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Genetic analysis of Tunisian fig (*Ficus carica* L.) cultivars using amplified fragment length polymorphism (AFLP) markers

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ABSTRACT

Genetic diversity of forty fig cultivars collected from five regions in Tunisia was investigated using amplified fragment length polymorphism (AFLP). A total of 342 reproducible bands amplified with six AFLP primer combinations were obtained. The high percentage of polymorphic bands (%PB) of 97.5 and the resolving power (Rp) collective rate value of 143 were scored. In addition, the polymorphism information content (PIC) values varied from 0.61 to 0.87 with an average of 0.77. Although cluster (UPGMA) and principal components analyses indicate that the cultivars' clustering made independently both from the geographical origin, horticultural classifications and/or from the sex of trees. In addition, the observed variation suggests considerable differentiation among fig cultivars. The present data supports the common origin of the fig cultivars. Analysis of molecular variance (AMOVA) revealed that average Φ_{ST} value overall loci was 0.026, and the overall distribution pattern of molecular variation indicated that about 97.43% of the total variance was accounted by the within-region variance component. The remaining 2.5% ($P < 0.001$) of the variation was founded among cultivars of the prospected regions. Our results proved that AFLP markers are useful for germplasm discrimination as well as for investigation of fig patterns variation. The information may be useful to define conservation management program.

1. Introduction

Common fig, *Ficus carica* L., is one of the oldest cultivated Mediterranean fruit (Zohary and Hopf, 2000). It is a member of the Moraceae family (Watson and Dallwitz, 2004). Fig fruit is well-known for its nutritive value, and is consumed fresh or dry worldwide. Fig fruits are also recognized for their mild laxative activity. The fig tree can be uniferous, produce only one harvest in autumn or biferous, and bear fruit twice: in spring (fig-flowers), then in summer and autumn (figs of autumn). In Tunisia, fig germplasm is highly diverse and provides a large array of genotypes. In addition, fig trees represent the principal component of several agro-ecosystems in the southern areas such as at the Jessours region (Matmata, Beni Khédache and Douiret) and constitute the second fruit crop in the Tunisian oases (Mars, 1995, 2003). However, abiotic and biotic stresses (urbanization, rainfall irregularities, plagues, etc.) are currently threatening the germplasm resources. Thus, it is imperative to examine the genetic diversity and to establish a reliable method for cultivars identification in order to preserve the local germplasm. In fact, genetic diversity of plant germplasm is the important basis of conservation biology and genetic improvement. In this scope, studies based on the use of the morphometric parameters and isoenzymes have been reported (Valizadeh et al., 1977; Ben Salah et al., 1995; Chessa et al., 1998; Mars and Marrakchi, 1998; Saddoud et al., 2008). However, these characters are sensitive to environmental conditions; the discriminant ones are limited in number and do not allow the separation of the phenotypes into distinct groups (Valdeyron, 1976). To overcome this problem, we investigated the development of molecular markers to characterize precisely the observed polymorphism. For this purpose, a large panel of more powerful DNA-based methods has been performed and their efficiency has been proven in the description of the polymorphisms within and between species. As a part of our study, we focused on the development of molecular methods as an attractive approach suitable in surveying the genetic polymorphisms at the fig's DNA level. The use of the random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR) and short sequence repeats (SSR) have been successfully designed to examine the ecotypes' phenetic relationships in Tunisian figs

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(Chatti et al., 2004; Saddoud et al., 2005; Salhi-Hannachi et al., 2004, 2005, 2006). Accurate germplasm characterization and revelation of the genetic relationships among the accessions maintained in a plant collection are essential links between the conservation and appropriate use of plant genetic resources (Rodrigues et al., 1999).

In the paper herein, AFLP markers were used in this scope in order to improve fig germplasm *ex situ* and *in situ* conservation. Indeed, AFLP is an efficient PCR based assay for plant DNA fingerprinting that reveals significant levels of DNA polymorphism (Vos et al., 1995). The advantages of this technique are reproducibility, high level of polymorphism detection, genome-wide distribution of markers, and no pre-requisite of knowledge of the genome being studied (Mueller and Wolfenbarger, 1999). AFLP has been used to study genetic relationships among several crops: *Hedysarum* (Marghali et al., 2005), soybean (Maughan et al., 1996), rice (Mackill, 1999), tea (Balasaravanan et al., 2003) and a wide range of fruit species such as date-palms (Rhouma et al., 2007), pomegranate (Jbir et al., 2008), apricot (Krichen et al., 2006), figs (Cabrita et al., 2001). This approach proven that the revealed markers are extremely sensitive to distinguishing closely related cultivars.

In the current paper, we used the amplified fragment length polymorphism (AFLP) approach to investigate genetic diversity, elucidate its structuration and clarify relationships among local fig resources representing the economically important cultivars. Such information will be of great interest for understanding the genetic improvement of Tunisian fig and for inspecting the implications of the preferment genotypes in management program for their conservation and rational utilization. However, in order to optimize the conservation and use of fig genetic resources in several countries, the carefully chosen primers combinations can be useful to standardize the molecular identification of fig cultivars in different germplasm collections and contribute to elucidate figs origin not clearly understood. Therefore, this approach can be useful to establish fingerprints of fig cultivars worldwide.

2. Materials and methods

2.1. Plant material

Forty cultivars (32 female and 8 male trees) of Tunisian fig (Table 1) were used in this study. These were collected from 5 regions. Plant material consisted of young leaves (approximately 10 g) sampled from adult trees.

Table 1
Tunisian fig cultivars studied and their geographical origin.

Accession name	Label	Horticultural classifications	Geographical origin	Region
Soltani 1	SL1	Uniferous	Ourdanine	Sahel
Kahli 1	KH1	Uniferous	Kalaa Kebira	
Hemri 1	HM	Uniferous	Enfidha	
Zidi 1	ZD1	Uniferous	Mesjed Aissa	
Baghali	BG	Uniferous	Mesjed Aissa	
Bidhi	BD	Uniferous	Kalaa Kebira	
Bither abiadh	BA	Biferous	Mesjed Aissa	
Besbessi	BS	Biferous	Mesjed Aissa	
Jrani	JR	Uniferous	Ghadhabna	
Assafri	AS	Uniferous	Ghadhabna	
Zergui	ZG	Uniferous	Degache	South West
Zidi 3	ZD3	Uniferous	Tozeur	
Hamri	HMR	Uniferous	Degache	
Khadhri	KDR	Uniferous	Degache	
Khartoumi	KR	Uniferous	Degache	
Tounsi	TS	Uniferous	Degache	
Wahchi	WH	Biferous	Degache	
Chetoui	CH1	Biferous	Degache	
Dhokkar 2	DH2	Uniferous	Degache	
Sawoudi	SD	Uniferous	Gafsa	
Gaa Zir	GZ	Uniferous	Gafsa	
Assal boudchiche	AB	Uniferous	Gafsa	
Khadhouri	KD	Uniferous	Gafsa	
Dhokkar 1	DH1	Uniferous	Gafsa	
Hammouri	HR	Uniferous	Medenine	South East
Widlani	WD	Uniferous	Medenine	
Zaghoubi	ZH	Uniferous	Medenine	
Rogabi	RG	Uniferous	Medenine	
Makhbech	MK	Uniferous	Medenine	
Dhokkar Zarzis	DZ	Uniferous	Medenine	
Zidi 2	ZD2	Uniferous	Utique	North East
Dhokkar 4	DH4	Uniferous	Utique	
Dhokkar 5	DH5	Uniferous	Raf Raf	
Soltani 3	SL3	Uniferous	Raf Raf	
Chetoui	CH2	Biferous	Raf Raf	
Soltani 2	SL2	Uniferous	Mornag	
Temri	TM	Uniferous	Kerkenah	Kerkenah
Baghli	BGL	Uniferous	Kerkenah	
Abiadh	ABD	Uniferous	Kerkenah	
Dhokkar 3	DH3	Uniferous	Kerkenah	

2.2. DNA isolation

Total genomic DNA was purified from frozen young leaves according to the procedure of Dellaporta et al. (1983). 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. AFLP analysis

The AFLP procedure was carried out using the AFLP Core Reagent Kit and AFLP Primer Kit (Invitrogen, Cergy Pontoise, France) based on Vos et al. (1995). Template DNA (250 ng) was double digested with 2.5 units of each *Eco*RI and *Mse*I restriction enzymes for 2 h at 37 °C in a final volume of 25 μL. Specific adapters were ligated overnight to the restriction fragments with 1 unit of T4 DNA ligase in 10 mM Tris–HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate and 0.4 mM ATP, at room temperature. Pre-amplification was carried out using *Eco*RI and *Mse*I primers with one additional nucleotide at the 3′, under the following conditions: 20 cycles of 94 °C for 30 s, 56 °C for 60 s and 72 °C for 60 s. Pre-amplified DNA was analysed by 1% agarose gel electrophoresis. Further selective amplification was performed, using two primers with two additional selective nucleotides at the 3′ end. Amplifications were carried out using a touch-down PCR program: 1 cycle of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 60 s, then 13 cycles with the annealing temperature lowered by 0.7 °C per cycle, followed by 23 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s. AFLP products were resolved in denaturing 6% polyacrylamide gels in 1× TBE buffer as follows: the amplified DNAs were mixed with 5 μL 6× loading sequence buffer [STR; 98% (v/v) formamide, 10 mM EDTA pH 8.0, 1% (v/v) xylene cyanol and 1% (v/v) bromophenol blue] before denaturation by heating for 2 min at 95 °C. Four microliters of each denaturated DNA mixture was loaded onto a pre-warmed polyacrylamide gel. Electrophoresis was performed at 1500 V for 3 h and the separated DNA bands were visualized by silver staining (Chalhoub et al., 1997). The gels were then dried overnight and photographed.

2.4. Data analysis

For all primers combination, the total number of bands was determined and only the polymorphic ones were taken into account in this study to estimate the percentage of polymorphic bands (%PB). The ability of the most informative primers to