South African Journal of Botany 116 (2018) 207–215

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

# Diversity analysis and genetic relationships among local Algerian fig cultivars (*Ficus carica* l.) using SSR markers

R.H. Boudchicha [a,b], J.I. Hormaza [c], H. Benbouza [d,*]

[a] Centre de Recherche en Biotechnologie, Ali Mejli Nouvelle Ville, UV03 Bp E73, Constantine, Algeria
[b] Département de Biologie et Environnement, Université Mostapha Benboulaid-, Batna 2, Fesdis, Batna, Algeria
[c] IHSM La Mayora – CSIC - UMA, 29750 Algarrobo-Costa, Málaga, Spain
[d] Département des Sciences Agronomiques, Université Hadj Lakhdar Batna 1, Algeria

ARTICLE INFO

*Article history:*
Received 17 February 2018
Received in revised form 19 March 2018
Accepted 28 March 2018
Available online xxxx

Edited by E Balázs

*Keywords:*
*Ficus carica* L.
Microsatellite
Genetic diversity
Population structure
Algeria

ABSTRACT

The common fig (*Ficus carica* L.) is an emblematic fruit tree in Mediterranean basin with complicated misidentification. A good management and conservation of local cultivars involve a prior genetic assessment of the variability presented in germplasm collections. This study characterizes for the first time the genetic variability of Algerian fig cultivars. In total, 34 fig cultivars from a local germplasm collection and a private orchard were analyzed using 24 SSR markers in order to evaluate the genetic diversity and the relationship among local Algerian cultivars. The polymorphic markers amplified a total of 79 fragments with a mean number of 3.59 alleles per locus. The observed heterozygosity ranged from 0.039 to 0.84, with a mean of 0.46. The expected heterozygosity varied from 0.074 to 0.74, with a mean of 0.42. The total value of probability of identity was $3.24 \times 10^{-8}$. Unweighted Pair Group Method with Arithmetic Averages (UPGMA) cluster analysis showed cases of synonymies and homonymies in the cultivars analyzed and some groups were related to fruit production type. The combination of only three SSR allowed to unequivocally distinguish 13 cultivars. Bayesian analysis showed a subtle genetic structure and depicted an admixture origin for most accessions.



## 1. Introduction

The characterization of genetic resources and a proper conservation of agrodiversity will strongly contribute, through a continuous supply of new cultivars and improved varieties, to ensure food security for future generations. Algeria, due to its strategic geographic location and large geographic area, possesses a rich genetic diversity of crop genetic resources, however most of them still poorly studied. Common fig (*Ficus carica* L.) is a rustic and traditional fruit tree crop in Algeria, and its adaptation capabilities allowed its propagation in all bioclimatic stages from the Mediterranean coast to the extreme south of the country and from Tunisian to Moroccan borders (Bourayou et al., 2005). The fig fruit is also known for its nutritive and ethno medical values. In Algeria, the fig tree constitutes an important income in the rural areas and marginalized regions.

The common fig (*Ficus carica* L.; 2n = 2x = 26) belongs to the Moraceae, a family with over 1400 species distributed in about 40 genera. The genus *Ficus* L. contains about 750 species divided into six subgenera that share a unique inflorescence, the syconium (Berg, 2003). It is an ancient fruit tree crop associated with the beginning of horticulture in the Mediterranean basin (Khadari et al., 2005a; Kislev et al., 2006). Although the common fig is a gynodioecious species with male trees (caprifigs) that produce syconia with male and female flowers, and female trees (the edible figs), that produce syconia with only female flowers, since only the male trees produce pollen, the common fig is functionally dioecious. Three types of fig female trees are cultivated: the i) common type that develops fruits parthenocarpically with one (unifera) or two (bifera) crops, the ii) Smyrna type that requires pollination with pollen from caprifigs (caprification) to develop fruits, and the iii) San Pedro type that produces a first crop (brevas) parthenocarpically and a second crop (main crop) only after pollination (Giraldo et al., 2008).

World fig production reached more than 1 million tons in 2014 (FAO, 2014). Algeria with around 129,000 tons is ranked third among the most important producing countries of figs in the world, after Turkey (300,000 tons) and Egypt (176,000 tons) (FAO, 2014). Most of the Algerian fig production comes from mountainous regions (Bejaia, Tizi Ouzou and Sétif) where it is considered as the most important fruit crop after olive (*Olea europaea*). However, the production of fresh or dried figs has been continuously reducing for more than a decade. Furthermore, although numerous cultivated varieties are present, this large genetic diversity is threatened by a significant genetic erosion (Bourayou et al., 2005). Nevertheless, since the launch of the fruit crop development program by the Algerian Ministry of Agriculture,

* Corresponding author.
*E-mail addresses:* r.boudchicha@crbt.dz, (R.H. Boudchicha), ihormaza@eelm.csic.es, (J.I. Hormaza), halima.benbouza@univ-batna.dz, benbouza@hotmail.com. (H. Benbouza).

https://doi.org/10.1016/j.sajb.2018.03.015
0254-6299/

an increasing interest in improving fig production, including dry fig for exportation, is taking place in Algeria.

The first morphological description of local Algerian fig cultivars was made by Mauri (1942). Then, different surveys have been undertaken to describe the most frequent and cultivated local cultivars (Bourayou et al., 2005). Both in situ and ex situ germplasm collections have been established but neither morphological nor molecular studies have been carried out in order to assess the genetic diversity preserved in those collections. Consequently, it raises the need to set up and perform proper and lasting identification and characterization programs in order to optimize Algerian fig diversity conservation.

The fig tree has not been subjected to intensive plant breeding programs and, thus, many fig tree populations exhibit a rich genetic diversity, that can only be fully exploited once it is properly identified and classified (Perez-Jiménez et al., 2012). The identification of fig cultivars has usually been carried out using morphological descriptors such as those developed by IPGRI and CIHEAM (2003). However, relying only on morphological description is problematic due to environmental influences and a limited number of phenotypic discriminating traits. To overcome these difficulties, various biochemical and molecular markers have been used for fig germplasm characterization and diversity analysis such as isozymes (Cabrita et al., 2001), random amplified polymorphic DNA (RAPD) (Papadopoulou et al., 2002), inter simple sequence repeats (ISSR) (Khadari et al., 2005b), amplified fragment length polymorphism (AFLP) (Cabrita et al., 2001; Baraket et al., 2011) and microsatellites or simple sequence repeats (SSR) (Bandelj et al., 2008; Giraldo et al., 2008; Achtak et al., 2009; Caliskan et al., 2012). Due to their codominant nature, intraspecific polymorphism and reproducibility, SSRs have become the marker of choice for fingerprinting and analysis of genetic diversity in most plant species (Gupta and Varshney, 2000). Many genomic microsatellites have been developed for common fig and related species (Khadari et al., 2001; Giraldo et al., 2005; Zavodna et al., 2005; Ahmed et al., 2007; Bandelj et al., 2008; Achtak et al., 2009) and some of them have been used in this study.

To our knowledge, only seven Algerian cultivars, maintained in a Moroccan fig germplasm collection, have been studied using molecular markers (Khadari et al., 2005b). In order to fill this gap, the aim of this work is to use SSR markers to assess the genetic diversity of 34 local Algerian fig cultivars maintained at the fig germplasm collection of Skikda, Bejaia and private orchards from the Tizi Ouzou region as well as to shed light on the genetic relationship among the studied cultivars.

## 2. Materials and methods

### 2.1. Plant material

A total of 34 fig tree cultivars were analyzed (Table 1): 25 cultivars from the Algerian National Germplasm collection maintained at the "Institut de l'Arboriculture Fruitiére et de la Vigne, ITAFV" [16 located in Béjaia (Tekerietz) and 9 in Skikda (Mzedj Edchiche)]; and 7 cultivars conserved, *in situ*, in private orchards in Tizi Ouzou, Ait Sidi Larbi including a male tree (caprifig) (Fig. 1). For some cultivars, more than one tree were analyzed making a total of 77 fig trees analyzed.

### 2.2. Genomic DNA extraction and PCR amplification

Genomic DNA extraction was performed on dried young leaves using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). DNA quality check and quantification were performed in a Nanodrop 2000C (Thermo Scientific, Waltham, MA, USA); extracted DNA was diluted to 10 ng·μl$^{-1}$ in modified TE buffer (10 mM Tris–HCl, 0.1 mM EDTA). Among the SSRs developed for fig fingerprinting in previous works (Khadari et al., 2001; Giraldo et al., 2005; Ahmed et al., 2007; Achtak et al., 2009; Caliskan et al., 2012), 24 loci were selected on the basis of their high polymorphism and high amplification quality (Table 2). PCR reactions were performed in a total volume of 15 μl reactions containing 16 mM $(NH_4)\ 2SO_4$, 67 mM Tris–HCl, pH 8.8, 0.01% Tween 20, 2 mM $MgCl_2$, 0.1 mM each dNTP, 0.4 μM each primer, 25 ng genomic DNA and 0.5 Units of BioTaq DNA polymerase (Bioline, London, UK). Amplification was carried out in an I-cycler (Bio-Rad Laboratories, Hercules, CA, USA) thermocycler using the following temperature profile: an initial step of 5 min at 94 °C, 35 cycles of 30 s at 55 °C–60 °C (depending on the SSR locus) and 1 min at 72 °C, and a final step of 5 min at 72 °C. Forward primers were labelled with D2, D3 and D4 WellRED fluorescent dyes (Sigma-Aldrich, MO, USA) on the 5-end and PCR products were detected and sized with a CEQ™ 8000 capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA). Samples were denaturalized at 90 °C for 120 s, injected at 2.0 kV for 30 s and separated at 6.0 kV for 35 min. Each PCR and capillary electrophoresis was repeated at least twice to ensure the reproducibility of the results.

**Table 1**
Cultivar name, origin, number of analyzed accessions, type or production and type of reproduction.

| Cultivar name | Origin | Number of accessions | Production type | Reproduction type |
|---|---|---|---|---|
| Roudane | Skikda | 3 | Biferous | San Pedro |
| Avouacou | Skikda | 3 | Uniferous | / |
| Bakkor noir | Skikda | 3 | Biferous | San Pedro |
| Hameri | Skikda | 3 | Uniferous | Common type |
| Abiarous SK | Skikda | 3 | Uniferous | Smyrna |
| Zreka | Skikda | 3 | Biferous | San Pedro |
| El fessi | Skikda | 3 | Uniferous | / |
| Bezoul El khadem | Bejaia | 3 | Uniferous | Smyrna |
| Bifer ta'al amara (BTA) | Bejaia | 3 | Biferous | San Pedro |
| Alekak sk | Skikda | 3 | Uniferous | Smyrna |
| Hafer lebghal | Bejaia | 3 | Uniferous | / |
| Bakkor Blanc sk | Skikda | 3 | Biferous | San Pedro |
| Chetoui | Bejaia | 3 | Uniferous | Common type |
| Azendjer | Bejaia | 2 | Uniferous | Smyrna |
| Abgaiti | Bejaia | 2 | Uniferous | Common type |
| Avouhvoul | Bejaia | 3 | Uniferous | / |
| Taarlit | Bejaia | 3 | Uniferous | Smyrna |
| Bakkor blanche Be | Bejaia | 3 | Biferous | San Pedro |
| Alekak Be | Bejaia | 2 | Uniferous | Smyrna |
| Taranimt | Bejaia | 3 | Uniferous | Smyrna |
| Tameriout | Bejaia | 3 | Uniferous | Smyrna |
| Azegouagh | Bejaia | 1 | Uniferous | / |
| Azegzaou | Bejaia | 2 | Uniferous | / |
| Abiarous Be | Bejaia | 3 | Uniferous | Smyrna |
| Aghelbengoure | Tizi Ouzou | 1 | Uniferous | / |
| Tkenvert Beznik | Tizi Ouzou | 1 | Uniferous | / |
| Avoyohriche | Tizi Ouzou | 1 | Uniferous | / |
| Avoghnime | Tizi Ouzou | 1 | Caprifig | / |
| Avoyehvole | Tizi Ouzou | 1 | Uniferous | / |
| Thguenguert | Tizi Ouzou | 1 | Uniferous | / |
| Tharanimt | Tizi Ouzou | 1 | Uniferous | Smyrna |
| Agungour | Tizi Ouzou | 1 | Uniferous | / |
| Thavournet | Tizi Ouzou | 1 | Uniferous | / |
| Bakkor blanc Be | Bejaia | 2 | Biferous | San Pedro |

Fig reproduction type according to Condit, 1955. /: Unknown origin reproduction type.

### 2.3. Data analysis

To evaluate the genetic diversity and informativeness of the SSR markers used, several genetic indexes were calculated: number of alleles per locus, allele frequencies, observed heterozygosity ($H_o$, calculated as the number of heterozygous genotypes over the total number of genotypes analyzed for each locus), expected heterozygosity ($H_e = 1 - \sum pi^2$, where pi is the frequency of the *i*th alleles in the cultivars; (Nei, 1973), effective number of alleles [$Ne = 1 / (1 - H_e)$], Wright's fixation index ($F = 1 - H_o / H_e$) (Wright, 1965), estimated frequency of null alleles (r) and the probability of identity [$PI = 1 - \sum pi^2 + \sum\sum (2pipj)^2$, where *pi* and *pj* are the frequency of the *i*th and *j*th alleles, respectively] that measures the probability of two