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Assessing the genetic diversity and population structure of Tunisian apricot germplasm

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ABSTRACT

Tunisian apricot germplasm consists of two kinds of material: grafted cultivars and seed-propagated Bargougs. A total of 144 accessions were selected for this study, including 109 cultivars accessions and 35 Bargoug accessions. A comparative analysis of the genetic structure was carried out utilizing morphological characters and AFLP molecular markers separately and in combination, with the aim of estimating the genetic diversity and identifying relationships with the geographical origins of the accessions. The hierarchical clustering structure obtained with each dataset revealed that the accessions could be divided into two different genetic groups, indicating two distinct gene pools. This result highlighted the existence of two distinct genetic origins of apricot genotypes in Tunisia. Moreover, Tunisian apricot diversity was classified according to the geographical origins of the accessions, with four regions being distinguished: North, Center, South-East and South-West (Oasis). The perfect accession distribution was based on the four predefined regions, as confirmed by the discriminant analysis. Furthermore, a correspondence analysis revealed a structure in two distinct groups and the existence of accessions from the four predefined regions in each group, which is evidence that Bargougs and grafted cultivars share the same genetic origin. Both markers (morphological and AFLPs markers) were complementary and contributed substantially to the diversity analysis with the best structure obtained from the combined dataset.

1. Introduction

Apricot (*Prunus armeniaca* L.) is widely distributed across five continents. It is mainly cultivated for fresh or processed fruit consumption in the Mediterranean Basin, for foresting in China, dried fruits in Turkey, flowered ornamental trees in Japan and nut consumption and oil extraction in Central Asia (Faust et al., 1998; Dauthy, 1995). Two main propagation strategies are used, depending on the fruit's potential uses: in Europe and North America, all cultivated apricots are propagated asexually by grafting, whereas in Asia and North Africa both seed propagation and grafting are used (Faust et al., 1998; Kostina, 1969).

In Tunisia, apricots are cultivated in various climatic areas, ranging from subhumid areas (500 < rainfall < 600 mm) in the Northern region, to arid (100 < rainfall < 200 mm) and Saharan areas (rainfall < 100 mm) in the South-West oasis region. Currently, among the traditional cultivars and breeding selections, two kinds of apricot material coexist in Tunisia, i.e. seed-propagated Bargougs and grafted 'MechMech' cultivars. Bargougs are an integral part of the oasis cropping system and are mainly cultivated for the shade they provide under the palm tree storey and over other fruit tree storeys (olive, citrus, fig, pomegranate, apple, etc.), while the lowest storey includes Fabaceae, Solanaceae, and Poaceae annual crops.

Several local apricot cultivars are found only in traditional orchards and old plantations and are unknown outside of their area of origin. Therefore, some of these cultivars are selected for regional cultivation as they are specifically adapted to local conditions while being able to overcome environmental constraints in different areas. Each of these cultivars has therefore likely been propagated by grafting for many years in the same region (Carraut and Crossa-Raynaud, 1974), for instance cultivars used in Testour (Northern Tunisia) are propagated by grafting and not adapted to conditions outside that area. Otherwise, few cultivars are encountered in different regions and are widely adapted to different climatic conditions.

For a long time, apricot cultivars were identified on the basis of pomological, morphological and horticultural traits (Maghuly et al., 2005). Various studies have focused on the variability of European

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apricot cultivars, with traditional and interesting cultivars being morphologically characterized and subsequently used to generate new selections that include traits of interest to breeding programs (Couranjou, 1977; Forte, 1971; Fideghelli and Monastra, 1977; Guerriero, 1982; Della Strada et al., 1989; Lichou and Audubert, 1989; Lichou, 1998). The assessment and description of trait variations are essential in the initial phase of plant breeding programs, allowing the selection of genotypes with high field performance, and the determination of qualitative traits that could eventually enhance apricot marketability and germplasm management.

In Tunisia, preliminary morphological and physiological characterization of apricot was carried out by Valdeyron and Crossa-Raynaud (1950), Crossa-Raynaud (1960) and Carraut and Crossa-Raynaud (1974). These studies focused on a subset of cultivars from the principal apricot cultivation areas in northern and central Tunisia. Not all of these cultivars have been preserved. Severe cases of genetic erosion have unfortunately occurred over the last decades and over 50% of the previously identified autochthonous cultivars have now disappeared (Krichen et al., 2009). However, many local cultivars have not yet been characterized, for instance the Bargoug oasis population was unknown and consequently not studied until a few years ago (Bourguiba et al., 2010, 2012; Krichen et al., 2010).

DNA based markers offer new opportunities for evaluating biodiversity among plant genomes (Maghuly et al., 2005). Today, different molecular marker techniques are available. Their effectiveness has been proven for studying and estimating genetic diversity and polymorphism, as well as determining relationships among accessions within several species such as peach (Sosinski et al., 2000; Testolin et al., 2000; Aranzana et al., 2002, 2003; Dirlewanger et al., 2002; Martínez-Gómez et al., 2003; Wünsch et al., 2005), cherry (Tavaud et al., 2004), vitis (Di Gaspero et al., 2000; Siret et al., 2000; Snoussi et al., 2004), date palm (Zehdi et al., 2004), coffee (Lashermes et al., 1999; Steiger et al., 2002) and melon (Garcia-Mas et al., 2000). It is widely acknowledged that molecular markers are powerful tools for investigating and determining genetic relatedness and for plant breeding (Maccaferri et al., 2007).

Several studies on apricot genetic diversity have been based on molecular marker techniques, including restriction fragment length polymorphism (RFLP) (De Vicente et al., 1998), random amplified polymorphic DNA (RAPD) (Gogorcena and Parfitt, 1994; Takeda et al., 1998; Hurtado et al., 1999), microsatellite, or simple sequence repeat (SSR) (Hormaza, 2002; Zhebentyayeva et al., 2003) and amplified fragment length polymorphism (AFLP) (Hagen et al., 2002; Hurtado et al., 2002; Geuna et al., 2003) analyses. AFLP markers have been extensively used for studying genetic diversity in different plant species (Vos et al., 1995; Maughan et al., 1996; Ellis et al., 1997; van Treuren and van Hintum, 2001; Sustar-Vozlic et al., 2006; Tamiru et al., 2007). They represent a powerful tool for characterizing the genetic diversity and structure, as well as for characterizing and differentiating morphologically similar varieties. Moreover, AFLPs are widely distributed throughout the genome, thus facilitating the assessment of genome-wide variation (Meudt and Clarke, 2007). In addition, it has been shown that AFLP markers are more stable and repeatable than RAPD markers, which are random and whose primers amplify fragments randomly (Powell et al., 1996; Meudt and Clarke, 2007).

It is essential to be able to detect genetic variation and determine genetic relationships between individuals and populations for efficient conservation and utilization of plant genetic resources (Powell et al., 1996).

Comparative analysis of morphological variability and genetic diversity via molecular markers is also very useful to determine the degree of correlation and complementarity between these two diversity analysis tools (Geleta et al., 2006; Ye et al., 2008; Tantasawat et al., 2010; Duc Pham et al., 2011).

As very few comparative studies of *Prunus* species have been conducted using different marker classes, the main objective of this work was to investigate the genetic diversity of Tunisian apricot germplasm using morphological characters and AFLP molecular markers. We also compared the efficiency of these two kinds of data for determining the genetic diversity structure and identifying relationships with the geographic origin of the studied accessions.

2. Material and methods

2.1. Plant material

Surveys were carried out at 14 sites representing the most important apricot production areas in Tunisia. These 14 sites were assigned to the 4 following regions: (1) North, including Ras Jbel and Testour (subhumid climate); (2) Center, including Kairouan, Mahdia and Sfax (highly arid climate); (3) South East, including Gabes, Mareth and Jerba (slightly arid climate); (4) South West, including the Oases of Gafsa, Tameghza and Midess (slightly arid climate); Tozeur, Nefta, Degache (highly Saharan climate) (Table 1, Fig. 1).

These surveys focused on traditional apricot landraces, including grafted cultivars and seed-propagated accessions. Some accessions with the same nomenclature were sampled from different areas, leading to the collection of 109 grafted accessions representing 47 cultivars. In addition, 35 seed-propagated Bargoug seedlings were collected from the South-West oasis region (Table 1, Fig. 1). Thus, a total of 144 apricot accessions were used in this study. The plant material was collected directly from the trees in the surveyed fields.

For the analyses in these studies, we opted to name the regions as follows: 'North' (48 accessions), 'Center' (36 accessions), 'South' (23 accessions) and 'Oasis', for oases in the South-West region (37 accessions).

2.2. Morphological characterization

Morphological characterization was carried out using the International Union for the Protection of New Varieties of Plants descriptor (UPOV, 1979, release 2005 http://www.upov.int/restrict/fr/index.html). The studied characters concerned fruits, leaves, and trees and were distributed as follows: 16 qualitative ordinal variables and 12 qualitative nominal variables. In addition, 13 quantitative morphometric parameters were measured on fruits and leaves. Morphological characterization was thus conducted on a set of 41 quantitative and qualitative morphological traits according to the modalities defined in Table 2.

All fruit observations were conducted on 25 tree-ripened fruits. All leaf observations were conducted on fully developed leaves from the central third of growing shoots (UPOV, 1979) and the representative samples selected to carry out this study were defined according to the criteria described in Table 2. Leaves were therefore collected in the autumn from the median part of long shoots of the current year, while fruits were collected according to the procedure described by Monestiez et al. (1990), whereby each tree was divided into four sides (north, south, east and west) and each side was divided into three levels (upper, middle and lower). In one collected sample, each unit was represented by 3–4 fruits. Consequently, the collected samples consisted of around 40 fruits, corresponding to 9–10 fruits from each side of the tree. The number of fruits exceeded that required by the descriptor because there was a risk of fruit damage during transport. The same procedure was adopted for leaves, whereby 30 leaves were collected from each tree in order to select the most representative leaves according to the specific characters of each variety.