Efficient Use of Coffee Genetic Resources: Molecular Analyses of Genome Interactions in the Arabusta Hybrid (*Coffea arabica* x *C. canephora*)

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INTRODUCTION

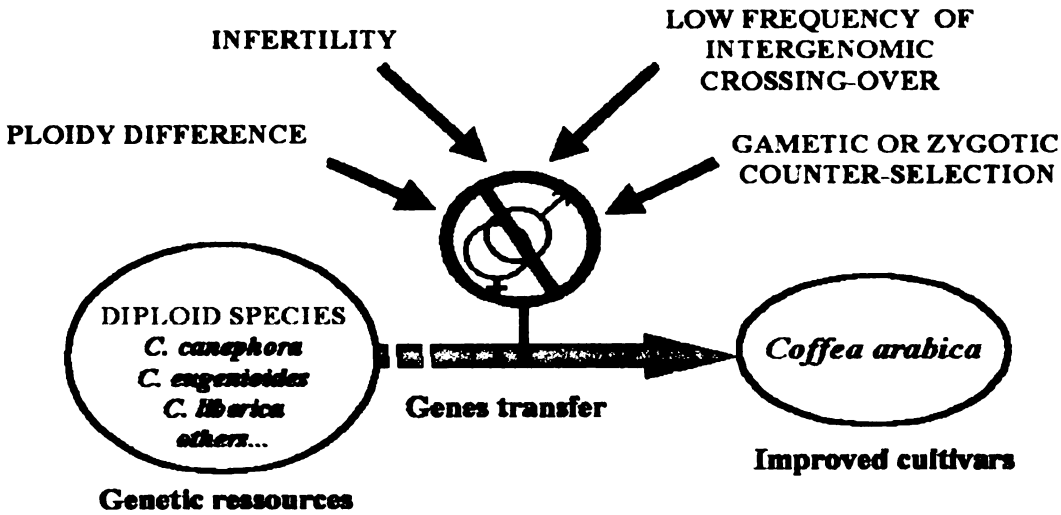
Only a minimum fraction of the genetic ressources has been exploited so far in coffee. Selection of improved cultivars and breeding work have been restricted to the original plant material (Typica and Bourbon, mainly) from which coffee had been stablished, and to occasional new introductions (Carvalho, 1988). Wild relative *Coffea* species constitute the most valuable gene reservoir for breeders, but its utilisation requires more knowledge about inherents problems regarding interspecific hybridization in coffee. Molecular markers are invaluable tools for genome analysis of polyploid species and have resulted in a better understanding of polyploid evolution and genetics (Da Silva and Sorrells, 1996). Particularly, factors affecting genetic exchange between parental genomes in polyploid hybrids could be adressed by marker analysis in segregating populations or from patterns of introgression (Garcia et al., 1995; Parkin and Lydiate, 1997). Recent molecular analyses in coffee showed that particular chromosomes of *C. arabica* only pair homogenetically in spite of the minor differentiation among its two constitutive genomes. Accordingly, a diploid-like segregation has been observed. Unlike *C. arabica*, in the interspecific tetraploid hybrid arabusta (*C. arabica x C. canephora* 4x) the four sets of chromosomes not display any preferential pairing (Lashermes et al., 1999; Lashermes et al., 2000). Actually we are interested to study the behaviour of the *C. canephora* genome and its interaction with the *C. arabica* genome in the context of the tetraploid arabusta hybrid. Therefore, the purpose of this study was to analyse the allele segregation and chromosome recombination in a population of BC_1 individuals derived from tetraploid arabusta hybrids. The results are discussed in relation to the mechanism of introgression into *C. arabica* and the efficient use of genetic ressources in arabica breeding.

MATERIAL AND METHODS

Plant material

Plant material consisted of two BC_1 populations resulting from the backcross of two interspecific arabusta tetraploid F_1 plants (Et 30 x IF 181T) to *C. arabica* (accession Et30). The tetraploid plant of *C. canephora* (IF 181T) was previously obtained following colchicine treatment of the clone IF 181 (Figure 1).

Possible reproductive barriers affecting gene exchange between the diploid species and *C. arabica*



Molecular marker assay and data scoring

Segregation and cosegregation of both restriction fragments (RFLP) and microsatellite polymorphic markers were studied in the BC_1 populations. Part of the analysed RFLP and microsatellite loci have been previously mapped in *C. canephora* and are distributed on 7 of the 11 identified linkage groups (Lashermes et al., 2001). Restriction fragments (i.e. RFLP locus) as well as PCR-amplified products (i.e. microsatellite locus) of different sizes were identified and easily interpreted as either canephora or arabica specific markers by comparing the parental accessions. When two different canephora specific markers were identified in the arabusta hybrid, the markers (i.e. RFLP or microsatellites) were interpreted as alleles of the considered locus and designed arbitrary by the letters C_1 and C_2 (Figure 2). Allelic interpretation of microsatellite loci was undertaken only when two different canephora specific markers were presents in the arabusta hybrids. In contrast, for RFLP loci, variations in banding intensity within the same lane were considered to represent differences in allele copy number and were designed by the letter C in single or double dose. Statistical analysis compared observed vs expected segregation frequencies of canephora alleles assuming random chromosome segregation in the hybrid.

RESULTS AND DISCUSSION

Analysing segregation patterns of 24 polymorphic loci (11 RFLP and 13 microsatellites) we scored for the presence of the specific canephora markers in the BC1 plants. Comparison between the two BC1 populations (i.e. P1 and P2) showed almost equal frequencies of plants with canephora markers. Overall analyses including both populations revealed that proportion of plants with canephora markers were consistent ($p<0.05$) with the expected proportion (i.e. 0.83) assuming random chromosome segregation.

On the other hand, and for almost all loci analysed, segregation of canephora alleles transmitted by the arabusta hybrids conformed to the expected ratio (i.e. 0.25) assuming random chromosome segregation and the absence of selection (Figure 3). Recombination fractions were analysed for seven marker intervals on four different linkage groups. Only two of the seven intervals analysed, exhibited a significant difference in recombination frequency

(Table 1). Although local differences in recombination may exist, these results suggest that overall recombination in the arabusta hybrid is not significantly restricted by genetic differentiation between chromosomes belonging to the different constitutive genomes (i.e. *C. arabica* and *C. canephora* genomes).

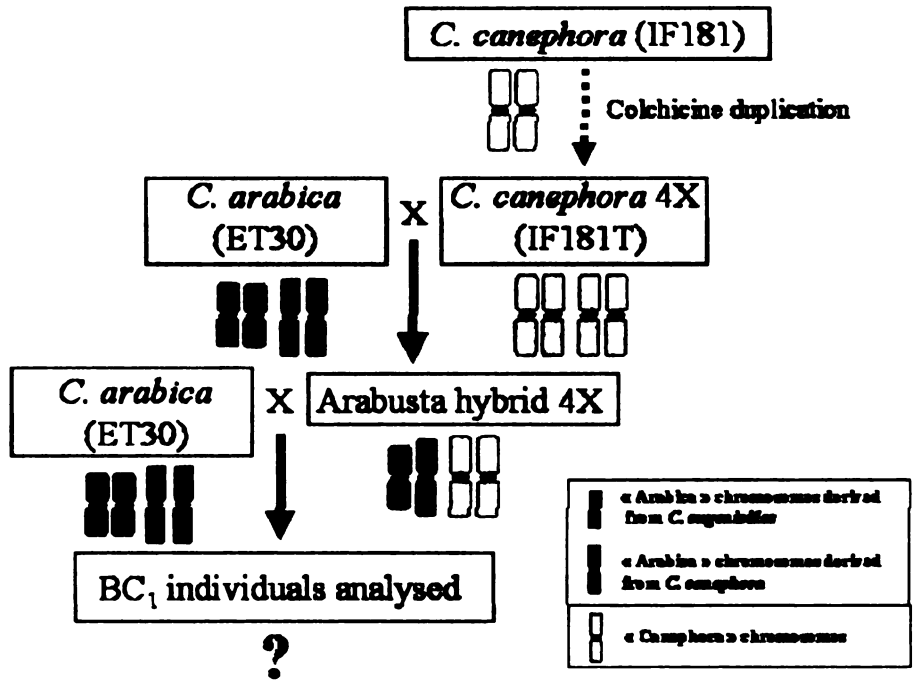


Figure 1. Origin of the surveyed populations BC_1

CONCLUSIONS

Enlarging the genetic base and improvement of arabica cultivars have become a priority for coffee breeders. Likewise, understanding of introgression mechanism could provide new perspectives to develop suitable strategies of field selection. In this report we present new evidence about the particulary favorable disposition of the arabusta hybrid (*C. arabica* x *C. canephora* 4x) to the introgression. Our results suggest that gene transfer from *C. canephora* (and probably from other diploid related species) to the cultivated *C. arabica*, should not be limited by differences either in sequence homology or in chromosome structure.

These preliminar results provides an optimistic view on major utilisation of coffee genetic ressources in coffee breeding programs. Although further investigations about genome interactions between *C. arabica* and others diploid related species are needed, monitoring of gene introgression using molecular tools, represent a first step towards real implementation of marker-assited selection in coffee.

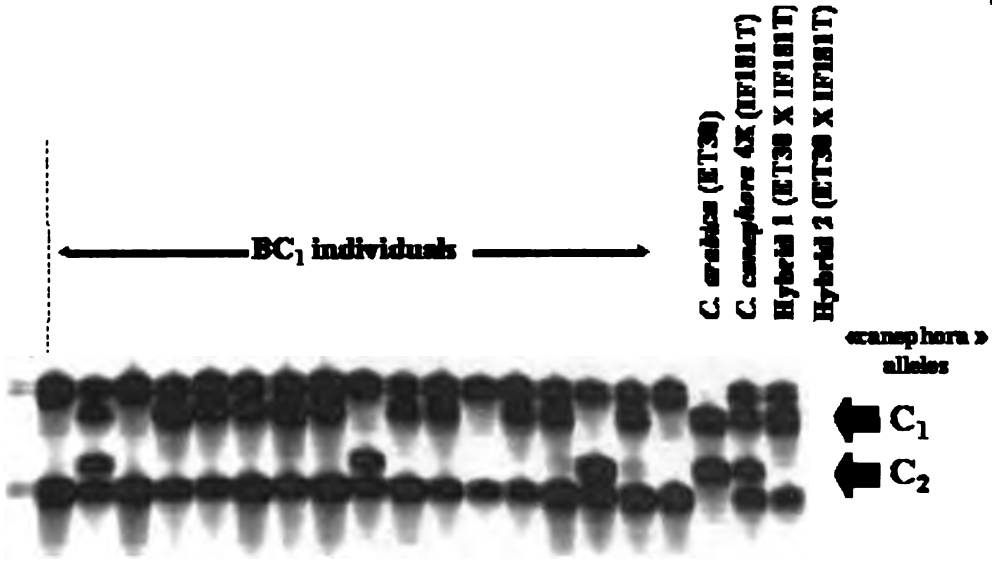
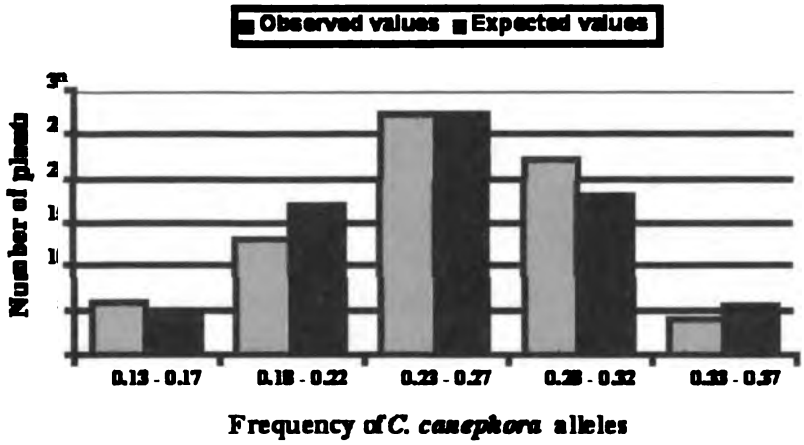


Figure 2. DNA marker analysis of introgression: Example of a microsatellite locus showing "canephora" allele segregation among BC_1 individuals resulting from the backcross of arabusta hybrids to *C. arabica*



Note: Expected values were calculed from a theoretical binomial distribution assuming random segregation at all loci.

Figure 3. Frequency distribution of *C. canephora* alleles: Histograms of the number of BC_1 individuals in which particular frequency of *C. Canephora* alleles were detected

Table 1. Genome recombination: Comparison for different chromosome segments of the recombination frequencies estimated in the arabusta hybrid an in *C. canephora*

Linkage group of the canephora map	Marker Intervals	Recombination fractions	
		Arabusta	*C. canephora*
3	M41-gA71	0.24	0.31
	gA71-M157	0.37	0.32
	M157-M42	0.13 *	0.04
	M41-M42	0.27	0.40
4	M47-cR167	0.10	0.07
7	gA72-gA61	0.37 **	0.20
9	gA1-gA19	0.35	0.49

*, ** indicate statistical significant differences in the proportion of parental and recombinant gametes at $P < 0.05$ and $P < 0.01$, respectively, as indicated by the chi-square test.

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