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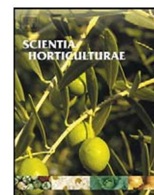

Cyto-nuclear discordance in the genetic relationships among Tunisian fig cultivars (*Ficus carica* L.): Evidence from non coding *trn*L–*trn*F and ITS regions of chloroplast and ribosomal DNAs

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ABSTRACT

Genetic diversity studies were conducted to evaluate intra-specific relationships in Tunisian fig *(Ficus*: *Moraceae*) using sequences of the ITS regions of nuclear ribosomal DNA and the chloroplast non-coding region *trn*L–*trn*F (*trn*L intron, and *trn*L [UAA]–exon–*trn*F [GAA] intergenic spacer). All data sets suggest that the sequences obtained showed variations either on their lengths or on their nucleotide compositions. The mean size of these nuclear and cytoplasmic non-coding regions is 697.5 and 1035 base pairs for ITS and *trn*L–*trn*F chloroplast DNA, respectively. Our results suggest that the substitution rate estimated for the ITS sequences of nuclear DNA is greater than the one unregistered for the non-coding regions of chloroplast DNA. Therefore, chloroplast DNA shows more homoplasy than the nuclear ribosomal DNA. In fact, the consistency (CI) and retention (RI) indexes calculated for nuclear and cytoplasmic DNAs show values of 0.420 and 0.490 for chloroplast DNA and 0.573 and 0.387 for ribosomal DNA, respectively. The nuclear DNA has more resolution of genetic relationships between cultivars. In addition, the result suggests that the cultivars studied are clustered independently from their geographical origin and the male trees did not thoroughly diverge from the common figs. The discordance between chloroplast and the nuclear topology is revealed. The lack of congruence between the two data sets may be a result of hybridization or introgression. Our result showed that the nuclear and cytoplasmic sequences are useful for germplasm discrimination as well as for investigation of patterns of variation in fig.

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1. Introduction

Ficus (Moraceae) constitute one of the largest genera of angiosperms (Frodin, 2004), with almost 800 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers occurring in the tropics and subtropics worldwide. All members of the genus share the distinctive fig inflorescence (syconium), which is the site of an extreme mutualism with pollinating fig wasps of the family Agaonidae (Cook and Rasplus, 2003). *Ficus* are important genetic resources with high economic and nutritional value. They are also an important part of the biodiversity in the rainforest ecosystem by setting fruit throughout the year and providing an important source of food for fruit-eating animals in the tropics. In Tunisia, fig (*Ficus carica* L.) was cultivated in several restricted areas extending from the North to the South of the country and was adapted to diverse climatic levels. An important variability among Tunisian fig landraces is revealed on the basis of morphological characterization. Fig material enclosed traditional varieties propagated by clonal propagation throughout cuttings are located in several regions of the country. However, diverse abiotic and biotic stresses like urbanization, rainfall irregularities, and plagues are currently threatening the germplasm resources. As a consequence, a lack of landraces has occurred during the recent decades and constituted a constraint in the improvement of the fig cultivation (Mars and Marrakchi, 1998). Moreover, the precise number of cultivars is still unknown since problems of mislabelling (homonymy and synonymy) are often detected. In order to preserve the local genetic resources, several studies focused on characterization and genetic diversity study. The Tunisian fig accessions were assessed using morphometric and pomological parameters as well as isozyme markers to discriminate fig cultivars (Mars and Marrakchi, 1998; Hedfi et al., 2003; Chatti et al., 2004a). Unfortunately, these parameters are highly influenced by environmental conditions and the stage of tissue development. To overcome these difficulties, molecular markers were successfully used. Among these, Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR),

Abbreviations: ITS, internal transcribed spacer; DNA, deoxyribonucleic acid; CI, consistency index; RI, retention index; MCL, maximum composite likelihood; CpDNA, chloroplast DNA; NJ, neighbor-joining; MPTs, maximum parsimony trees; bt, bootstrap.

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Random Amplified Microsatellite Polymorphism (RAMPO), Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP) have been applied on fig, and have been proven to be suitable to survey molecular polymorphism and to examine relationships among fig cultivars (Salhi Hannachi et al., 2004, 2005, 2006; Chatti et al., 2004b, 2007; Saddoud et al., 2007).

The objectives of the present study are to (1) assess the genetic relationships among *F. carica* cultivars using the chloroplast *trn*L–*trn*F non-coding region (*trn*L intron, and *trn*L [UAA]–exon–*trn*F [GAA] intergenic spacer) and the ribosomal internal transcribed spacer (ITS); (2) compare informations provided by the cpDNA to those given by nuclear DNA; also, a comparison of genetic inferences based on chloroplast and nuclear markers may provide important insights into relationships and patterns of evolution within *F. carica*. In fact, at lower taxonomic levels (genus and below) such comparisons have revealed high levels of concordance in several studies as suggested by Baldwin (1992), Kim and Jansen (1994), Bayer et al. (1996, 2000), and Choi and Wen (2000). It is important to note that a significant discordance has been found by authors Soltis and Kuzoff (1995) and Soltis et al. (1996). Such discordance may suggest hybridization or introgression (Soltis and Kuzoff, 1995; Renoult et al., 2009).

Angiosperm chloroplast DNA studies have focused mainly on substitution patterns in genes such as *rbc*L (Albert et al., 1994; Kellogg and Juliano, 1997; Manen et al., 1999), *ndh*F (Catalan et al., 1997), and *mat*K (Hilu and Liang, 1997) and have outnumbered studies on non-coding chloroplast regions such as the *trn*L (UAA) 5′ exon *trn*F (GAA) exon region. Yet, this so-called “*trn*L–F” region is being used increasingly for species level phylogenetic reconstruction (Compton et al., 1998; Bakker et al., 1999; Bayer and Starr, 1999; McDade and Moody, 1999; Wikström et al., 1999), making a better understanding of its substitution pattern desirable. The ITS regions are more variable than most other nuclear or cytoplasmic DNA sequences (White et al., 1990; O’Donnell, 1992; Fujiwara et al., 1994; Schlöttere et al., 1994; Chen et al., 1996). Also, the 18S and 28S rDNA sequences which flank the ITS region are highly conserved and can be used to design primers that are specific to a range of taxa. Therefore, analysis of sequence variation in the ITS region is widely used in population and systematic studies of a variety of organisms.

2. Materials and methods

2.1. Plant accessions

A total of 49 accessions of Tunisian *F. carica* cultivars were considered in this study. These consist of 41 domestic trees and 8 male trees or ‘Dhokkars’ collected from five regions: Sahel, South West, South East, North East and Kerkenah Islands (Table 1). The genetic diversity of ITS sequences was performed in only 31 trees represented by 25 female and 6 fig pollinators (Table 2).

Table 1
Tunisian fig cultivars studied and their geographical origin.

Cultivar	Horticultural classifications	Geographic origin	
		Region	Locality
Soltani 1	*Uniferous*	Sahel	Ourdanine
Kahli 1	*Uniferous*		Kalaa Kebira
Hemri 1	*Uniferous*		Enfidha
Zidi 1	*Uniferous*		Mesjed Aissa
Baghali	*Uniferous*		Mesjed Aissa
Bidhi	*Uniferous*		Kalaa Kebira
Bither abiadh 1	*Bifèrous*		Mesjed Aissa
Besbessi	*Bifèrous*		Mesjed Aissa
Jrani*	*Uniferous*		Ghadhabna
Assafri*	*Uniferous*		Ghadhabna
Dchiche Assal	*Uniferous*		Ghadhabna
Kahli 2	*Uniferous*		Enfidha
Zidi 4	*Uniferous*		Ghadhabna
Dhokkar 1*	*Uniferous*	South West	Gafsa
Zidi 3	*Uniferous*		Tozeur
Hamri	*Uniferous*		Dégache
Khadhri	*Uniferous*		Dégache
Khartoumi	*Uniferous*		Dégache
Tounsi	*Uniferous*		Dégache
Wahchi	*Bifèrous*		Dégache
Chetoui 1	*Bifèrous*		Dégache
Dhokkar 2*	*Uniferous*		Dégache
Sawoudi 1	*Uniferous*		Gafsa
Gaa Zir	*Uniferous*		Gafsa
Assal boudchiche	*Uniferous*		Gafsa
Khadhouri	*Uniferous*		Gafsa
Sawoudi 2	*Uniferous*		Dégache
Grichy	*Uniferous*		Tozeur
Khalt	*Uniferous*		Tozeur
Khzami	*Uniferous*		Tozeur
Bither	*Bifèrous*		Tozeur
Hammouri	*Uniferous*	South East	Medenine
Widlani	*Uniferous*		Medenine
Zaghoubi	*Uniferous*		Medenine
Rogabi	*Uniferous*		Medenine
Makhbech	*Uniferous*		Medenine
Dhokkar Zarziz*	*Uniferous*		Medenine
Bither abiadh 2	*Uniferous*		Tataouine
Tayouri Assfar	*Uniferous*		Douiret
Zidi 2	*Uniferous*	North East	Utique
Dhokkar 4*	*Uniferous*		Utique
Dhokkar 5*	*Uniferous*		Raf Raf
Soltani 3	*Uniferous*		Raf Raf
Chetoui 2	*Bifèrous*		Raf Raf
Soltani 2	*Uniferous*		Mornag
Temri	*Uniferous*	Kerkenah	Kerkenah
Baghli	*Uniferous*		Kerkenah
Abiadh	*Uniferous*		Kerkenah
Dhokkar 3*	*Uniferous*		Kerkenah

* Male tree.

2.2. DNA extraction, PCR amplification, and sequencing

Total DNAs were extracted from fresh leaves according to Dellaporta et al. (1983) procedure. The DNA concentration was spectrophotometrically estimated and their integrity was checked by analytic agarose minigel electrophoresis as described by Sambrook et al. (1989).

ITS region was amplified as reported by Weiblen (2000) and the composition of PCR reaction was used as mentioned by Baraket et al. (2009a). The *trn*L–*trn*F region of chloroplast DNA was amplified using the appropriate primers designed by Taberlet et al. (1991). Conditions for PCR amplification were demonstrated by Baraket et al. (2009c). PCR products were cheeked by agarose gel electrophoresis (1.5%). Excess of primers and dNTPs after amplification were removed by purification using the Wizard SV Gel PCR Clean-Up System kit according to the manufacturer’s instructions (Promega, WI, USA). PCR products were directly sequenced in both the strands, by the automated fluorescent cycle sequencing method using the Big Dye Terminator Ready Reaction Kit according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). The primers used for sequencing were those used for PCR reactions.

2.3. Sequence analysis

The identity of *trn*L–*trn*F region and ITS sequences was confirmed through a BLASTN search in NCBI data base (Altschul et al., 1997). Nucleotide sequences were aligned using the Clustal W to the DAMBE program (Xia, 2000). The alignment was corrected man-

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