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Morphological characterization and pollen evaluation of some Tunisian *ex situ* planted caprifig (*Ficus carica* L.) ecotypes

A. Essid [a,*], F. Aljane [a], A. Ferchichi [a,b]

[a] Laboratoire d'Aridoculture et Cultures Oasiennes Institut des Régions Arides de Médenine, Médenine 4119, Tunisie
[b] Institut National Agronomique de Tunisie, 43 Avenue Charles Nicolle, 1082 Cité Mahrajène, Tunis, Tunisie

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ABSTRACT

Caprifig has a particular interest in plant conservation due to their important role in fig pollination. In this work, 53 morphological traits and four pollen descriptors were studied in order to select the most discriminating traits. Significant difference among ecotypes was revealed for almost quantitative traits except for anther number/flower and pollen viability. Qualitative data showed morphological variation within ecotypes (Blastophaga richness, external color, internal color, leaf color, central shape lobe, number lobe, tree growth habit, density branch, aspect branch and size tree). The results of the principal component analyses (PCA) for the morphological traits and pollen descriptors indicated that the first three PCs explained 54.23% of the total variation. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on the similarity matrix grouped studied ecotypes in five clusters. It was possible to discriminate seven distinct ecotypes (Magouli1, Magouli2, Dhokkar1, Dhokkar4, Bouharrag1, Bouharrag2, and Bithri1). Similarly, some ecotypes sharing some fruit and shoots traits (Dhokkar2 and Dhokkar3) or some leaves and shoots characteristics (Bouharrag1, Assafri and Jrani) were grouped together. Some homonyms were detected between ecotypes such as Magouli, Bithri, Bouharrag and Dhokkar. Our study suggests among the 53 morphological traits and the four pollen descriptors 40 characters showed a good discriminating power and can be used for the discrimination of Tunisian caprifig trees.

1. Introduction

In Mediterranean countries, fig is one of the most important fruit species (Polat and Özkaya, 2005; Çalişkan and Polat, 2012). It's widely distributed in Tunisia (Trad et al., 2013) and many niches are well adapted for fig production (Baraket et al., 2011; Gaaliche et al., 2012a). Local cultivars are numerous (Chatti et al., 2004; Mars et al., 2008). Three different types of female figs can be distinguished: the common type (cultivars no require obligatory caprification), the Smyrna type (cultivars need pollination) and the San Pedro type (cultivars with obligatory caprification in the second crop). Several of them have a good quality and there are very appreciate by consumers. However, they need pollination (caprification). In fact, artificial caprification is a common practice used by farmers in Tunisians fig cultivations (Mars et al., 2008) in order to conserve fig crop.

Hundreds of genotypes have been described worldwide, in which high number of homonyms and synonyms can be observed. The selection of the most discriminating traits is especially important for this crop (Khadivi-Khub and Anjam, 2014). Morphological descriptors remain the first step in the conservation process of plant (Podgornik et al., 2010) and the most suitable tool in genetic diversity (Darjazi, 2011). Traditionally, they are used in the plant germplasm characterization (Giraldo et al., 2010) and suitable in the assessment and identification of fig genotypes (Salhi Hannachi et al., 2003; Çalişkan and Polat, 2008; Şimşek and Yildirim, 2010; Saddoud et al., 2011; Aljane et al., 2012; Almajali et al., 2012; Gaaliche et al., 2012a).

As pollen source, caprifig tree (*Ficus carica* L.) plays a major role in female fig tree breeding programs (Rostami and Rahemi, 2013). Prospects carried out in different regions of the country have identified numerous caprifig ecotypes that have been chosen on the basis of fruit characteristics and pollen maturity date (Mars et al., 2009).

Some works have been interested to study the genetic diversity using morphological traits of Tunisian caprifig tree (*Ficus carica* L.,) cultivated in the southeast (Aljane and Ferchichi, 2007) or in center-east and northwest of Tunisia (Gaaliche et al., 2012b). Therefore, there are few published data about the study of genetic diversity of Tunisian caprifig ecotypes collected from different regions of Tunisia and planted *ex situ* using morphological traits and pollen descriptors. For this purpose, the main objectives of this research were: to study the morphological characterization and to determine the relationship between 15 Tunisian caprifig ecotypes cultivated from four traditional fig cultivation areas, which include a high concentration of plantations and an important varietal richness, as well. The results obtained from this

* Corresponding author.
E-mail address: awatef.essid@yahoo.fr (A. Essid).

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Table 1
Caprifig ecotypes names and their original geographic localities.

Ecotype name	Geographic locality	Region	Climate stage
Magouli1	Douiret (Tataouine)	South-east	Arid inferior
Magouli2	Bir Amir (Tataouine)		
Bouharrag1	Bir Amir (Tataouine)		
Bithri2	Bir Amir (Tataouine)		
Bouharrag2	Toujen (Gabés)	South-east	Arid superior
Limi	Kébéli	South-west	Saharan superior
Tebessi	Kébéli		
Sawoudi	Kébéli		
Dhokkar2	Tamaghza (Tozeur)		
Dhokkar3	Dégâche (Tozeur)		
Dhokkar4	Gafsa	South-west	Arid inferior
Bithri1	Kerkennah (Sfax)	Centre-east	Arid superior
Assafri	Kerkennah (Sfax)		
Jrani	Ghadhabna (Mahdia)	Centre-east	Semi-arid
Dhokkar1	Djebba (Béja)	North-west	Sub-humid

study will provide information about the suitable caprifig ecotypes for caprification or for exploitation in breeding programs.

2. Materials and methods

2.1. Plant material

Fifteen Tunisian caprifig ecotypes collected from four traditional fig cultivation areas belong to several bioclimatic stages (Table 1) and planted in the fig germplasm collection of the Arid Land Institute of Médenine established in El Gordhab, Tataouine, were studied. Plant materials were propagated by hardwood cuttings. The experimental orchard was 8 years old and established in 3 replicates of 5 × 5 m. It was installed on sandy soils, cultivated under irrigated conditions and received only the organic fertilization and standard cultural practices. The climate is an arid inferior Mediterranean type with mild winter and hot summer. The annual average temperature of the hottest months (June, July and August) is between 20 and 30 °C, and within 10 and 20 °C for the annual average of the coldest months (December, January and February). Average annual rainfall varied from 100 to 200 mm.

2.2. Morphological traits

Morphological traits of fruit (Fig. 1), leaves (Fig. 2) and trees were chosen according to the fig descriptors (IPGRI and CIHEAM, 2003). Overall 53 morphological traits were described. Measurements and observations of fruit, leaves, and trees were performed during three consecutive years (2011, 2012 and 2013). Caprifig ecotypes have a harvest date ranged from middle May to middle June, so they were grouped as early or middle. According to number of gall in the fruit, ecotypes were grouped as having low, medium or high Blastophaga richness.

2.3. Caprifig pollen evaluation

Caprifig male flowers have been fixed by FAA solution (70% ethanol: glacial acetic acid: formalin [18:1:1; v/v/v]) (Johansen, 1940). For each ecotype, pollen grain numbers have been counted from twenty flowers, using a hemocytometer, as a protocol that has been used by Niesenbaum (1992) with minor modification. Each flower was placed in Micro centrifuge tube, crushed with a glass rod in 100 µl of water and vortexing. Viability test was performed with acetocarmine stain (acetic acid 45% and aceto-carmine 1%). The percentage of pollen viability has been determined as the ratio of stained pollen grains to the total number of pollen grains. For each ecotype, six repetitions have been used.

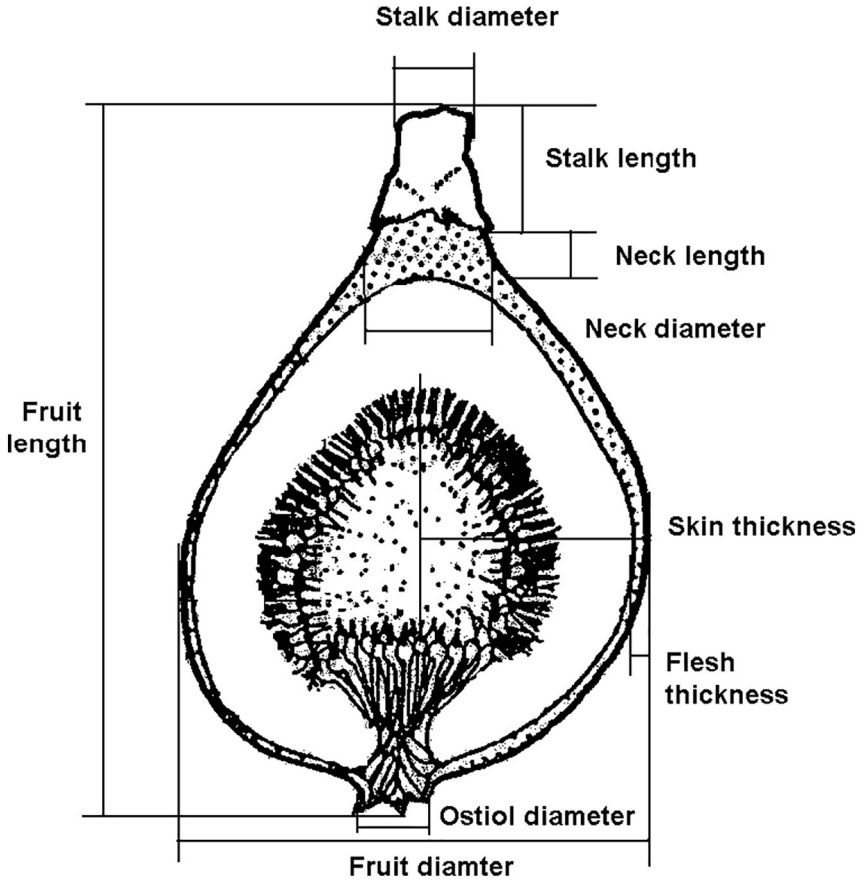

Fig. 1. Different parameters measured on caprifig fruit according to Storey (1975), IPGRI and CIHEAM (2003).