

Regular Article

Maternal inheritance of chloroplast DNA in *Coffea arabica* hybrids

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Intergenic spacers of chloroplast DNA (cpDNA) are very useful in phylogenetic and population genetic studies of plant species. Their integration in phylogenetic analysis leads to a deep understanding of species relationships. Mode of chloroplast DNA inheritance was investigated in the genus *Coffea arabica* (Rubiaceae) by polymerase chain reaction (PCR) amplification of cpDNA fragments using universal primer pair *rrn23* - *trnR* (ACG) region of chloroplast genome. A total of 30 F₁ plants from five different *C. arabica* parent varieties were examined. Two crosses involving *C. arabica* var. Sln.10 and *C. arabica* var. Devamachy, and *C. arabica* var.S.881 and another cross involving *C. arabica* var. Agaro with *C. arabica* var. Tafari-kela were analyzed. Length polymorphism was observed in all three hybrids at *rrn23* - *trnR* (ACG) region of cpDNA. In each case, it was the maternal cpDNA marker that appeared in the F₁ individuals. Further it was observed that the length polymorphism observed within parental plants led to the identification of five different banding patterns at *rrn23* - *trnR* (ACG) region of coffee cpDNA. The possible reasons for the observed differences are discussed.

Keywords: Rubiaceae, *Coffea arabica*, cpDNA, Maternal inheritance, Hybrid analysis.

Coffee plants belong to the genus *Coffea*, of the family Rubiaceae and more than 100 species are presently reported under the subgenus *Coffea*. All species are woody, ranging from small shrubs to robust trees and most of them originate in the inter-tropical forests of Africa and Madagascar. *Coffea arabica* L. (2n=4x=44) and *Coffea canephora* Pierre (2n=2x=22) are the only cultivated species of economic importance. *C. arabica* is a natural allo-tetraploid and is self-fertile, while other species are diploid and generally

self-incompatible (Charrier and Berthaud 1985).

Chloroplast genes have been extensively used to reconstruct the phylogeny of related species. Because of the low frequency of structural changes in the chloroplast DNA molecule (cpDNA) and low rates of sequence evolution, the plastome (plastid genome) is considered a useful tool for plant phylogenetic studies, especially above the species level (Chiang and Schaal, 2000; Ingvarsson et al., 2003; Neves et al., 2005). In order to gain more information for

phylogenetic reconstruction many chloroplast regions including genes and intergenic spacers have been analyzed. The sequences of the chloroplast *rbcL* gene (coding the large subunit of Rubisco) as well as a few other genes like *matK*, *ndhF*, *psaB*, *rbcL-accD* and *trnL-trnF* have been widely used for inferring phylogeny in plants (Chiang and Schaal., 2000; Oxelman et al., 1999; Soltis et al., 2000; Miz et al., 2008; Yuji et al., 2005). Supposed to evolve more rapidly than coding regions, chloroplast non-coding sequences such as the inter-genic spacers between the *trnL* (UUA) 3' exon and the *trnF* (GAA) gene (Neves et al., 2005; Miz et al., 2008; Cros et al., 1998), the *atpB-rbcL* spacer (Chiang and Schaal., 2000; Soltis et al., 2000), *trnC-trnD* spacer (Lee and Wen, 2004) and *trnE-trnT* spacer (Danila et al., 2008) have been used to address questions concerning relationships among related species and related genera. Clarkson et al. (2004) reported the phylogenetic relationships in *Nicotiana* (Solanaceae) inferred from the plastid DNA regions *trnL* intron, *trnL-F* spacer, *trnS-G* spacer and two genes, *ndhF* and *matK*. In some cases the phylogenetic information contained in just one chloroplast genetic marker does not have enough resolving power to separate closely related taxa, especially at lower taxonomic levels. Increase in the number of characters usually improves the resolution of phylogenetic analysis. Since the chloroplast genome does not usually undergo systematic recombination, all characters in different chloroplast markers can be pooled in a single analysis as belonging to the same haplotype.

For using cpDNA as an evolutionary marker in the genus *Coffea*, some of the features such as the mode of inheritance and the importance of intra-specific variation should be understood (Lashermes et al., 1996). Maternal inheritance is the commonly observed mode of plastid inheritance in angiosperms although there is evidence for

the inheritance of paternal plastids in some species (Hageman and Schröder, 1989; Reboud and Zeyl, 1994). Plastid maternal inheritance in *C.arabica* was suggested on the basis of cytological evidence from epifluorescence microscopy (Corriveau and Coleman, 1988). Restriction analysis of cpDNA, (Berthou et al., 1983, Lashermes et al., 1995, 1996) also indicated maternal inheritance in an interspecific hybrid between *C. canephora* and *C. arabica*. The complete chloroplast genome size of *Coffea arabica* is reported to be about 155189 bp, which comprises of LSC (large single copy) and SSC (small single copy) regions separated by IRA and IRB inverted repeat (IR) regions comprising 25943 bp each (Samson et al, 2007). The present study aims at understanding the inheritance pattern of *rrn23-trnR* (ACG) region of the coffee chloroplast DNA. This region comprises of partial 23S rRNA, full length 4.5S rRNA, 5S rRNA and tRNA coding for the amino acid Arginine *trnR*(ACG). The three intergenic spacers between these genes are variable and can be effective markers to study chloroplast inheritance.

Materials and Methods

Plant material

Thirty inter-varietal arabica F₁ hybrids derived from three crosses and their respective parents constitute the material for the present study. These materials were grown in the experimental plots of Central Coffee Research Institute, India. The detail of the plant material and their parentage is given in Table 1.

DNA extraction

Genomic DNA was extracted from fresh young leaves using a modified CTAB method (Murray and Thomson, 1980). About 200 mg of leaf tissue was ground to fine powder in liquid nitrogen, and then transferred to 5 mL extraction buffer (2%

CTAB, 100mM Tris HCl (pH 8.0), 25mM EDTA, 2M NaCl, 0.1% beta-Mercaptoethanol). The suspension was incubated at 60°C for one hour with occasional shaking, cooled down to room temperature and centrifuged at 6000 rpm for 20min. The supernatant was transferred into a new tube and extracted twice with an equal volume of chloroform-isoamyl alcohol (24:1) followed by precipitation with 0.7 vol. of isopropanol at room temperature for 30min and centrifugation at 8000 rpm for 20min at

4°C. The pellet formed was washed with 75% (v/v) ethanol for 10min and resuspended in TE buffer. The concentration of DNA was measured using 0.8% w/v agarose gel stained with ethidium bromide and quantified by UV spectrophotometry at 260nm and 280nm. Then purity of DNA was determined by calculating the ratio of absorbance at 260/280nm. For PCR templates, DNA were diluted to 10ng/ μ L and stored at -20°C until use.

Table1. Parents and hybrid combinations analyzed by using rrn23 - trnR (ACG) primer

Parents and hybrid codes	Cultivars / Hybrids	Detailed description on the origin of parents and hybrids
P1	Sln.10	A double cross hybrid derived (Caturra x Cioccie) x (Caturra x S.795) released as Selection.10 by CCRI
P2	Devamachy	Putative robusta-arabica hybrid from India
P3	S.881	Exotic coffee variety from Rume Sudan
P4	Agaro	Exotic coffee variety of Ethiopian origin released as Selection - 4 by CCRI
P5	Tafarikela	Exotic coffee variety of Ethiopian origin
H1 (P1 X P3)	Sln.10 x S.881	New F ₁ hybrid under evaluation
H2 (P2 X P3)	Devamachy x S.881	Commercial cultivar developed at CCRI and released as Sln.5A
H3 (P4 X P5)	Agaro x Tafarikela	Inter-varietal hybrid
T1	<i>C. arabica</i> (S.881/29)	A Rume Sudan Collection received during 1942
T2	<i>C. arabica</i> (S.882)	A wild collection from Barbak plateau of Anglo Egyptian Sudan
T3	<i>C. arabica</i> (S.881/26)	A Rume Sudan Collection received during 1942
T4	<i>C. arabica</i> (S.1662)	An world collection received during 1954
T5	<i>C. arabica</i> (S.1495)	A collection received from USDA, USA during 1953

PCR amplification

Amplifications were performed in 25 μ L reactions containing 2.5 μ L of 10x KCl buffer, 2.0 μ L of 25mM MgCl₂, 2.5 μ L of 2mM dNTPs, 0.5 μ L each of forward (F) and reverse (R) primer (F: 5'-ctg ctg aaa gca tct aag tag taa gc-3' and R: 5'-ggg tgt ggg cga gga ggg att cga ac-3'), 10ng DNA template and 1 Unit of Taq DNA polymerase enzyme in an Eppendorff

master cycler gradient PCR machine. The following touchdown PCR conditions with an initial denaturation at 94°C for 4 minutes followed by 10 cycles of denaturation, annealing and extension at 94°C, 55°C-0.5°C/cycle and 72°C for 40 seconds respectively. A cycle containing 25 repeats with 94°C, 50°C and 72°C for 40 seconds each for denaturation, annealing and extension

respectively preceded the final extension at 72°C for 8 minutes (Dhingra and Folta, 2005). The amplification products were analyzed by electrophoresis on a 2% (w/v) agarose gel using 1x TAE running buffer and detected by staining with ethidium bromide. The gel profiles were viewed under UV-transilluminator (SYNGENE) and documented using the Gene Snap software.

Results and Discussion

The *rrn23 - trnR* (ACG) region of coffee chloroplast was selected to study these hybrids because this region of chloroplast DNA was duplicated in the inverted repeat and shown to possess enough polymorphism between parents without restriction digestion analysis, whereas earlier reports on the chloroplast DNA and its regions were based

only on using restriction digestion of chloroplast DNA (Berthou et al, 1983, Lashermes et al, 1995, 1996; Cros et al., 1998). It was found all the three hybrid progenies analyzed are showing maternal inheritance of cpDNA. These results are depicted in Figure.1. In the present study it was also observed that the *rrn23 - trnR* (ACG) region is exhibiting length polymorphisms among the parental samples analyzed. Therefore, the study was extended to different *C. arabica* collections and *Coffea* species (data not presented here) to know the variation of this region across the genus. Finally five different patterns were found in this region among the samples screened. Figure.2 shows the length polymorphic differences observed in the present study.

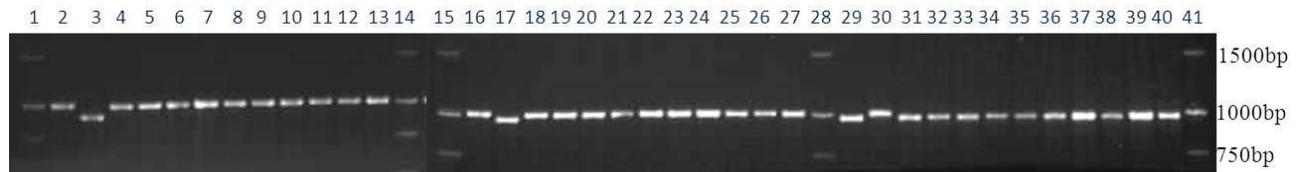


Figure 1: Maternal Inheritance pattern of *rrn23 - trnR* (ACG) region in hybrids. 1, 14, 15, 28, 41 - 1kb DNA ladder; 2 - Devamachy (P2); 3, 17 - S.881(P3); 4-13 - Devamachy X S.881 (H2); 16 - Sln.10 (P1); 18-27 - Sln.10 X S.881(H1); 29 - Agaro(P4); 30 - Tafarikela(P5); 31-40 - Agaro X Tafarikela(H3).

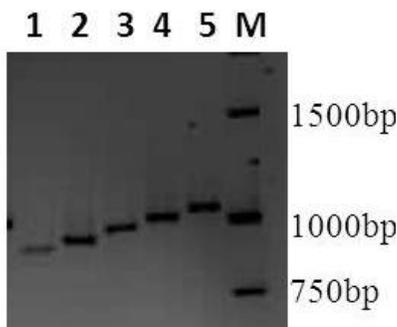


Figure 2: Five different length polymorphic bands observed at *rrn23 - trnR* (ACG) region. 1-*C. arabica* (S.881/29-T1), 2-*C. arabica* (S.882-T2), 3-*C. arabica* (S.881/26-T3), 4-*C. arabica* (S.1662)-T4, 5-*C. arabica* (S.1495)-T5, M - 1kb ladder.

Maternal inheritance of chloroplast DNA was deduced from a comparison of nine species of *Coffea* at an early stage in the development of coffee genomics (Berthou et al., 1983). Since then there is considerable research on this aspect that contributed significantly to an understanding of the inheritance and phylogenetic relationships among the many species of *Coffea* (Lashermes et al., 1995; Cros et al., 1998). Even though the present study is based on a single primer combination, it draws support from all the earlier reports and strongly emphasizes the maternal inheritance of chloroplast DNA in coffee. Present study also indicates the

usefulness of *rrn23* - *trnR* (ACG) region and the single primer pair combination in analyzing the chloroplast heredity of hybrids derived from parents of diverse origins. The primer pair used in this study can be efficiently utilized to study the maternal inheritance of chloroplast DNA in coffee hybrids without involving restriction digestions.

The polymorphisms observed in this region have parallels in other species of *Coffea* (Cros et al., 1993, 1998; Lashermes et al., 1996) as well as several related genera of Solanaceae as well as Rutaceae and may be due to indels that form in the course of evolution of the genotype (Samson et al., 2007; Bausher et al., 2006). Also, this region was reported to be having two direct repeats of 64 bp between Inter-genic spacer (IGS) at *rrn5*-*trnR* (ACG) and a 32 bp repeat at IGS between *rrn4.5*-*rrn5* that appear to have a role in rearrangement of genes (Samson et al., 2007). Repeat regions are generally more variable and that also can be one of the possible reasons for the observed differences in band sizes. From the foregoing discussion, it can be suggested that the study of *rrn23* - *trnR* (ACG) region offers the potential to gain further phylogenetic insights in the case of genus *Coffea*.

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