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Microsatellite polymorphism in Tunisian pomegranates (*Punica granatum* L.): Cultivar genotyping and identification

Jbir Rania [a], Zehdi Salwa [a], Hasnaoui Najib [a], Ben Dhiaf Amal [a], Mars Messaoud [b], Salhi Hannachi Amel [a,*]

[a] *Laboratory of Molecular Genetics, Immunology & Biotechnology, Faculty of Sciences, University El Manar, Campus Universitaire, 2092 El Manar, Tunis, Tunisia*
[b] *U.R. Agrobiodiversity, High Agronomic Institute, 4042 Chott-Mariem, University of Sousse, Tunisia*

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ABSTRACT

Specific microsatellites (SSRs) markers were used to characterize a set of 32 Tunisian pomegranate (*Punica granatum* L.) cultivars. Using 13 SSR primers, a total of 40 alleles and 46 genotypes have been identified. As a result, data proved that a high level of polymorphism characterizes the Tunisian pomegranate germplasm at the DNA level. The derived Neighbour-joining (NJ) dendrogram constructed using DAS genetic distances exhibited a genetic diversity structured independently from the geographical origin of cultivars and their denomination. This result suggested that a common genetic basis may characterize Tunisian pomegranate cultivars despite their phenotypic divergences. Furthermore, based on the multilocus genotypes a cultivar's identification key has been established and permitted to unambiguously differentiate between varieties. The obtained results are discussed in term of establishment and management of a national collection of pomegranate varieties, conformity checks, identification of homonyms and synonyms, and screening of the local resources. Furthermore, this microsatellite-based key is a first step towards a marker-assisted identification pomegranate database.

1. Introduction

The pomegranate, *Punica granatum* L. (2n = 16), included in the family of Punicaceae, is one of the oldest known fruit trees (Guarino et al., 1990; Melgarejo Moreno and Martínez Valero, 1992). Pomegranates have widely spread, since Roman period, in different Mediterranean countries where a large number of cultivars have been identified (Ozguven, 1996; Mars, 1996, 2001; Mars and Marrakchi, 1998). This area was, therefore, considered as secondary centre of diversification of this crop. Since it is a monoecious and preferably allogamous, species develop male and perfect flowers (Mars and Marrakchi, 2004). The pomegranate has been described variously as self-pollinated, self and cross-pollinated, highly cross-pollinated or often cross-pollinated (Karale et al., 1993). Pomegranate cultivars, selected traditionally by farmers, were maintained through vegetative propagation and in course of time, have received names referring mainly to geographical origin and/or fruit colour (Mars and Marrakchi, 2004). Genetic erosion due to biotic and abiotic stresses was also reported and concerned many cultivars that are well adapted to local and regional conditions (Levin, 1995; Mars et al., 1994; Mars and Marrakchi, 1998). Establishment of genetic resources conservation strategies has, therefore, become imperative in order to preserve local pomegranate germplasm. In Tunisia, prospections were conducted in all pomegranate growing areas and permitted the collection of numerous cultivars (Mars and Marrakchi, 1998; Mars, 2001). Identification and characterization of the collected genotypes constitutes an attractive

* Corresponding author. Tel.: +216 71 87 26 00; fax: +216 70 86 04 32.
E-mail address: Amel.SalhiHannachi@fsb.rnu.tn (S.H. Amel).

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task to examine level and distribution of genetic diversity in this crop. For this purpose, studies have reported the use of morpho-phenological and pomological traits to characterize pomegranate cultivars (Mars and Marrakchi, 1999, 2004). As suggested by Mars and Marrakchi (1999) and Mars and Marrakchi (2004) a high phenotypic variability characterize local resources regarding their leaf, flower and fruit characteristics. Accessions are often classified as sweet, sweet-sour and sour, early, mid season and late, juicy and table fruit, soft-seeded and hard-seeded or major and minor. Also, chemical and biochemical parameters have been used for Tunisian pomegranates and permitted to discriminate cultivars according to fruit constituents (Ben Nasr et al., 1996; Hasnaoui et al., 2010). However, these analyses are less rewarding since they were based on parameters limited in number and/or highly influenced by the environmental conditions. To overcome such inconvenience, AFLP markers were developed and successfully used to characterize cultivars and to establish genetic relationships between varieties (Jbir et al., 2008, 2009). Recently, in order to provide necessary additional information on pomegranate genetic resources, SSR (Simple Sequence Repeats or microsatellites) primers were developed and designed for *P. granatum* L. (Hasnaoui et al., 2010; Pirseyedi et al., 2010; Ebrahimi et al., 2010). In fact, SSRs are of several benefits over other available DNA-based methods. They are highly frequent, uniformly dispersed over genomes, easily transferable, of co-dominance inheritance and able to generate high levels of polymorphism. Here, microsatellite markers were used to examine the level and structure of genetic diversity and develop an identification key for this fruit crop.

2. Materials and methods

2.1. Plant material

Thirty two Tunisian pomegranate cultivars maintained in *ex situ* collection were used in this study. Table 1 illustrates the denomination and origin of the studied cultivars. According to their geographical origin, considered cultivars were ranged into two groups namely: South and North (Table 1). The plant material consisted of young leaves sampled from adult trees. The sampled plant material was freshly used or frozen at −80 °C for nucleic acids purification.

2.2. DNA isolation

Total cellular DNA was purified from young leaves as described by Dellaporta et al. (1984). DNA concentration was estimated by analytic agarose gel electrophoresis according to Sambrook et al. (1989).

Table 1
Denomination and origin of the 32 studied Tunisian pomegranate cultivars.

Label	Cultivar	Geographical origin	Group
TN9-2	Tounsi 9	Sedaghiane (Jerba)	South
JR2	Jerbi 2	Sedaghiane (Jerba)	
TN10	Tounsi 10	Srandi (Jerba)	
TN17	Tounsi 17	El May (Jerba)	
CH17	Chelfi 17	Srandi (Jerba)	
BY1	Beyounsi 1	Sedaghiane (Jerba)	
CH7	Chelfi 7	Srandi (Jerba)	
CH8-2	Chelfi 8-2	Sedaghiane (Jerba)	
CH8-3	Chelfi 8-3	Sedaghiane (Jerba)	
CH9	Chelfi 9	Sedaghiane (Jerba)	
CH15	Chelfi 15	Sedaghiane (Jerba)	
CH16	Chelfi 16	El May (Jerba)	
GB11	Gabsi 11	Sedaghiane (jerba)	
ZH11	Zehri 11	Sedaghiane (jerba)	
RF1	Rafrafi 1	Zerkine (Mareth)	
GS1	Garsi 1	Tozeur	
GB19	Gabsi 19	Sbikha	North
JB8	Jebali 8	Sbikha	
CH13	Chelfi	Sbikha	
ZH4	Zehri 4	Sidi Bou Ali	
CH4	Chelfi 4	Sidi Bou Ali	
ZH5	Zehri 5	Beni Khalled	
JB5	Jebali 5	Mehrine	
JB2	Jebali 2	El Alia	
JB4	Jebali 4	El Alia	
CHT1	Chetoui	El Alia	
GB6	Gabsi 6	Ghar El Meleh	
NB1	Nebli	Esslouguia (Testour)	
CH3	Chelfi 3	Testour	
TN2	Tounsi 2	Testour	
FP2	Double Flowers Variegated	Tunis	
FP1	Double Flowers Red	Tunis	