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# Genetic diversity and molecular evolution of the internal transcribed spacer (ITSs) of nuclear ribosomal DNA in the Tunisian fig cultivars (*Ficus carica* L.; Moracea)

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ABSTRACT

Nuclear ribosomal DNAs were explored to establish genetic relationships among *Ficus carica* cultivars and elucidate its molecular evolution. Results suggest the occurrence of haplotypic and nucleotide diversity. The neighbour-joining dendrograms show a continuous diversity that characterize local resources. Furthermore, our results demonstrated that the ITS2 spacer is seating to a larger number of substitutions than the ITS1 spacer. Sequence analysis demonstrates that the ITS2 spacer is evolving 1.12 times faster than the ITS1 one. The ratio of transition/transversion of 0.278 suggests that the 5.8S gene is evolving 2.84 and 3.20 times less rapidly than the spacers ITS1 and ITS2, respectively. Molecular evolution analysis confirmed an explicit rejection of the null hypothesis in *F. carica*. ITS1, ITS2 spacers and the 5.8S gene evolved under a strictly neutral model of molecular evolution. A scenario of positive selection and recent expansion of *F. carica* genotypes across Tunisia seems to be retained.



## 1. Introduction

The fig, *Ficus carica* L., (Moraceae) is a traditional fruit tree of olden times associated with the beginning of horticulture in the Mediterranean basin (Zohary and Spiegel-Roy, 1975). It is known to have been domesticated from a group of different spontaneous figs found in the South and East of the Mediterranean region sometime in the Early Neolithic period (Zohary and Hopf, 1993). The cultivated fig is gynodioecious, but is functionally dioecious, with pollination facilitated by the mutuality between pollinator wasps (*Blastophaga psenes* L.) and the two different fig types, Caprifig and edible fig (Kjellberg et al., 1987).

In Tunisia, the fig germplasm consists of numerous landraces mainly selected by farmers for their fruit qualities and maintained in orchards. They are widely spread through different eco-geographical areas of the country and are threatened by genetic erosion due to biotic and abiotic stresses. In recent years, several works have focused on the identification and characterization of Tunisian fig cultivars, to elaborate a national core collection and to preserve these genetic resources (Chatti et al., 2007; Salhi-Hannachi et al., 2005; Saddoud et al., 2007; Baraket et al., 2010).

This paper aims to explore the diversity encountered in 31 traditional fig cultivars and establish a molecular evolution history using nuclear ribosomal DNA. Eukaryotic nuclear ribosomal DNA (rDNA) has two internal transcribed spacers ITS1 and

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ITS2. The two spacers and the 5.8S subunit are collectively known as the internal transcribed spacer (ITS) region and have become an important nuclear locus for molecular systematic investigations of flowering plants (Baldwin et al., 1995). One of the remarkable properties of nrDNA (including ITS) genes is that their paralogs within individuals are quite homogenous, resulting from concerted evolution. The popularity of the ITS region can be attributed to the relatively high rate of nucleotide substitution in the transcribed spacers, permitting the systematic comparison of relatively recently diverged taxa.

## 2. Material and methods

### 2.1. Plant material

Thirty one cultivars (25 female and 6 male trees) of Tunisian fig (Table 1) were used in this study. These were collected from 5 regions. Plant material consisted of young leaves sampled from adult trees.

### 2.2. DNA isolation

Total genomic DNA was purified from frozen young leaves according to the procedure of Dellaporta et al. (1984). The DNA concentration was estimated spectrophotometrically and its integrity was checked by analytical [1% (w/v)] agarose minigel electrophoresis (Sambrook et al., 1989).

### 2.3. PCR amplification, purification and sequencing

The entire ITS region was amplified using external 'ITS4' and 'ITS5' primers designed by White et al. (1990). The procedure followed is that previously described in Baraket et al. (2009a). Cycle Sequencing and the Big Dye Terminator Ready Reaction Kit (Applied Biosystems Foster City, CA, USA) were used.

### 2.4. Sequence analysis

All sequence information has been deposited in the GenBank database (accession nos. GQ395445–GQ395467 and EF579611–EF579622). The derived ITS nucleotide sequences were aligned using the DAMBE program (Xia, 2000) and

**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 |
| Zidi 1 | *Uniferous* | | Mesjed Aissa |
| Baghali | *Uniferous* | | Mesjed Aissa |
| Bidhi | *Uniferous* | | Kalaa Kebira |
| Besbessi | *Bifêrous* | | Mesjed Aissa |
| Jrani* | *Uniferous* | | Ghadhabna |
| Assafri* | *Uniferous* | | Ghadhabna |
| Zidi 3 | *Uniferous* | South Ouest | Tozeur |
| Hamri | *Uniferous* | | Dégache |
| Khadhri | *Uniferous* | | Dégache |
| Khartoumi | *Uniferous* | | Dégache |
| Tounsi | *Uniferous* | | Dégache |
| Wahchi | *Bifêrous* | | Dégache |
| Chetoui 1 | *Bifêrous* | | Dégache |
| Sawoudi 1 | *Uniferous* | | Gafsa |
| khadhouri | *Uniferous* | | Gafsa |
| Hammouri | *Uniferous* | South East | Medenine |
| Widlani | *Uniferous* | | Medenine |
| Zaghoubi | *Uniferous* | | Medenine |
| Makhbech | *Uniferous* | | Medenine |
| Dhokkar Zarziz* | *Uniferous* | | Medenine |
| Bither abiadh 2 | *Uniferous* | | Tataouine |
| Zidi 2 | *Uniferous* | North East | Utique |
| Dhokkar 4* | *Uniferous* | | Utique |
| Dhokkar 5* | *Uniferous* | | Raf Raf |
| Soltani 3 | *Uniferous* | | Raf Raf |
| Chetoui 2 | *Bifêrous* | | Raf Raf |
| Soltani 2 | *Uniferous* | | Mornag |
| Baghli | *Uniferous* | Kerkenah | Kerkenah |
| Dhokkar 3* | *Uniferous* | | Kerkenah |