol/L citrate buffer (sodium citrate – citric acid, pH 4.5). A single root tip was placed in the center of a pre-cleaned glass slide and incubated at 37 °C for 40–50 min in 25 μL of an enzymatic mixture (1% cellulase RS, 1% macerase R10, 1% pectolyase Y23, and 0.2% driselase in citrate buffer, pH 4.6). Remaining enzymatic solution was removed and the meristematic portions of the roots were subjected to a hypotonic treatment by addition of a drop of distilled water. After 15 min, water was removed and meristems were macerated in a drop of 3:1 (v/v) ethanol : glacial acetic acid using fine-pointed forceps. Slides were air-dried and then stored at 4 °C until utilization. Before hybridization, slides were incubated with 100 μL of RNase (100 μg/mL) for 45 min at 37 °C under a plastic cover slip in a humid chamber and then washed 2 × 5 min in 2× SSC (1× SSC: 0.15 mol/L NaCl, 0.015 mol/L sodium citrate) and BT buffer (1 mol/L sodium bicarbonate, 0.5% Tween 20), respectively. Chromosome preparations were fixed in paraformaldehyde (4% in PBS (phosphate-buffered saline)) for 10 min at room temperature, washed 3 × 5 min in 2× SSC, and then dehydrated

Fig. 1. GISH on mitotic metaphase chromosomes of an interspecific triploid hybrid between *C. arabica* and *C. canephora* using total genomic DNA from the *C. arabica* parent (*a*), detected with Texas Red (in red), and from the *C. canephora* parent (*b*), detected with fluorescein isothiocyanate (in green). (*c*) After superimposition of *a* and *b*. GISH revealed 11 chromosomes (arrows) belonging to the E^a subgenome present in *C. arabica*. Scale bar = 5 μm.
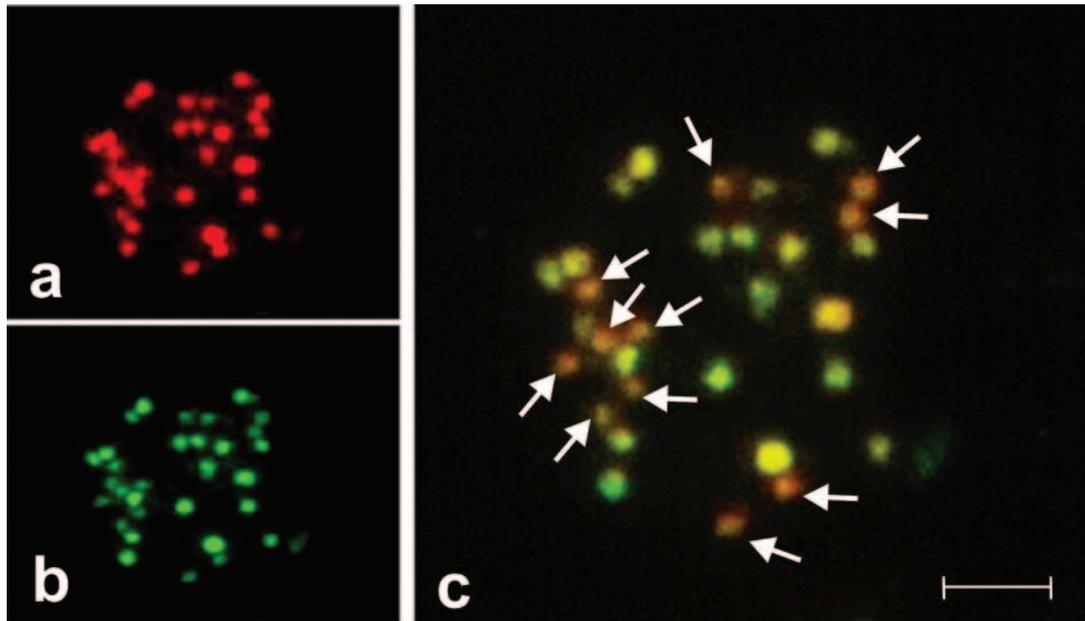
by submerging for 5 min in 50%, 70%, and 100% (*v/v*) ethanol and air-dried.

## Genomic probes and BAC clone screening

Genomic probes were generated from total genomic DNA isolated from young leaves of *C. arabica*, *C. canephora*, and *C. liberica* through a nuclei isolation step as described by Agwanda et al. (1997). Before labeling, genomic DNA was purified using the illustra GFX^TM PCR purification kit (Amersham). Furthermore, selected chromosome-specific BAC clones were analyzed by dot blot to eliminate clones with a high proportion of repeated sequences. The chromosome-specific BAC clones were previously identified by hybridizing high-density colony filters from a BAC library of *C. arabica* with an RFLP probe (gA67A) corresponding to linkage group 1 (i.e., chromosome 1) of the *C. canephora* genome (Noir et al. 2004). The DNA from each selected BAC clone was isolated using the NucleoBond® AX kit for plasmid DNA purification (Macherey-Nagel). For dot blot hybridization analysis, approximately 250 ng of each BAC DNA was transferred to a Hybond^TM-N+ nylon membrane and fixed by UV-crosslinking. ^{32}P-labeled total *C. arabica* genomic DNA was used as probe. Hybridization and washing were carried out under high-stringency conditions. Two BAC clones (81-13H and 72-2D) exhibiting no or very low hybridization signals and belonging to the C^a subgenome were selected for further BAC-FISH identification of chromosome 1.

Fluorescence in situ hybridization analysis

Double genomic in situ hybridization experiments were carried out by simultaneously using genomic DNA from either *C. arabica* and *C. canephora* or *C. arabica* and *C. liberica* as probe. To determine the chromosome localization of *C. liberica*–introgressed fragments present in the S.288

line, both genomic DNA (from *C. liberica*) and a chromosome-specific BAC clone (81-13H or 72-2D) were used as probes in a combined GISH/BAC-FISH analysis. Whenever 2 probes were used together, each one was labeled with a different system. Probe labeling as well as GISH and BAC-FISH analyses were conducted according to methods described by Jiang et al. (1995), with some modifications. In short, 1 μg of total genomic DNA or BAC DNA was labeled with biotin-14-dATP (BioNick^TM DNA labeling system, Invitrogen) or digoxigenin-11-dUTP (DIG-Nick Translation Mix, Roche) in accordance with the manufacturer's protocol. The hybridization mixture for each slide included 50% deionized formamide (Sigma product No. F9037), 10% dextran sulphate, 2× SSC, 1% sodium dodecyl sulphate (Sigma product No. L4509), 30 μg of sheared herring sperm, and either 250 ng of labeled genomic probe or 150 ng of labeled BAC probe. Genomic DNA from the S.288 line was used as a blocking agent prior to BAC probe hybridization. Therefore, genomic DNA was autoclaved for 5 min to obtain genomic fragments of about 100–500 bp and added at a concentration of about 0.5–1 μg/μL. The hybridization mixtures were centrifuged briefly, denatured at 85 °C for 10 min, and then transferred to ice for 5 min before applying them to slides. Slide-bound chromosomal DNA was denatured separately for 2 min 30 s at 72 °C in a solution of 70% formamide in 2× SSC, dehydrated in a 2× SSC washing solution at 4 °C followed by a graded ethanol series (50%, 70%, and 100%, 5 min each) at –20 °C, and air-dried. The previously denatured probe mixture was placed on the slide. Approximately 30 μL of each probe was applied per slide and a cover slip (24 mm × 40 mm) was placed over. After overnight incubation at 37 °C in a humid chamber, the cover slips were carefully removed and

Fig. 2. GISH on mitotic metaphase chromosomes of the introgressed line S.288 of *C. arabica*, using labeled genomic DNA from *C. liberica* as probe (detected with fluorescein isothiocyanate, in green). All individual chromosomes carrying introgressed fragments are indicated by arrows and enlarged at the right. Chromosomes were counterstained with DAPI, in blue. Scale bar = 5 μm.
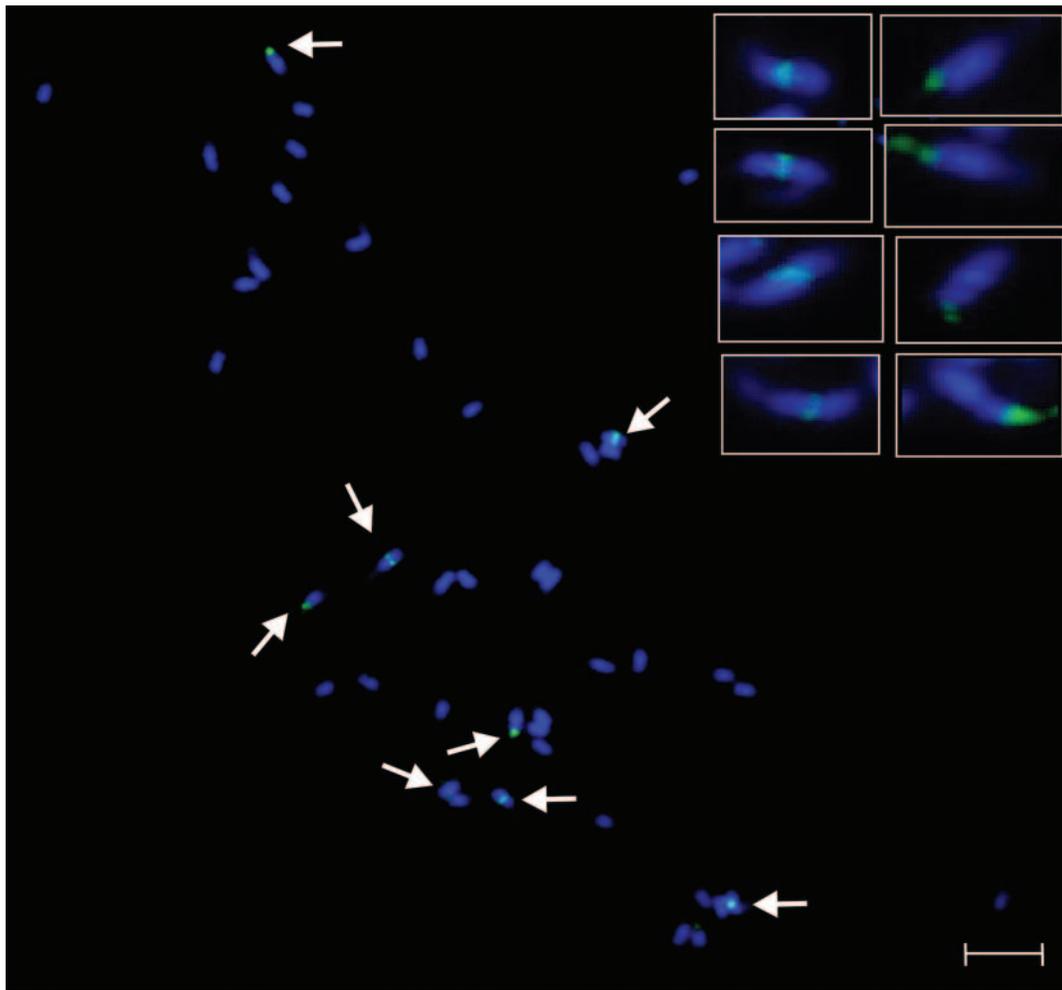
the slides were washed. To remove the hybridization mixture and the excess unbound probe, slides were washed in 2× SSC, then in formamide in 2× SSC (50% *v/v*), and finally in 2× SSC and then transferred to 4× SSC plus 0.2% Tween 20. All washes were carried out at 40 °C for 5 min. After rinsing, slides were incubated in PBS for 30 min at room temperature.

Probe detection and analysis

The biotinylated probes were detected with Texas Red® avidin DCS (Vector Laboratories, 10 μg/mL in 5% BSA) and the signal was amplified with biotinylated anti-avidin D (Vector Laboratories, 25 μg/mL in 5% (*v/v*) normal goat serum block solution). Digoxigenin-labeled probes were detected using the fluorescent antibody enhancer set for DIG detection (Roche). 4′,6-Diamidino-2-phenylindole (DAPI, 1 μg/mL) was used as a chromosome counterstain. Images were obtained with a Leica epifluorescence microscope equipped with filter blocks for DAPI, fluorescein, and Texas Red. Images were captured with a digital cooled CCD camera (Sensys). Numerical image acquisition and analysis were implemented with the Leica QFISH program.

Results and discussion

GISH detection of alien chromosomes in *C. arabica* × *C. canephora* interspecific hybrids

In situ hybridization using total genomic DNA from *C. arabica* and *C. canephora* as probes showed considerable cross hybridization to the hybrid chromosome preparations (Fig. 1). The fact that all chromosomes were moderately to highly labeled with both probes after GISH analysis confirms a close genetic affinity between *C. arabica* and *C. canephora* as well as between the C genome of *C. canephora* and the E^a subgenome (from *C. eugenioides*) present in *C. arabica*. Chromosome origin in interspecific hybrids or allopolyploid species may be distinguished on the basis of divergent dispersed repetitive sequences between parental genomes. Nevertheless, when parental species share too similar families of dispersed repeats or extensive homogenization of these sequences has occurred already within the hybrids, the chromatin of chromosomes belonging to the parental complements of synthetic interspecific hybrids (or presumed allopolyploid species) can no longer be discriminated reliably by GISH (Schubert et al. 2001). In coffee,

Fig. 3. (*a–c*) Root-tip metaphases of the introgressed line S.288 after FISH with BAC clone 81-13H as probe. (*a*) FISH signals detected with fluorescein isothiocyanate (in green). (*b*) Same preparation after chromosome counterstaining with DAPI (in blue). (*c*) BAC-FISH signals on chromosomes counterstained with DAPI on a second chromosome preparation. Chromosomes exhibiting BAC signals (in green) are indicated with arrows. (*d*) Same preparation as in (*c*) after GISH hybridization using total genomic DNA from *C. liberica* (detected with Texas Red, in red). GISH signals co-localize with BAC signals in 2 homologous chromosomes (indicated by arrowheads and enlarged). Scale bars = 5 μm.
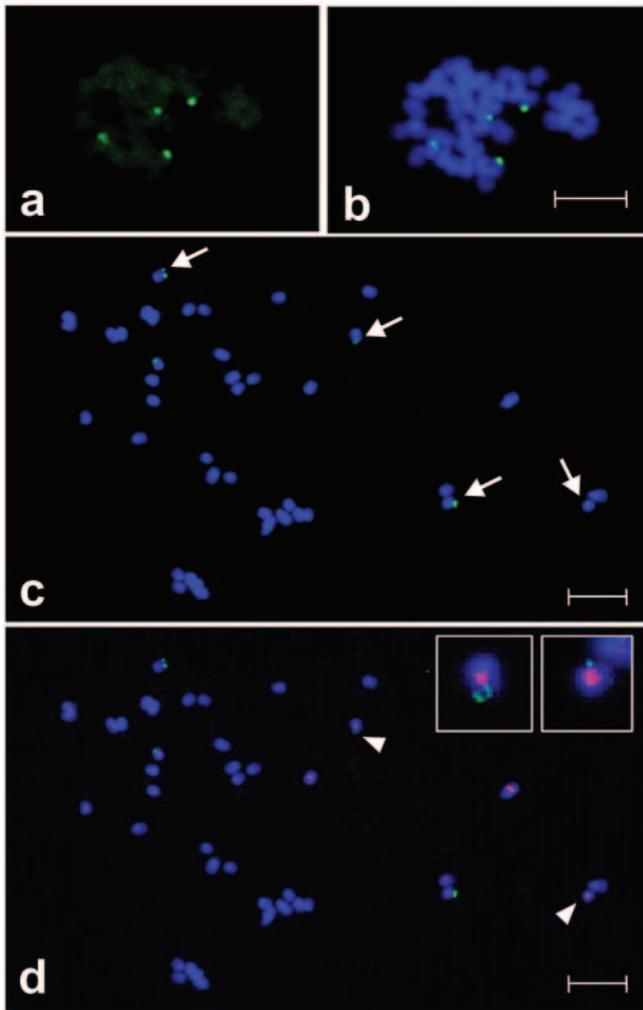

the close genomic relationship between *C. arabica* and *C. canephora* has been evaluated by comparing chloroplast as well as nuclear ribosomal DNA sequences (Cros et al. 1998; Lashermes et al. 1996, 1997). Further, previous molecular analyses involving tetraploid interspecific hybrids between these 2 species showed high recombination frequencies between homoeologous chromosomes, suggesting very low differentiation between chromosomes belonging to the 2 different subgenomes, E^a and C^a (Lashermes et al. 2000; Herrera et al. 2002). Therefore, it is not surprising that the overall frequency and distribution of repetitive sequences were found be quite similar between the *C. arabica* and *C. canephora* genomes. Although GISH was not able to clearly differentiate the parental chromosome origin in

the triploid hybrid genomes, it allowed enough discrimination between E^a and *C. canephora*–related chromosomes (Fig. 1*c*). In fact, using GISH we were able to detect 11 red chromosomes (corresponding to chromosomes belonging to the E^a subgenome of *C. arabica*) and 22 greenish yellow chromosomes (corresponding to both the chromosomes from *C. canephora* and the constitutive chromosomes of *C. arabica* belonging to the C^a subgenome). Closeness between the modern *C. canephora* genome (C) and the *C. canephora*–derived subgenome of *C. arabica* (C^a) appears rather considerable, at least regarding the frequency and distribution of dispersed repetitive sequences. This observation suggests that there was limited genome reorganization during the evolution of the tetraploid archetype and the present amphidiploid *C. arabica*, and it is also in concordance with the hypothesis that speciation of the *C. arabica* genome took place recently: from historical times to 1 million years ago (Lashermes et al. 1999). Although it is not the rule, slow genome reorganization has also been observed in other allopolyploid species such as cotton. Interestingly, molecular analysis in this species showed that genome formation was not necessarily causally connected to rapid structural stabilization among the constitutive genomes (Cronn et al. 1999; Liu et al. 2001). In this sense, coffee species would represent an alternative model to study the mechanisms involved in polyploid genome evolution. Difficult discrimination of chromosome origin in interspecific hybrids has been favored by incomplete labeling of chromosomes following GISH. In most cases we observed that mostly the pericentromeric parts of chromosomes were well hybridized. This phenomenon has already been observed in coffee (Barre et al. 1998; Lashermes et al. 1999) and in other species such as *Brassica*, *Rubus*, *Arabidopsis*, and *Musa* spp. (Fahlesen et al. 1997; Lim et al. 1998; Kamm et al. 1995; D'Hont et al. 2000). It has been suggested that low abundance of dispersed repetitive DNA sequences within the coffee genome and the small size of the genome are likely to be responsible for little or no GISH hybridization at the distal regions of chromosomes (Raina and Rani 2001). This finding is also consistent with the hypothesis that plant repetitive DNA sequences are often organized in clusters and predominantly concentrated in either centromeric or telomeric regions of the chromosomes (Schmidt and Heslop-Harrison 1998).

Detection and chromosomal location of *C. liberica* chromatin introgressed into the *C. arabica* genome

GISH using total genomic DNA of *C. liberica* as probe consistently showed 8 strong hybridization signals on different chromosomes of the introgressed line S.288 (Fig. 2). These hybridization signals were observed at both the pericentromeric region and the telomeric region of the putative introgressed chromosomes. Use of unlabeled blocking DNA from the S.288 line enabled clear differentiation of the *C. liberica*–introgressed fragments among the *C. arabica* background, thanks to the reduction of nonspecific probe hybridization. Most of the disease resistance genes used in coffee breeding have been transferred into the *C. arabica* gene pool via natural or artificial hybridization involving *C. canephora* or *C. liberica* (Berthaud 1978; Bettencourt

Fig. 4. Comparison between the genetic (left) and cytogenetic (center) maps of the chromosome that includes the BAC clone 81-13H (signal in green) and the introgressed fragment carrying the S_H3 factor from *C. liberica* (signal in red). Genetic distances between marker loci are indicated in centiMorgans (cM).
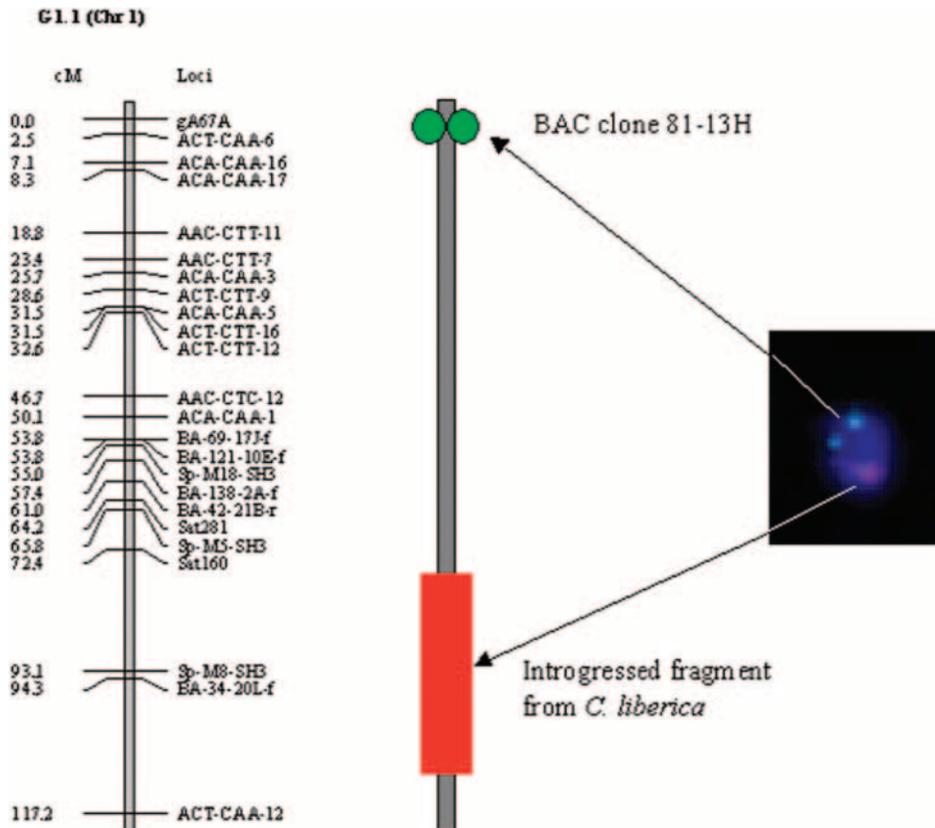
and Rodrigues 1988). Therefore, identification of alien chromatin from these 2 species in introgressed *C. arabica* genotypes would represent a very important tool for breeding strategies in *C. arabica*. Here we demonstrate the potential of GISH analysis to identify alien chromosome segments from *C. liberica* in an introgressed line of *C. arabica*. Unlike the cross hybridization signals observed in GISH analysis of interspecific hybrids between *C. arabica* and *C. canephora*, unambiguous discrimination of *C. liberica* fragments in the S.288 line clearly suggests an important degree of divergence between this species and *C. arabica*. Using a molecular marker approach, Prakash et al. (2004) identified 3 genetically independent *C. liberica* alien fragments in the genome of the introgressed line S.288. These authors estimated that alien introgression represented a half-chromosome equivalent of *C. liberica* distributed over at least 3 chromosomes in the S.288 line. Our results using GISH consistently showed the presence in this line of 8 fluorescent signals corresponding to 4 introgressed fragments distributed on 4 different homoeologous chromosomes (i.e., 2 signals for each homologous set of chromosomes). Therefore, it is conceivable that AFLP marker screening failed to detect one relatively small introgressed fragment present in the S.288 genome. Further, highly repetitive or hyper-methylated regions in chromosomes carrying introgressed fragments would also limit detection of alien chromatin by AFLP analysis. Current development of additional markers surrounding introgressed regions will provide more precise de-

tection of alien chromatin from *C. liberica*. Identification of the homoeologous chromosome group corresponding to linkage group 1 in the coffee genome was carried out using the 81-13H BAC clone as probe for FISH. We observed 4 signals corresponding to the 4 homoeologous chromosomes of linkage group 1 (Figs. 3a–3c). BAC probe signals were often located in the terminal region of hybridized chromosomes, as shown in Fig. 3c. Distal localization of BAC-FISH signals was in agreement with the genetic positions of the gA67A marker in the *C. canephora* map of chromosome 1 according to the linkage map established by Lashermes et al. (2001). It is acknowledged that under optimal hybridization and detection conditions, FISH sensitivity remains dependent principally on the accessibility of the targets and therefore on the extent of chromosome DNA condensation (Hans de Jong et al. 1999). Our results demonstrate for the first time that under the described conditions, coffee mitotic metaphase chromosomes seemed to be appropriate to detect the presence of specific BAC fragments of between 120 and 150 kb. Usually BAC-FISH experiments would result in both primary and secondary sites of hybridization. Primary signals are usually larger and brighter than secondary signals. When secondary signals are detected, it is important to determine whether they are constant and represented in both chromatids, since this would indicate that the signals arose from hybridization based on homology rather than from nonspecific binding of the probe (Kim et al. 2002). In this report

the 4 primary signals were clearly identified in both chromatids on each pair of homologous metaphase chromosomes (Fig. 3c). When weak secondary signals were occasionally detected on one of the sister chromatids, they were interpreted as nonspecific binding of the fluorochrome conjugate. Using a combined GISH/BAC-FISH analysis, chromosomes carrying both the *C. liberica*–introgressed fragments and the BAC clone signals corresponding to chromosome 1 were successfully identified (Fig. 3d). Indeed, 2 BAC signals were observed in 2 of the 8 *C. liberica*–introgressed chromosomes. As expected from genetic data, our results corroborate localization of introgressed fragments carrying the S_H3 resistance factor from *C. liberica* on 2 homologous chromosomes belonging to linkage group 1. Further, the physical position of BAC signals and the *C. liberica*–introgressed fragments was in agreement with the expected localization based on the genetic map of chromosome 1 (Fig. 4). In future studies we will study the exact localization of the S_H3 factor for rust resistance on chromosomes and its relationship with the presence of resistance gene analogue families in coffee.

Conclusions

This report offers a starting point for new applications of FISH techniques in coffee (particularly *C. arabica*) breeding. For instance, our results demonstrated that GISH analysis appears to be a useful tool for detection of alien chromosomes or chromosome fragments in introgressed genotypes derived from diploid species displaying substantial genomic differentiation from *C. arabica*. Future positional mapping of BAC clones carrying interesting genes could be expected by FISH on either mitotic metaphase or meiotic prophase (i.e., pachytene) chromosomes. Detailed morphology of pachytene chromosomes has already been described in *C. arabica* species (Pinto-Maglio and Da Cruz 1998). Choosing pachytene chromosomes as targets in FISH is particularly interesting because of better spatial resolution. Indeed, separation of 2 tightly linked markers flanking a target gene is currently difficult and time-consuming because of the uneven distribution of recombination along the physical length of chromosomes (Hans de Jong et al. 1999). It is known that coffee chromosomes are small and very difficult to identify individually on the basis of morphological differences (Bouharmont 1959). In this sense, FISH analysis using genomic clones involving chromosome-specific markers should provide a useful approach for chromosome identification in coffee. As demonstrated in this study, different chromosome-specific BAC clones can be isolated from libraries and used as specific probes for chromosome identification. Current evidence of similar chromosome architecture and genome structure between coffee and solanaceous species such as tomato or pepper (Lin et al. 2005) will provide new genomic information useful for development of physical and cytogenetic maps for coffee.

Acknowledgements

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