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Molecular based assessment of genetic diversity within Barbary fig (*Opuntia ficus indica* (L.) Mill.) in Tunisia

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Abstract

In this work, we report for the first time on the analysis of genetic diversity within a set of 36 Tunisian *Opuntia ficus indica* (L.) Mill. ecotypes using RAPD markers.

Random decamer primers were screened to assess their ability to detect polymorphisms in this plant crop. Thirty-nine RAPD markers were revealed and used to survey the genetic diversity at the DNA level and to establish relationships.

Consequently, considerable genetic diversity was detected and the UPGMA analysis permitted the discrimination of all the genotypes and enabled their sorting into thirteen groups. The accession 'R Sbiba inerme' was significantly divergent from all tested genotypes. In addition, as shown by the clustering the tested genotypes did not significantly diverge, though originating from different localities.

Since RAPD markers proved to be useful for germplasm discrimination as well as for discovery of patterns of variation in Barbary fig, the opportunity of this study was discussed in relation to the setting up of rational decisions concerning the management of a national reference collection that ought to allow the preservation of all studied genotypes.

Keywords: *Opuntia ficus indica*; RAPD; Genetic diversity; Preservation

1. Introduction

The *Cactaceae* are a dicotyledonous angiosperm of about 2260 accepted taxa. They are most plentiful in the arid and semiarid regions between 35°N and 35°S (Taylor, 1997).

Cacti have been exploited since the pre-Columbian times and are mainly present in North and South America but also in the Mediterranean basin, Middle-East, South Africa, India, Thailand and Australia (Britton and Rose, 1919; Anderson, 2001). For instance, this species reached the Mediterranean basin during the 16th century (Le Houérou, 1992; Barbera, 1995). Nevertheless, only towards the end of the 20th century have large plantations been established.

Fruits are eaten raw, cooked or fermented into alcoholic beverages and some species produce substances of pharmaceutical or industrial interest (Boyle and Anderson, 2001).

The absence of updated statistical data from countries in which this species is cultivated allows only a rough estimate of the worldwide land area. For instance, in Tunisia about 500,000 ha are cultivated (Nefzaoui and Ben Salem, 2001).

In Tunisia, *Opuntia ficus indica* has proved to be an important fodder crop mainly for sheep, particularly during periods of drought and seasons of low feed availability (Nefzaoui and Ben Salem, 2000, 2001) in central and southern regions of the country. The cladodes can be fed either as fresh forage, or stored as silage for later feeding (Castra et al., 1977).

Cactus is also planted to reduce water and wind erosion, rangeland degradation, sand movement and to enhance the restoration of the vegetation cover.

Recently, large plantations have been established near the region of Kasserine (Inglese et al., 2001); the most specialized cactus-growing region, where the highest morphological diversity of this plant crop is maintained. Nevertheless, this species does not play a basic role in the human diet as it is, for the animals. In fact, only late ripening fruits produced through scollozatura (removal of spring flush of flowers and cladodes) are promoted, as in the Island of Sicily (Barbera et al., 1992).

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Despite from these aforementioned advantages, no collections of Barbary fig were set up in the country in order to preserve this crop. In fact, the conservation of biodiversity is one of the major issues facing humankind.

Due to the multiple uses and the ability of cacti to thrive in arid and semiarid environments, it has become increasingly important to describe and characterize these valuable resources. The latter is a challenging goal since up to now; knowledge regarding the amount of genetic variation and genetic relationship by means of molecular tools is missing in Tunisian *Opuntia ficus indica*. In fact, though this crop is widely cultivated in the country, the majority of research works were especially oriented towards the characterization of the nutritional value of the cladodes as an important fodder crop in arid areas, independently from their genetic potential (Nefzaoui and Ben Salem, 2000, 2001). In addition, most of the published information available on biodiversity of the cultivated cacti in the world comes from allozyme studies (Chessa et al., 1997; Uzun, 1997; Boyle and Anderson, 2001).

At present, molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Powell et al., 1996; Russell et al., 1997). The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques (Saïki et al., 1998). These molecular markers had been successfully used in *Opuntia* genus for detecting genetic diversity and relationships (Wang et al., 1998; Arnholdt-Schmitt et al., 2001; Labra et al., 2003). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost, and the use of small amount of plant material, etc.

The aims of this study were to produce suitable markers for the investigation of DNA polymorphism in Tunisian Barbary fig ecotypes useful in the analysis of genetic diversity and ecotype identification, and to set up rational decisions concerning the establishment of a national reference collection. Indeed, though this crop is widely cultivated in the country, collection repositories are missing.

2. Materials and methods

2.1. Plant material

Thirty-six Barbary fig (*Opuntia ficus indica*) accessions, well representative of the *Opuntia ficus indica* germplasm in Tunisia were used in this study (Zemni, personal communication). They were sampled from 15 localities in the regions of Kairouan and Kasserine (Fig. 1); the most well known regions sheltering various different morphological sorts of Barbary fig in the country (Table 1).

2.2. DNA extraction

Within Barbary fig, Isolation of DNA from cladodes is difficult, especially due to the presence of high amounts of mucilage that interfere with DNA extraction.

In this study, a DNA method extraction technique for cacti which helps to overcome the difficulties caused by mucilage has been used. Thus, for each accession, an external slice of the cladode was taken for analysis. The cuticle was removed and a piece of about 1 g of the chlorenchyma (mesophyll cells; which is the interior of the cladode, between the upper and the lower layers of epidermis) was cut using a scalpel and taking care not to include areolar meristems (light to dark coloured bumps, out of which grow clusters of spines).

The protocol of DNA extraction used here is that of Bowers et al. (1993) later modified by This et al. (1997) and Zoghlami et al. (2001).

DNA was quantified by visual comparison with lambda DNA molecular marker on ethidim bromide stained agarose gels.

2.3. Primers and PCR assays

Twenty-two universal decamer oligonucleotides, purchased from the University of British Colombia were used for the amplification of random DNA banding patterns (Table 2). They were tested on three ecotypes (labeled 5, 10 and 29 in Table 1) for their ability to produce polymorphic, unambiguous and stable RAPD markers (Table 2).

PCR reactions were performed in a 10 μl reaction mixture containing: 2.5 ng of template DNA, 2.0 μl of Go Taq buffer (Promega), 0.4 M dNTPs (0.1 M of each: dATP, dGTP, dTTP and dCTP (Promega), 0.35 μl of 25 mM $MgCl_2$, 2.5 μM of primer and 0.5 U of Go Taq DNA polymerase (Promega).

The PCR was performed in a Thermoblock thermocycler (Genius), as described by Burrow et al. (1996).

Products of the PCR were separated by electrophoresis in 1.6% agarose gels with 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) using a volt range of 3 V cm^{-1} during 3 h. Lambda DNA *Eco*RI/*Hin*dIII digested (Boehringer Mannheim, Germany) was used as a molecular size standard.

Amplifications were performed at least twice and only reproducible (stable) products were taken into account for further data analysis.

2.4. Data analysis

Photographs were taken with a Biometra Bio-doc II™ system.

In order to ensure the absence of artifacts, bands were carefully selected from replicated amplifications. Amplified bands were designated by their primer code and their size in base pairs.

Polymorphic DNA bands were scored as discrete variables: 1 for the presence and 0 for the absence of a similar band.

For each primer, the number of bands and the polymorphic ones were calculated as well as the percentage of polymorphic bands (PPB). The latter was determined as the percentage of polymorphic bands over the total number of the yielded bands. The number of RAPD banding profiles (profiles generated by all the accessions per primer) has been also calculated.