Usefulness of Microsatellites for Positioning the Tunisian almond germplasm in its Mediterranean Geographic Context

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Abstract

In Tunisia, the almond tree, (*Prunus dulcis* Mill.) dating back to ancient times, has been grown extensively since the Carthaginian era. In the framework of a national project on the characterisation and the conservation of the local almond germplasm many morphological and molecular markers (RAPD and SSR) have been used in order to analyse the genetic diversity of the main almond cultivars and to seek for the genetic position of these resources in the Mediterranean basin. For this study, 10 SSRs were used to analyse 82 almond accessions from different origins. Most of them originated from Tunisia (50), the others included in the National collection were from France (9), Italy (7), Morocco (1), Spain (8), USA (3), or were of unknown origin (4). The dendrogram based on UPGMA analysis using the similarity matrix generated by the Nei and Li (1979) coefficient presented four main clusters (A, B, C and D). In group A were present 40 of the 50 local genotypes that originate from the centre and the south contrarily, all cultivars from the north were in group C and clustered with the European and American genotypes. Accordingly, our study stressed the large diversity of the Tunisian almonds and revealed the presence of two distinct genetic groups. One located in the north genetically close to the gene pool of the Northern border of the Mediterranean and the second in the central and southern part that is highly adapted to different abiotic stress mainly drought.

INTRODUCTION

The almond tree, (*Prunus dulcis* Mill.) dating back to ancient times, has been grown extensively in Tunisia since the Carthaginian era. Occupying the second position after olive with approximately 22 millions of trees covering more than 250.000 ha, Tunisian almond plantations are located throughout all the country in different climatic conditions.

In fact recent prospecting efforts have pointed out a large adaptation of this species to different climatic conditions varying from the humid in the north with a rainfall of more than 700 mm/year to the arid and harsh climate with rainfall less than 150 mm/year. Furthermore many authors (Felipe, 2000; Gradziel, 2011) have underlined the contribution of this region of the world to the dissemination of almond in between both shores of the Mediterranean region.

Microsatellites or SSRs are currently being employed for molecular characterization, for estimation of genetic diversity and genetic relationships among almond cultivars and related *Prunus* species. As little information is available about the origin of the genetic diversity of the Tunisian germplasm, the relatedness within Tunisian almond cultivars and their relationship with the others originated from other countries, the aims of this work are to identify by SSR analysis the cultivars preserved in the Tunisian National Collection and the landraces collected directly from different sites of the country (Sidi Bouzid and Bizerte), to determine their relatedness to European and American cultivars and to estimate the origin of the existent diversity.

MATERIALS AND METHODS

Plant material: 82 almond genotypes most of them originated from Tunisia (50), the others included in the National collection were from France (9), Italy (7), Morocco (1), Spain (8), USA (3), or were of unknown origin (4) (Table 1). Leaves were sampled in early summer and were immediately ground to a powder in liquid nitrogen before storage at -80°C.

DNA extraction: Total DNA was extracted from young and healthy leaf tissue following the protocol of Doyle & Doyle (1987).

DNA amplification: DNA was amplified by PCR using ten primer pairs of microsatellite , nine pairs derived from a library enriched for AG/TC motifs, constructed with the almond cultivar 'Texas' (Mnejja et al. 2005) and one pair previously cited by Joobeur et al. (2000).

Data analysis: Data were analyzed as discrete variables (1) for the presence and (0) for the absence of a similar band. Cluster analysis was done using SAHN procedure of NTSYS software ver. 2.1 (Rohlf 2000), which uses the unweighted pair group method with arithmetic averages (UPGMA) to cluster the genotypes. Obtained results were used to construct a final dendrogram showing all accessions. Bootstrap support values were obtained from 2000 replicates using TREECON 1.3b (Van de Peer and De Wachter 1994).

RESULTS AND DISCUSSIONS

The majority of the Tunisian genotypes (80%) were clustered together but they showed several minor groups, which revealed their high heterogeneity (cluster A, Fig. 2). This is probably due to the traditional method of propagation of this species all over the country which was mainly done by seeds (open-pollinated), until the more extensively use of grafting in the Mediterranean at the beginning of the 20th century (Grasselly and Crossa Raynaud, 1980). In addition, the need of out-crossing of this species as self incompatible confirmed for the majority of the local genotypes by Gouta et al., 2012 is assumed to be one of the main causes of the existing genetic diversity. The clear distinction between the majority of local cultivars from the central and Southern part and all of the other groups which was not previously demonstrated using RAPD (Gouta et al., 2008) is a proof of the higher discrimination power of SSRs compared to RAPD.

In contrast to what has been observed in group A (Fig. 1), the local cultivars from Bizerte (north of Tunisia), were clustered in the group C with some European and all the North American cultivars. These last originated from material of the Languedoc region of

France (Kester 1994). In fact, the position of this area in the extreme north of Tunisia probably favored the exchange of genotypes between both shores of the Mediterranean Sea (Fig.3). The presence in group C of the two cultivars: 'Porto Farina' as was the old name of Ghar El Melh (a city in Bizerte) and 'Faggoussi' could be another fact in favor of this hypothesis. Moreover, the high bootstrap values observed in the sub cluster grouping cultivars from Bizerte (85% for 'Abiodh Ras Djebel' and 'Khoukhi' and 93% for 'Dillou' and 'Blanco') support the specificity of this site.

CONCLUSIONS

SSRs analysis has been successfully used to examine the crop origin, geographic divergence and distributions of the Tunisian germplasm. This study reveals the high diversity and the distinct origin of the Tunisian almond germplasm and can be considered as a first step in understanding the origin of local and traditional cultivars. Thus, all the Tunisian genotypes except the northern cultivars from Bizerte were genetically distant from the European and American cultivars studied. This supposes the existence in Tunisia of two gene pools. One in the North that may originates from materiel exchange with countries of the Northern border of the Mediterranean and a second in the Central and Southern part probably issued from the ancient silk route of Timbuktu. The great diversity found in the almond germplasm supports the idea that Tunisia has a valuable source of almond genes to be exploited in further international breeding programs.

Literature Cited

Doyle, J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochem. Bull. 19:11-15.

Felipe, A.J. 2000. Variedades de almendro. In: El Almendro. Volume I El Material Vegetal. Integrum, Saragosse, Spain. 461 pp.

Gouta, H., Ksia, E. Zoghlami, N. Zarrouk, M. and Mliki, A. 2008. Genetic diversity and phylogenetic relationships among Tunisian almond cultivars revealed by RAPD markers. J. Hort. Sci. Biot. 83:707-712.

Gouta, H. 2012. Morphological and molecular characterization of Almond genotypes cultivated in Tunisia. Ph.D Thesis, Faculty of Sciences, Sfax, Tunisia. 144 pp.

Gradziel, T.M. 2011. Origin and dissemination of almond. In: Horticultural Reviews, ed. J. Janick Wiley-Blackwell 38:23-81.

Grasselly, C. and Crossa-Raynaud, P. 1980. Les variétés d'amandier In : L'Amandier. Maisonneuve G.R. et Larose, Paris XII, France. 446 pp.

Joobeur, T., Periam, N., De Vicente, M.C., King, J. and Arús, P. 2000. Development of a second generation linkage map for almond using RAPD and SSR markers. Genome 43:649-655.

Kester, D.E. 1994. Almond cultivar and breeding programmes in California. Acta Hort. 373:13-28.

Mnejja, M., Garcia-Mas, J., Howad, W. and Arús, P. 2005. Development and transportability across Prunus species of 42 polymorphic almond microsatellites. Mol. Ecol. Notes 5:531-535.

Rohlf, F.J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Publishing, Setauket, NY.

Van De Peer, Y. and De Wachter, R. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Computer Application in the Biosciences 10:569-570.

Tables

Table 1. List of origin, location and description of the 82 almond genotypes studied.

Cultivar	Origin and Location	Description
Abiodh Ras Djebel	Bizerte (Tunisia) - E. C.	Old local cultivar
Faggoussi	Bizerte (Tunisia) - E. C.	Old local cultivar
Khoukhi	Bizerte (Tunisia) - E. C.	Old local cultivar
Harth Nefta	Nefta (Tunisia) - E. C.	Seedling selection
Achaak M.	Sfax (Tunisia) - E. C.	Seedling of Achaak
Abiodh de Sfax	Sfax (Tunisia) - E. C.	Old local cultivar
Achaak	Sfax (Tunisia) - E. C.	Old local cultivar
Elloumi	Sfax (Tunisia) - E. C.	Old local cultivar
Fekhfekh	Sfax (Tunisia) - E. C.	Old local cultivar
Grosse Tendre de Sfax	Sfax (Tunisia) - E. C.	Old local cultivar
Guerneghzel	Sfax (Tunisia) - E. C.	Old local cultivar
Guerneghzel CH.	Sfax (Tunisia) - E. C.	Old local cultivar
Ksontini B	Sfax (Tunisia) - E. C.	Old local cultivar
Mahsouna	Sfax (Tunisia) - E. C.	Old local cultivar
Sahnoun CH.	Sfax (Tunisia) - E. C.	Old local cultivar
Triki	Sfax (Tunisia) - E. C.	Old local cultivar
Zahaaf	Sfax (Tunisia) - E. C.	Old local cultivar
Tozeur 1	Tozeur (Tunisia) - E. C.	Seedling selection
Tozeur 2	Tozeur (Tunisia) - E. C.	Seedling selection
Tozeur 4	Tozeur (Tunisia) - E. C.	Seedling selection
B200	Unknown - E. C.	Unknown origin
B202	Unknown - E. C.	Unknown origin
B203	Unknown - E. C.	Unknown origin
B204	Unknown - E. C.	Unknown origin
Forme en Boule	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Forme en Poire	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Houcine B.N. 2	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Lakhdhar	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Port retombant	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 1	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 2	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 3	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 4	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 5	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 6	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 7	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 8	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Tlili 9	Ben Aoun (Sidi Bouzid - Tunisia)	Chance seedling
Belgacem N.2	Regueb (Sidi Bouzid - Tunisia)	Chance seedling
Guernghzel B.N.	Regueb (Sidi Bouzid - Tunisia)	Chance seedling
Cheikh Sadok 1	Regueb (Sidi Bouzid - Tunisia)	Unknown origin
Cheikh Sadok 3	Regueb (Sidi Bouzid - Tunisia)	Unknown origin
Cheikh Sadok 4	Regueb (Sidi Bouzid - Tunisia)	Unknown origin

Table 1 (continued):

Cultivar	Origin and Location	Description
Ancetre 1	Ouled Haffouz (Sidi Bouzid - Tunisia)	Unknown origin
Bouchouka B.S.	Ouled Haffouz (Sidi Bouzid - Tunisia)	Unknown origin
Bouchouka. K.F.	Ouled Haffouz (Sidi Bouzid - Tunisia)	Unknown origin
K.F.3	Ouled Haffouz (Sidi Bouzid - Tunisia)	Chance seedling
K.F.4	Ouled Haffouz (Sidi Bouzid - Tunisia)	Chance seedling
Merghad H.1	Ouled Haffouz (Sidi Bouzid - Tunisia)	Chance seedling
Nabil F.	Ouled Haffouz (Sidi Bouzid - Tunisia)	Chance seedling
Porto Farina*	Ouled Haffouz (Sidi Bouzid - Tunisia)	Unknown origin cultivar
Blanco	Bizerte (Tunisia)	Old local cultivar
Dillou	Bizerte (Tunisia)	Unknown origin cultivar
Khoukhi Bizerte	Bizerte (Tunisia)	Old local cultivar
Bruantine	France - E. C.	Old local cultivar
Doree	France - E. C.	Old local cultivar
Ferraduel	France - E. C.	Cristomorto x Aï ♣
Ferragness	France - E. C.	Cristomorto x Aï ♣
Fournat de Breznaud	France - E. C.	Marie (1901) ♣
Languedoc	France - E. C.	Old local cultivar
Lauranne	France - E. C.	Ferragness x Tuono ♣
Pointue d'Aureille	France - E. C.	Old local cultivar
Soucaret	France - E. C.	Old local cultivar
Avola	Italy - E. C.	Old local cultivar
Cristomorto	Italy - E. C.	Unknown origin
Fasciuneddu	Italy - E. C.	Unknown origin
Genco	Italy - E. C.	Genco G. (1910)♣
Mazetto syn. Tuono	Italy - E. C.	Old local cultivar
Pizzuta	Italy - E. C.	Old local cultivar
Super Nova	Italy - E. C.	Induced mutation from Fascionello♣
Desmayo Larguetta.	Spain - E. C.	Old local cultivar
Desmayo Rojo	Spain - E. C.	Unknown origin
Guara	Spain - E. C.	Old local cultivar
Malagueña	Spain - E. C.	Old local cultivar
Marcona	Spain - E. C.	Old local cultivar
Mas Bovera	Spain - E. C.	Primorskiy x Cristomorto ♣
Moncayo	Spain - E. C.	Tardive de la verdiere x Tuono
Tarragona	Spain - E. C	Unknown origin
Nec Plus Ultra	USA - E. C.	Hatch A.T. (1884)♣
Non Pareil	USA - E. C.	Hatch A.T. (1884)♣
Peerless	USA - E. C.	Unknown origin

♣A.J. Felipe, (2000).

*This cultivar was identified in Sidi Bouzid but Porto Farina is the native name of a city (actually Ghar El Melh) in Bizerte.

E.C: Accessions are from Ettaous National Collection.

Figures

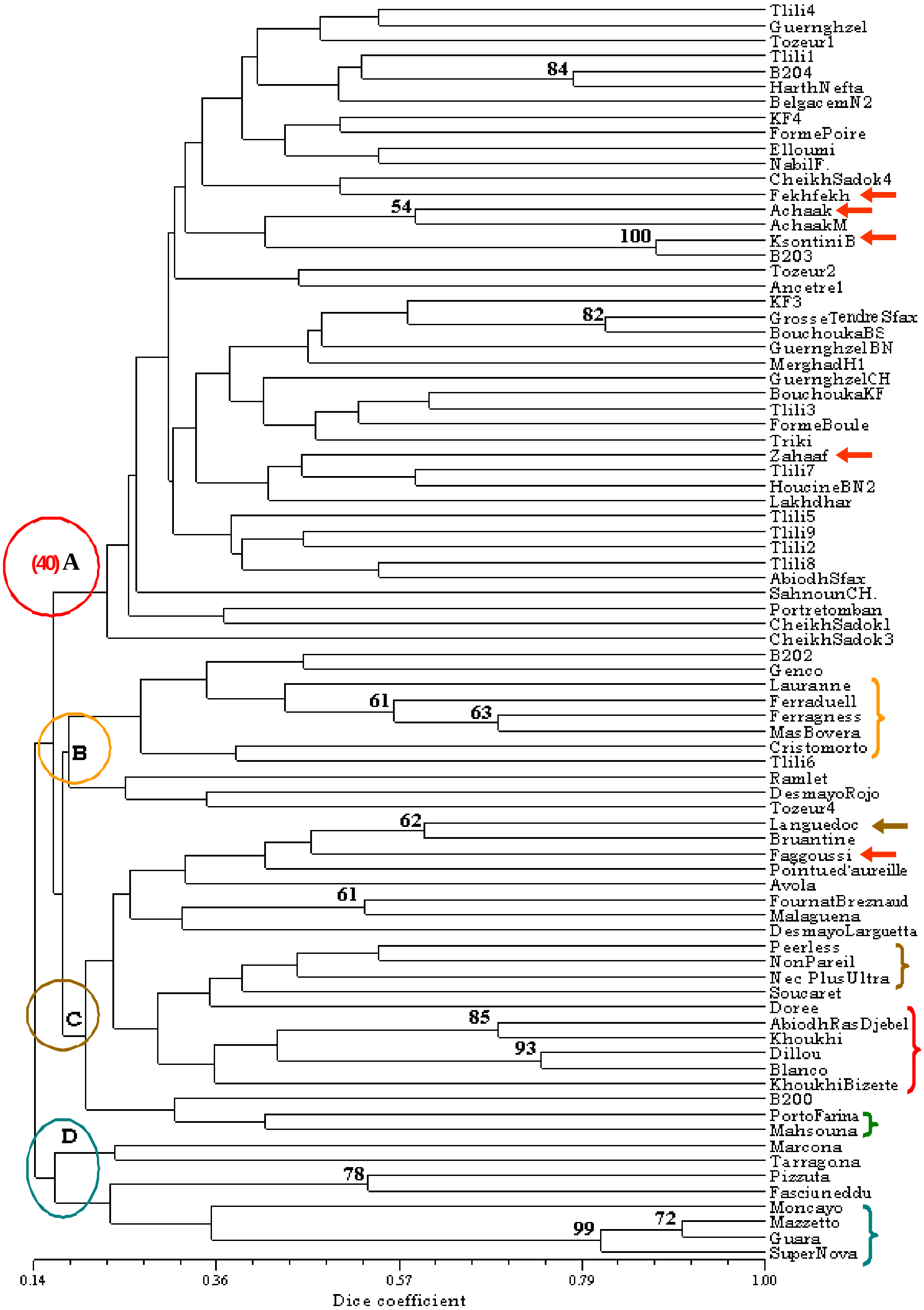

Fig. 1. Dendogram based on Dice coefficient illustrating the genetic similarities among 82 almond genotypes obtained by 10 SSR Primers data.