Development of an SSR-based identification key for Tunisian local almonds

Hassouna Gouta[1], Elhem Ksia[2], Tatiana Buhner-Zaharieva[3], Ahmed Mliki[4], Yolanda Gogorcena[5]*

[1]Unité des Ressources Génétiques et de l'Amélioration de l'Olivier, de l'Amandier et du Pistachier/Institut de l'Olivier, P.O. Box 014 – 4061 – Sousse – Tunisia.
[2]Faculty of Sciences, Campus Universitaire, 1060, Tunis – Tunisia.
[3]Universidad de Zaragoza – Dept. of Organic Chemistry, Pedro Cerbuna, 12 – 50009 – Zaragoza – España.
[4]Centre de Biotechnologie Borj-Cedria, P.O. Box 901 – 2050 – Hammam-Lif – Tunisia.
[5]CSIC/Estación Experimental de Aula Dei – Depto. de Pomología, Apdo. 13034 – 50080 – Zaragoza – España.
*Corresponding author <aoiz@eead.csic.es>

Edited by: Antonio Costa de Oliveira

Received March 03, 2011
Accepted September 15, 2011

ABSTRACT: Ten simple sequence repeat (SSR) loci were used to study polymorphism in 54 almond genotypes. All genotypes used in this study originated from almond-growing areas in Tunisia with different climatic conditions ranging from the sub-humid to the arid and are preserved in the national collection at Sidi Bouzid. Using ten SSR, 130 alleles and 250 genotypes were revealed. In order to develop an identification key for each accession, the data were analysed separately for each microsatellite marker. The most polymorphic microsatellite (CPDCT042) was used as a first marker. Two microsatellite loci (CPDCT042 and CPDCT025) were sufficient to discriminate among all accessions studied. Neighbour-joining clustering and principal coordinate analysis were performed to arrange the genotypes according to their genetic relationships and origin. The results are discussed in the context of almond collection management, conformity checks, identification of homonyms, and screening of the local almond germplasm. Furthermore, this microsatellite-based key is a first step toward a marker-assisted identification almond database.

Keywords: *Prunus dulcis* Mill, genetic diversity, genetic resources, microsatellites, molecular characterization

Introduction

Almond [*Prunus dulcis* (Miller) D.A. Webb, syn. *Prunus. amygdalus* Batsch] belongs to the Prunoideae subfamily of Rosaceae, which includes several other species producing fruits of economic importance. Almond is a diploid species with 2n = 16 and a small genome size of ~0.3 pg/1C (Dickson et al., 1992). Traditional almond plantations in Tunisia are characterised by old selected cultivars such as 'Achaak', 'Abiodh', 'Blanco', 'Fekhfekh', 'Khoukhi', 'Ksontini' and 'Zahaaf'. Local germplasm consists of numerous ecotypes (most unknown to consumers) selected by farmers for high production and adaptation to specific agro-ecosystems (Gouta et al., 2010a). Thus, it was necessary to identify these genotypes as a first step in their conservation and protection against potential genetic erosion.

Worldwide, many markers have been used to identify almond genotypes such as isozymes (Arulsekar et al., 1986; Cerezo et al., 1989; Hauagge et al., 1987; Viruel et al., 1995), AFLP (Martins et al., 2001), ISSR (Martins et al., 2003), RAPD (Gouta et al., 2008) and SNP (Wu et al., 2008). Simple sequence repeat (SSR) or microsatellite markers, which are co-dominant and highly reproducible (Powell et al., 1996), have been used for genetic diversity and phylogenic analysis (Sánchez-Pérez et al., 2006; Shiran et al., 2007; Xie et al., 2006; Xu et al., 2004; Zeinalabedini et al., 2007), to study the origin of cultivated genotypes (Zeinalabedini et al., 2009), and to distinguish genetic lineages and characterise an extensive and largely unexploited inter-species gene pool available to peach and almond breeding programs (Martínez-Gómez et al., 2003).

SSR markers have been used for a genetic diversity assessment of Tunisian almond germplasm and to determine its allocation in comparison to some European and American cultivars (Gouta et al., 2010b). However, the relatedness among local cultivars was very briefly described. In this study, we examined the relatedness among this germplasm more closely and develop an identification key based on microsatellite polymorphism.

Materials and Methods

Fifty-four almond accessions of different origins were analyzed (Table 1). Most were identified in various Tunisian growing regions, while others were already preserved in the national collection at Ettaous. Four were of unknown origin and were included in the study set to clarify their origin. All 50 Tunisian local genotypes originated from the regions of Bizerte, Sidi Bouzid, Sfax, Nefta, and Tozeur (Figure 1). Leaf samples for DNA extractions were collected from farmers' fields, but all genotypes were preserved in a new national germplasm collection at Sidi Bouzid.

Young leaves were collected from all accessions for DNA extraction. Total genomic DNA was isolated as described (Doyle and Doyle, 1987). DNA quality was examined by electrophoresis in 0.8 % agarose gel and DNA concentration was quantified by spectrophotometer. Extracted DNA was diluted to 5 ng μL^{-1} with Tris-EDTA buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and stored at -20°C for polymerase chain reaction (PCR) amplification.

DNA was amplified by PCR using ten microsatellite primer pairs (Table 2). Pairs one to nine were derived

Table 1 – Zone of origin and names of 50 Tunisian almond cultivars used to create the identification key.

Zone of origin	Cultivars[z]
North: Bizerte	'Abiodh Ras Djebel'; 'Blanco' (RP); 'Dillou' (RP); 'Faggoussi'; 'Khoukhi'; 'Khoukhi Bizerte' (RP).
Centre: Sidi Bouzid (RP)	'Ancetre 1'; 'Belgacem N.2'; 'Bouchouka K.F.'; 'Bouchouka B.S.'; 'Cheikh Sadok 1'; 'Cheikh Sadok 3'; 'Cheikh Sadok 4'; 'Forme en Boule'; 'Forme en Poire'; 'Guernghzel B.N.'; 'Houcine B.N. 2'; 'Lakhdhar'; 'Merghad H.1'; 'Nabil F.'; 'K.F.3'; 'K.F.4'; 'Port retonbant'; 'Porto Farina'; 'Tlili 1'; 'Tlili 2'; 'Tlili 3'; 'Tlili 4'; 'Tlili 5'; 'Tlili 6'; 'Tlili 7'; 'Tlili 8'; 'Tlili 9'.
South: Sfax	'Abiodh de Sfax'; 'Achaak'; 'Achaak M.'; 'Elloumi'; 'Fekhfekh'; 'Grosse Tendre de Sfax'; 'Guernghzel'; 'Guernghzel CH.'; 'Ksontini B.'; 'Mahsouna'; 'Sahnoun CH.'; 'Triki'; 'Zahaaf'.
Nefta and Tozeur	'Harth Nefta'; 'Tozeur 1'; 'Tozeur 2'; 'Tozeur 4'.

[z]All accessions were collected at the Ettaous National collection, except those signalled as RP. RP: from recent prospecting trips.

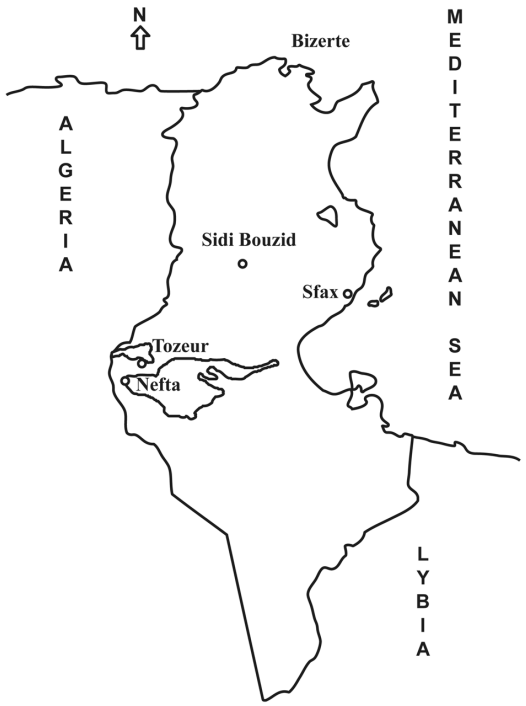

Figure 1 – Location of the collection areas for the cultivars used in this study.

from a library enriched for AG/TC motifs constructed from the almond cultivar 'Texas' (Mnejja et al., 2005). The final pair (number 10) was described previously (Joobeur et al., 2000). Amplification reactions were carried out in a final volume of 15 µL containing 10 ng of template DNA, 1 × reaction buffer (20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl, pH 8.8), 2 mM $MgCl_2$, 50 µM each of dATP, dGTP, dTTP, dCTP (Amersham Pharmacia Biotech, Spain), 0.15 mM forward and reverse primers, and 0.5 U Tth DNA Polymerase (Biotools Band M Labs, S.A., Spain). PCR amplifications were carried out in a Gene Amp 2700 thermocycler (Applied Biosystems, CA, USA) using the following temperature cycles: one cycle of 3 min at 95 °C; 35 cycles of 1 min at 94 °C, 45 s at the corresponding annealing temperature, and 1 min at 72 °C.

Table 2 – Locus name, number of alleles, allele sizes, and number of genotypes identified by using 10 SSR markers of *Prunus* species on 54 almond cultivars.

Locus	Number of alleles	Allele size bp	Number of genotypes
CPDCT022	10	133, 141, 151, 153, 155, 157, 161, 163, 171, 175	27
CPDCT025	15	162, 164, 172, 174, 176, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200	31
CPDCT027	11	156, 158, 160, 162, 164, 166, 172, 174, 176, 198, 180	19
CPDCT033	11	116, 120, 126, 128, 130, 132, 134, 136, 138, 142, 150	19
CPDCT038	12	147, 149, 155, 161, 163, 169, 171, 177, 179, 181, 185, 197	21
CPDCT040	11	138, 146, 156, 160, 162, 164, 166, 168, 170, 172, 174	19
CPDCT042	18	160, 162, 164, 166, 170, 172, 174, 176, 178, 182, 184, 186, 188, 190, 192, 194, 198, 200	36
CPDCT044	16	161, 165, 169, 171, 173, 175, 177, 179, 181, 189, 191, 193, 195, 197, 199, 205	21
CPDCT047	15	170, 174, 176, 182, 184, 186, 190, 192, 194, 198, 204, 206, 212, 214, 216	30
PS9f8	11	130, 132, 138, 144, 146, 148, 150, 154, 168, 170, 178	27
Means	13		25
Total	130		250

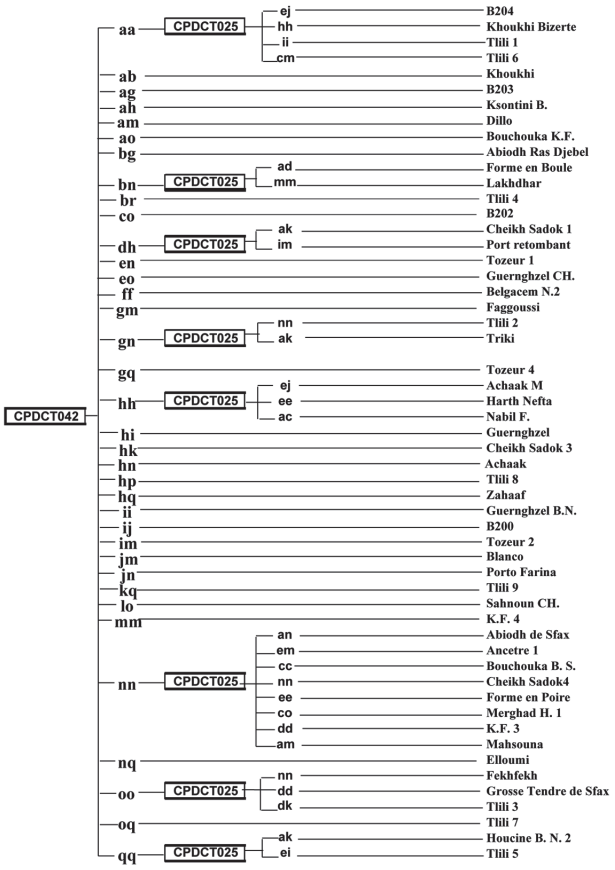

Figure 2 – Identification key and genotypes of 50 Tunisian and four unknown almond cultivars based on two microsatellite markers: CPDCT025 and CPDCT042.

The last cycle was followed by a final incubation for 7 min at 72 °C and the PCR products were stored at 4°C until analysis. Two independent SSR reactions were performed for each DNA sample. The DNA amplification products were loaded on 5 % polyacrylamide sequencing gels. Gels were run for 2 h at 65 W and then silver-stained as described (Bassam et al., 1983). Fragment sizes were estimated using 30-330 bp AFLP ladder DNA sizing markers (Invitrogen, Carlsbad, CA, USA) and analyzed by the Quantity One program (Bio Rad, Hercules, CA, USA).

The genetic relatedness among Tunisian almond cultivars was described using a phylogenetic analysis. To represent the differences among individuals and construct a phylogenetic tree, the simple matching distances $d_{ij} = 1 - \frac{1}{L}\sum_{l}^{L} = 1 \frac{m\,l}{\pi.}$ were calculated with d_{ij}: dissimilarity between units i and j; L: number of loci; p: ploidy; and ml: number of matching alleles for locus l. The individual distance tree was constructed using Darwin 5.0.148 software (Perrier and Jacquemoud-Collet, 2006) and the neighbor-joining method of Saitou and Nei (1987). The robustness of each node was evaluated by bootstrapping data over loci for 10,000 replications. A principal coordinate analysis based on the dissimilarity matrix was also performed with the same software.

Results and Discussion

The 10 microsatellite primer pairs revealed 130 alleles and 250 potential genotypes among the 54 almond accessions studied (Table 2). Overall, primer pairs showed alleles in size ranges larger than those reported (Mnejja et al., 2005). Unique genotypes for all 54 cultivars identified a subset of the best 10 microsatellite primers for almond cultivar differentiation. To develop the identification key, the data were analysed separately for each microsatellite marker. The most polymorphic marker was chosen as the principal marker. The remaining markers were used to separate the genotypes in groups created by the previous marker until all accessions were clearly identified. We began by selecting the most polymorphic loci that revealed the most different genotypes, CPDCT042 (36) and CPDCT025 (31). We based the identification key first on the primer CPDCT042 and then on CPDCT025. These two SSRs discriminated among all 50 Tunisian genotypes as well as the four of unknown origin. Theoretically, these two loci could encompass a total of 36 × 31 = 1116 possible genotypes, suggesting that there is room to expand our key to discriminate more genotypes. The most polymorphic SSR primer, CPDCT042, allowed unambiguous differentiation of 28 of the 54 studied cultivars (52 %). The use of the additional CPDCT025 primer pair was required to identify the remaining cultivars. An identification key was thus established for these local almond accessions (Figure 2).

A similar identification key was obtained for 49 Tunisian date palm cultivars (*Phoenix dactylifera* L.) based on three microsatellite primers, revealing 25 alleles and 57 genotypes (Zehdi et al., 2004). For the Tunisian apricot landraces, 26 *Prunus* microsatellite primers formed an identification key for 54 genotypes (Krichen et al., 2006). With only five primers, it was possible to discriminate among all landraces studied, identifying 103 alleles and 155 different genotypes. In fig (*Ficus carica* L.), it was not possible to discriminate among all 72 Tunisian local ecotypes with six SSR primers, but the identification key revealed 58 alleles and 124 genotypes, for a resolving power of 97.2 % (Saddoud et al., 2007). An identification key for 26 Tunisian olives (*Olea europea* L.) was successful in discriminating among all local cultivars using three of ten SSR markers (Taamalli et al., 2008). Our finding that in almond, the alleles of only two loci were sufficient to discriminate among all the accessions is probably because they were generated from an AG/TC-enriched library constructed from a cultivar of the same species. Thus, while transfer of SSRs among different species in the genus *Prunus* is well documented (Mnejja et al., 2005; Mnejja et al., 2010), our work stresses the greater efficacy of using SSRs generated from the same species for genotyping studies.

The identification key was used to detect differences among cultivars with the same name. For instance, three accessions of 'Guernghzel' ('Guernghzel', 'Guernghzel C.H.', and 'Guernghzel B.N.') that originated from two regions (Sfax and Sidi Bouzid) were distinguished on the basis of one microsatellite primer. In the same way, the accessions 'Khoukhi' and 'Khoukhi Bizerte' were identified as homonyms. Further investigation showed that the accession 'Khoukhi' originated from the region of Bizerte. Additional homonyms were detected for the cultivar 'Bouchouka' (K.F. and B.S. from Sidi Bouzid), which can be distinguished by one primer. Similar cases of homonymy were detected in Tunisian almond germplasm on the basis of RAPD analysis (Gouta et al., 2008), in apricot and grapevines using microsatellites (Krichen et al., 2006; Ulanovsky et al., 2002), and in a fig collection using morphological traits (Mars et al., 1998). Thus, the identification key for almond established in this study is a continuation of an effort that was started several years ago for the molecular characterisation of the main Tunisian fruit species.

A Neighbor-joining phylogenetic tree based on the simple matching distance clustered the 54 almond genotypes into five major groups: A, B, C, D, and E (Figure 3).

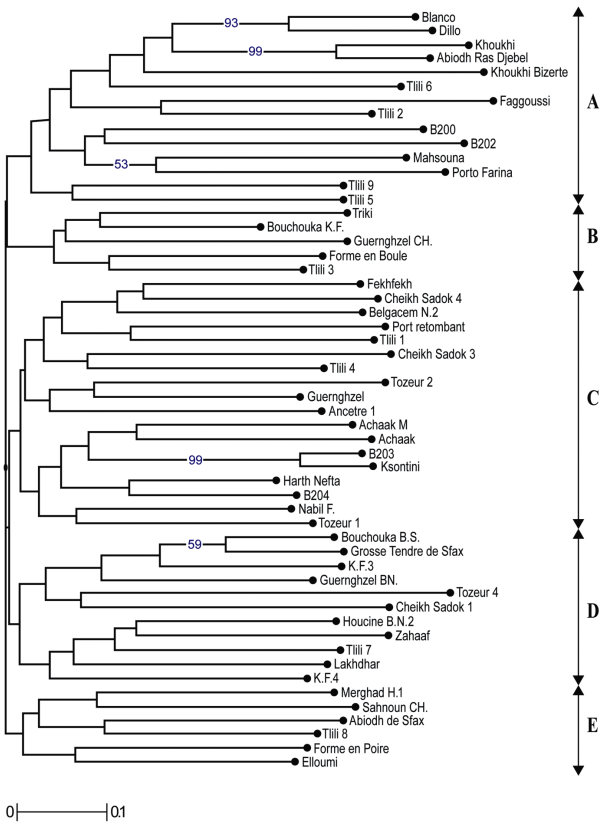

Figure 3 – Unweighted neighbor-joining tree based on the simple matching dissimilarity matrix of 10 SSR markers for 54 almond genotypes. The numbers on the tips indicate bootstrap values expressed in percentages and are shown for all clusters when ≥ 50 %.

Cluster A grouped 14 genotypes, all from northern Tunisia, with 'Mahsouna' from Sfax; 'Tlili 2', 'Tlili 5', 'Tlili 6', and 'Tlili 9' from Sidi Bouzid; and two of the unknowns, 'B200' and 'B202'. The close relatedness revealed between 'Blanco' and 'Dillou' from one side and 'Khoukhi' and 'Abiodh Ras Djebel' from the other side was supported by high bootstrap values of 93 and 98 %, respectively. Moreover, the genotypes 'Blanco' and 'Dillou' shared at least one allele for each SSR used in this work (14 of 20 total alleles) (data not shown). This supposes a common parentage for these two local cultivars of unknown pedigree. The presence of 'B200' and 'B202' in the group implies genetic closeness between these two genotypes and the local cultivars of cluster A. This also suggests a different origin for these genotypes from the two other unknowns, 'B203' and 'B204', which were included in cluster C. This cluster was the largest, with 18 cultivars. The relatedness between 'B203' and the local cultivar 'Ksontini B.', supported by both a bootstrap value of 99 % and 16 of 20 common alleles, supposes a common parentage and consequently a Tunisian origin for this unknown genotype.

Four of the five most commonly planted local cultivars, 'Achaak', 'Fekhfekh', 'Guerneghzel', and 'Ksontini', were included in cluster C; the exception was 'Zahaaf', present in group D. The low similarity (0.75 dissimilarity) between 'Achaak' and 'Zahaaf' observed in this study agrees with previous results (Fernández Marti et al., 2009). The repartition of the different ecotypes 'Tlili' 1 to 9, which originated from the same area (Sidi Ali Ben Aoun in Sidi Bouzid) into all five clusters highlights the importance of an underestimated local diversity and stresses the importance of a continuous collecting effort. The most distant cluster, E, includes three cultivars from Sfax: 'Abiodh de Sfax', 'Elloumi', and 'Sahnoun' and three from Sidi Bouzid: 'Forme en Poire', 'Merghad H.1' and 'Tlili 8'. There is no clear separation between cultivars originating from central and southern Tunisia. This can be explained by their close proximity, while Sfax and Sidi Bouzid have common borders (Figure 1) and their cultivars might have a common origin. Moreover, commercial exchanges between these regions are well documented since ancient times.

Principal coordinate analysis (PCA) generated two clearly important components, PC1 and PC2, which explained 11 and 7.3 %, respectively, of the total variation in SSR data (Figure 4). This analysis showed some well-defined distribution patterns and relationships among the accessions. The divergence of all northern cultivars (Bizerte) was clearly demonstrated by PC1. In addition, a group composed of 'Abiodh Ras Djebel', 'Blanco', 'Dillo', 'Khoukhi', and 'Khoukhi Bizerte' could be clearly separated from the other accessions. The PC2 principal coordinate separated two distinct groups. The first contained genotypes from Sfax and Sidi Bouzid ('Grosse Tendre de Sfax', 'Bouchouka B.S.', and 'KF.3'). The second had cultivars originating from

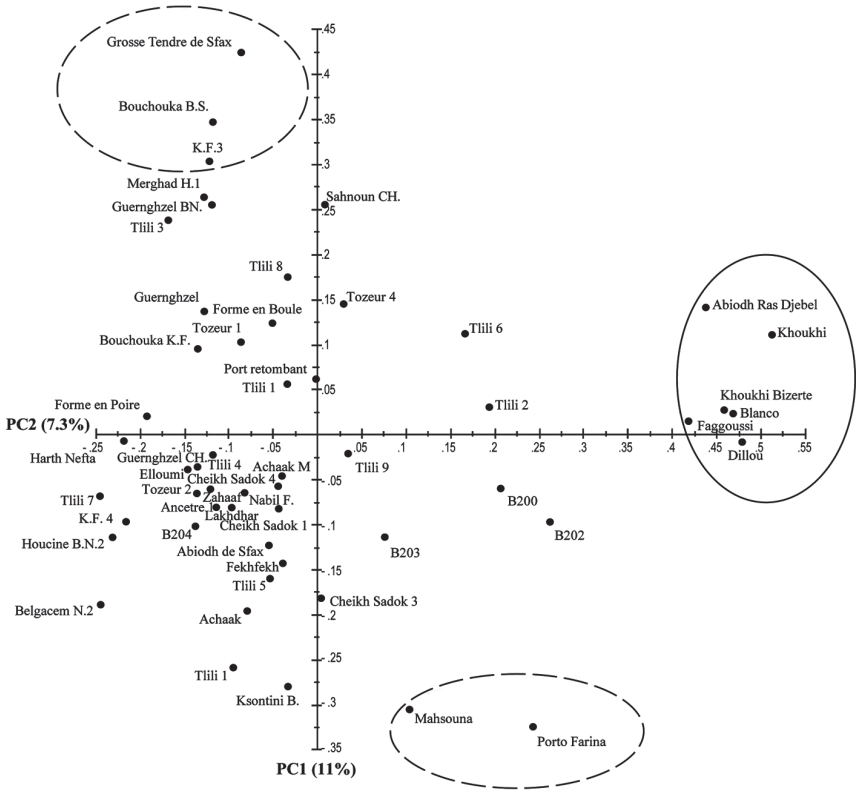

Figure 4 – Plot of the first two components (PC1 and PC2) of the principal coordinate analysis on the dissimilarity matrix obtained for 54 almond accessions using 10 SSR markers.

Sfax and Bizerte ('Mahsouna' and 'Porto Farina'). The neighbour joining analysis placed these two groups in cluster D and cluster A, respectively (Figure 3).

A clear distinction between almond genotypes from northern and southern Tunisia is clearly demonstrated in this paper and supported by previous results (Gouta et al., 2010b). This in turn suggests the presence of two almond gene pools in Tunisia, associated with the geographic position of the cultivars, and implying a different initial ancestry. Only two of 10 microsatellite loci were sufficient to distinguish among all Tunisian almond cultivars analysed in this study. The remaining microsatellite primers allow assessment of genetic relationships between the studied cultivars.

The homonyms elucidated through this paper illustrate the confusion in nomenclature that can be observed in a given region or even between regions in a species such as almond, which adapts well to different climates. Our identification key will aide the description, registration, and certification of plant material and facilitates the rational management and conservation of Tunisian almond germplasm. Moreover, since many ecotypes are cultivated world wide and exchanges of materials among breeders are common, the availability of an efficient SSR-based identification system is of interest.

Acknowledgements

Financial support was provided in part by the Tunisian Ministry of Higher Education, Scientific Research and Technology, the Spanish Ministry of Science and Innovation (AGL2008-00283/AGR co-financed by FEDER), the Aragon Government (Group A44), and the Agencia Española de Cooperación Internacional (A/5339/06 and A/8334/07). We gratefully acknowledge Dr. Mouna Mezghani Aïachi, Rosa Giménez Soro and Dr. M. Ángeles Moreno Sánchez for their valuable advice and support.

References

Arulsekar, S.; Parfitt, D.E.; Kester, D.E. 1986. Comparison of isozyme variability in peach and almond cultivars. Journal of Heredity 77: 272–274.

Bassam, B.J.; Caetano-Anoelles, G.; Gresshoff, P.M. 1983. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry 196: 80–83.

Cerezo, M.; Socias i Company, R.; Arús, P. 1989. Identification of almond cultivars by pollen isoenzymes. Journal of the American Society for Horticultural Science 114: 164–169.

Dickson, E.E.; Arumuganathan, K.; Kresovich, S.; Doyle, J.J. 1992. Nuclear DNA content variation within the Rosaceae. American Journal of Botany 79: 1081–1086.

Doyle, J.J.; Doyle, J.L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochemistry Bulletin 19: 11–15.

Fernández i Marti, Á.F.; Alonso, J.M.; Espiau, M.T.; Rubio-Cabetas, M.J.; Socias i Company, R. 2009. Genetic diversity in Spanish and foreign almond germplasm assessed by molecular characterization with simple sequence repeats. Journal of the American Society for Horticultural Science 134: 535–542.

Gouta, H.; Ksia, E.; Zoghlami, N.; Zarrouk, M.; Mliki, A. 2008. Genetic diversity and phylogenetic relationships among Tunisian almond cultivars revealed by RAPD markers. Journal of Horticultural Science and Biotechnology 83: 707–712.

Gouta, H.; Guenichi, H.; Rezgui, R.; Mars, M.; Zarrouk, M.; Mliki, A.; 2010a. Production system and genetic resources of almond (*Prunus dulcis* L.) in the central western part of Tunisia. Revue des Régions Arides 24: 235–241 (in French, with abstract in English).

Gouta, H.; Ksia, E.; Buhner, T.; Moreno, M.A.; Zarrouk, M.; Mliki, A.; Gogorcena, Y. 2010b. Assessment of genetic diversity and relatedness among Tunisian almond germplasm using SSR markers. Hereditas 147: 283–292.

Hauagge, R.; Kester, D.E.; Arulsekar, S.; Parfitt, D.E.; Liu, L. 1987. Isozyme variation among California almond cultivars. II Cultivar characterization and origins. Journal of the American Society for Horticultural Science 112: 693–698.

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

Krichen, L.; Mnejja, M.; Arús, P.; Marrakchi, M.; Trifi Farah, N. 2006. Use of microsatellite polymorphisms to develop an identification key for Tunisian apricots. Genetic Resources and Crop Evolution 53: 1699–1706.

Mars, M.; Chebli, T.; Marrakchi, M. 1998. Multivariate analysis of fig (*Ficus carica* L.) germplasm in southern Tunisia. Acta Horticulturae 480: 75–81.

Martínez-Gómez, P.; Arulsekar, S.; Potter, D.; Gradziel, T.M. 2003. An extended interspecific gene pool available to peach and almond breeding as characterized using simple sequence repeat (SSR) markers. Euphytica 131: 313–322.

Martins, M.; Farinha, A.; Ferreira, E.; Cordeiro, V.; Monteiro, A.; Tenreiro, R.; Oliveira, M. 2001. Molecular analysis of the genetic variability of Portuguese almond collections. Acta Horticulturae 546: 449–452.

Martins, M.; Tenreiro, R.; Oliveira, M. 2003. Genetic relatedness of Portuguese almond collection assessed by RAPD and ISSR markers. Plant Cell Report 22: 71–78.

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

Mnejja, M.; Garcia-Mas, J.; Audergon, J.M.; Arús P. 2010. *Prunus* microsatellite transportability across rosaceous crops. Tree Genetic and Genomes 6: 689–700.

Perrier, X.; Jacquemoud-Collet, J.P. 2006. DARwin software. Available at: http://darwin.cirad.fr/darwin [Accessed Aug. 14, 2007]

Powell, W.; Morgante, M.; Andre, C.; Hahafey, M.; Vogel, J.; Tingley, S.; Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2: 225–238.

Saddoud, O.; Chatti, K.; Salhi-Hannachi, A; Mars, M.; Rhouma, A.; Marrakchi, M.; Trifi, M. 2007. Genetic diversity of Tunisian figs (*Ficus carica* L.) as revealed by nuclear microsatellites. Hereditas 144: 149–157.

Saitou, N.; Nei, M. 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology Evolution 4: 406–425.

Sánchez-Pérez, R.; Ballester, J.; Dicenta, F.; Arús, P.; Martínez-Gómez, P. 2006. Comparison of SSR polymorphisms using automated capillary sequencers, and polyacrylamide and agarose gel electrophoresis: implications for the assessment of genetic diversity and relatedness in almond. Scientia Horticulturae 108: 310–316.

Shiran, B.; Amirbakhtiar, N.; Kiani, S.; Mohammadi, S.; Sayed-Tabatabaei, B.E.; Moradi, H. 2007. Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. Scientia Horticulturae 111: 280–292.

Taamalli, W.; Guena, F.; Bassi, D.; Daoud, D.; Zarrouk, M. 2008. SSR marker based DNA fingerprinting of Tunisian olive (*Olea europea* L.) varieties. Journal of Agronomy **7**: 176–181.

Ulanovsky, S.; Gogorcena, Y.; Martínez De Toda, F.; Ortiz, J.M. 2002. Use of molecular markers in detection of synonymies and homonymies in grapevines (*Vitis vinifera* L.). Scientia Horticulturae 92: 241–254.

Viruel, M.A.; Messeguer, R.; De Vicente, M.C.; Garcia-Mas, J.; Puigdomenech, P.; Vargas, F.J.; Arús, P. 1995. A linkage map with RFLP and isozyme markers for almond. Theoretical and Applied Genetics 91: 964–971.

Wu, S.B.; Wirthensohn, M.G.; Hunt, P.; Gibson, J.P.; Sedgley, M. 2008. High resolution melting analysis of almond SNPs derived from ESTs. Theoretical and Applied Genetics 118: 1–14.

Xie, H.; Sui, Y.; Chang, F.Q.; Xu, Y.; Ma, R.C. 2006. SSR allelic variation in almond (*Prunus dulcis* Mill.). Theoretical and Applied Genetics 112: 366–372.

Xu, Y.; Ma, R.C.; Xie, H.; Liu, J.T.; Cao, M.Q. 2004. Development of SSR markers for the phylogenetic analysis of almond trees from China and the Mediterranean region. Genome 47: 1091–1104.

Zehdi, S.; Trifi, M.; Billotte, N.; Marrakchi, M.; Pintaud, J.C. 2004. Genetic diversity of Tunisian date palm (*Phoenix dactylifera* L.) revealed by nuclear microsatellite polymorphism. Hereditas 141: 278–287.

Zeinalabedini, M.; Majourhat, K.; Khayam-Nekoui, M.; Grigorian, V.; Toorchi, M.; Dicenta, F.; Martínez-Gómez, P. 2007. Molecular characterization of almond cultivars and related wild species using nuclear and chloroplast DNA markers. Journal of Food and Agriculture Environment 5: 242–247.

Zeinalabedini, M.; Majourhat, K.; Khayam-Nekoui, M.; Grigorian, V.; Torchi, M.; Dicenta, F.; Martínez-Gómez, P. 2009. Study of the origin of the cultivated almond using nuclear and chloroplast DNA Markers. Acta Horticulturae 814: 695–700.