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Contribution in tracing the origin and evolutional history of almond around the Mediterranean basin

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Abstract. Results regarding the origin and domestication steps of almond and its dissemination around the Mediterranean basin are very controversial. The general belief is that the domestication was in the eastern side and then its dissemination to the western part was through the different trade routes. A recent study has proved using a combination of nuclear and chloroplast microsatellites a westward dispersal of almond and a long-standing presence of domesticated almond in northern Africa. In order to contribute to this discussion, new interpretation of the previous study using 10 SSRs to analyse 82 almond accessions from different origins was undertaken. The dendogram based on the similarity matrix presented four main clusters. In group A were present the majority of the 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. In fact, the Bayesian-based analysis endorses a two divergent population structure. Accordingly, our study supports the presence of two genetically distinct groups. One located in the north and a second in the central and southern part that may be issued from mutational processes. Thus, our work supports the hypothesis of the French team regarding a native existence of almond in North Africa and a possible westward dispersal.

Keywords. *Prunus dulcis* L. – Domestication – Gene pools – Bayesian analysis.

Contribution au traçage de l'origine et de l'histoire évolutive de l'amandier aux alentours du bassin méditerranéen

Résumé. *Les résultats relatives à l'origine et aux différentes étapes de domestication et de dissémination de l'amandier au alentour du bassin méditerranée sont très controversées. Les hypothèses avancées à ce jour évoquent une domestication dans la zone Est suivie d'une dissémination au coté Ouest à travers les différentes voies de commerce terrestres et maritimes. Des études récentes ont montré par combinaison de marqueurs microsatellites nucléaires et chloroplastiques l'existence plausible d'une étape de dissémination West-est et d'une présence très ancienne de l'amandier dans le nord de l'Afrique. Ainsi et dans le but de contribuer à cette discussion une nouvelle interprétation des résultats relatifs à l'utilisation des dix marqueurs microsatellites utilisées pour la caractérisation moléculaire de 82 génotypes d'amandier issus de différentes origines a été entrepris. Le dendrogramme basé sur la matrice de similarité montre la présence de 4 groupes distincts. Dans le groupe A on note la présence de toutes les variétés et écotypes locaux issus du centre et du sud tunisien alors que dans le groupe C existent toutes les variétés locales du nord regroupées avec celles originaires de l'Europe et de l'Amérique. De plus, l'analyse bayesienne approuve la présence en Tunisie de deux pools génétiques d'amandier très distincts. Ainsi, ce travail soutient l'hypothèse émise par l'équipe française quand à l'ancienne existence de l'amandier en l'Afrique du nord ainsi que la possibilité d'une dissémination west-est.*

Mots-clés. Prunus dulcis *L. – Domestication – Pools de gènes – Analyse bayesienne.*

I – Introduction

Almond trees belong to the subgenus *Amygdalus* (L.) Focke, an Irano-Turanian complex of *Prunus* including more than 30 species (Browicz and Zohary 1996) but only *P. dulcis* was domesticated to produce sweet almonds. The introduction of cultivated almond in the eastern Mediterranean area took place by the second millennium BCE and there is evidence of extensive of almond trade in the fourth century BC (Cerda, 1973). Kester *et al.* (1991) suggested the wide dissemination of modern almond and its cultivation in four separated phases: Asiatic, Mediterranean, Californian and Southern Hemisphere. In Tunisia the origin of the existing almond genotypes is very controversial being the granary of the Roman Empire it is considered as the main routes along witch almond was spread along the Mediterranean basin. It may be also a secondary centre of diversity for almond. In fact, Tunisian almond plantations are located throughout all the country in different climatic conditions. In the north with a rainfall more than 700 mm/year are located cultivars that are adapted to sub humid climate and fungus resistant. The prospecting effort that was done last few years in the central and southern part of the country permitted the discovery of large diverse ecotypes.

A previous study (Gouta, 2012) has supposed using microsatellites the presence in Tunisia of two genetic pools. The first in the north resulting from plant material exchange with the northern shore of the Mediterranean and the second pool existing in the central and southern part that is probably the result of the dissemination of almond from its origin to north Africa via one of the ancient silk road caravan routes crossing north-central Africa through Timbuktu as it was advanced by Gradziel (2011). Recent study dealing with chloroplast and nuclear genotyping (Delplancke *et al.*, 2012) sheds light on a long standing presence of domesticated almond in North Africa and considered that this area is outstanding with a divergent haplotype and a phylogeographic cluster differentiated from the others mostly by mutational processes. The aims of this work are to ensure the hypothesis evocated by previous studies, to analyse the population structure of the almond genotypes from different origins and to contribute to this discussion about the origin of the existent germplasm in North Africa and particularly in Tunisia.

II – Materials and methods

Eighty-two almond accessions from different origins were analyzed in this study. 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).

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

PCR amplification reactions were carried out according to Gouta *et al.* (2010).

Data analysis: Data were recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. A similarity matrix was generated with NTSYS software version 2.1 (Rohlf, 2000). Genetic similarity between pairs was estimated by the Dice coefficient (SD). Cluster analysis was done using the Sequential Agglomerative Hierarical Nested cluster analysis (SAHN) procedure of NTSYS. Bootstrap support values were obtained from 2000 replicates using TREECON 1.3b (Van de Peer and De Wachter, 1994).

The genetic population structures were accessed by using the admixture model of STRUCTURE 2.3.4 software (Pritchard *et al.,* 2000).

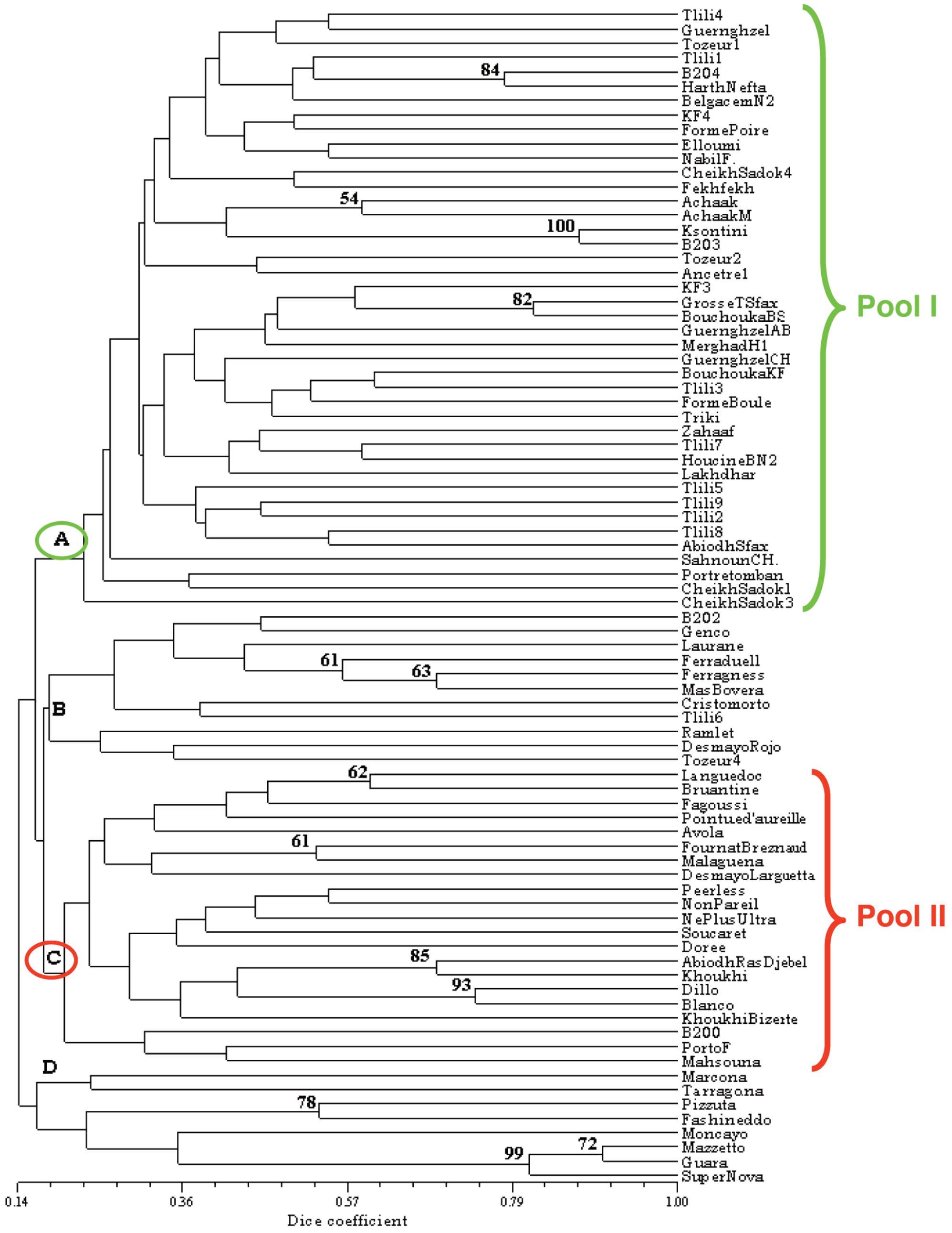

Fig. 1. Dendogram based on Dice coefficient illustrating the genetic similarities among 82 almond genotypes obtained by 10 SSR primers data.

III – Results and discussion

The dendrogram based on dice coefficient illustrating the genetic similarities among eighty two almond genotypes accessed by 10 genomic microsatellites (Fig. 1) showed that the majority of the Tunisian genotypes issued from the central and southern part (pool 1) were clustered together but they showed several minor groups, which revealed their high heterogeneity (cluster A, Fig. 1). 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 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 (pool 2) 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. 1). 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.

Furthermore and in order to strengthen the hypothesis regarding the genetic structure of the populations a Bayesian model based clustering was used. Results have moreover showed (Fig. 2) the clear existence of two distinguished populations that are geographically distinct. In fact it was clearly elucidated that a part of the local cultivars of the north (region of Bizerte) and another part of the local ecotypes were in the same cluster as the foreigner group (black colour). In this last were grouped all of the French, Spanish, Italian and American cultivars. The presence of the Californian cultivars in the same cluster with the French corroborates with results of Fernández i Martí *et al.* (2015) assuming that they belong all to the Mediterranean almond pool.

Thus, and tacking into account the results evocated by Delplancke *et al.* (2012) we can confirm that in Tunisia prevails two almond genetic pools: one in the northern part of the country that is historically recent issued from exchange of genotypes between both shores of the Mediterranean, and a second natively existing in the central and southern part that may be issued from mutational processes.

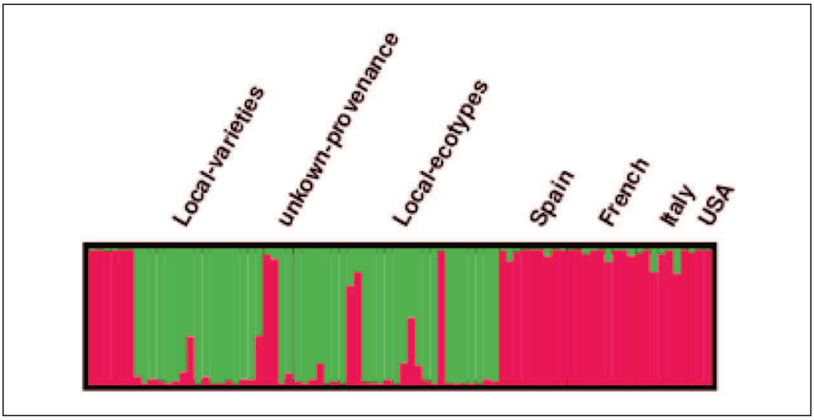

Fig. 2. Genetic structure of almond cultivars inferred from the admixture model.

IV – Conclusions

This study reveals the high diversity and the distinct origin of the Tunisian almond germplasm and can be considered as a first step forwards the understanding the origin of local and existing cultivars. The great diversity found in the almond germplasm supports the idea that Tunisia has a valuable source of almond genotypes that is tolerant to many biotic and abiotic stresses to be exploited in further breeding programs.

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