Electronic Journal of Biotechnology 18 (2015) 40–45

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Genetic diversity in two Italian almond collections

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ARTICLE INFO

Article history:
Received 27 March 2014
Accepted 29 September 2014
Available online 24 December 2014

Keywords:
Biodiversity
Genetic structure
Prunus amygdalus
SSR

ABSTRACT

Background: Sweet-seeded domesticated almonds were brought to the Mediterranean Basin from central Asia about 4000 years ago. In Italy, most of the almonds produced are cultivated in the southern part of the country. Local populations of the tree in Sardinia are largely seed-derived and mostly self-incompatible, so have developed extensive genetic diversity. The need to protect biodiversity has prompted a revived interest in local genetic materials in almond. Two Italian collections have been established, one in Sardinia and the other in Apulia. These collections were the focus of the present evaluation of genetic diversity.
Results: Eleven SSRs (microsatellites) were used for fingerprinting. The Sardinian germplasm was highly polymorphic, revealing a mean of 14.5 alleles per locus and a mean heterozygosity of 0.71. Using a model-based clustering approach, two genetic clusters were distinguished: one included all the commercial varieties and most of the Sardinian accessions, and the other most of the Apulian accessions. A similar structure was produced using a distance-based cluster analysis. The Sardinian accessions could still be distinguished from the commercial germplasm with few exceptions.
Conclusion: The extensive genetic variability present in the Sardinian and Apulian almond germplasm indicates that these materials represent an important source of genes for the improvement of the crop.

1. Introduction

Almond (*Amygdalus communis*), one of the most important nut crops worldwide [1], is a native of central Asia [2]. Based on an analysis of chloroplast DNA, the species has been shown to be very closely related to peach (*Prunus persica*) [3]. It is believed that, starting from a common progenitor, peach evolved in low elevation and high humidity regions of China, whereas almond was adapted to the drier climates prevalent in central Asia [4]. Wild forms of almond form bitter tasting seeds, a trait which was selected against during the domestication process. The domesticated tree appears in the literature as early as 2000 BCE [5], and was brought to Italy by Greek settlers during the 5th century BCE [6]. The crop is now grown on a large scale in the southern part of the country; annual production in Sicily and Apulia is currently more than 100 kt of shelled nuts [7]. Production relies on a small number of cultivars, although a few local varieties still persist. Smaller scale production also occurs in other Italian regions, such as Sardinia, Calabria, Abruzzo and Basilicata, mainly based on local varieties. Sardinia in particular, harbours a considerable number of such local varieties, which have evolved in isolation from the mainland populations. However, most local varieties are susceptible to frost damage, and thus tend to be low yielding. Nevertheless, a growing recognition of the importance of conserving biodiversity has reawakened the interest in these traditional varieties.

Robust methods which allow for discrimination between non-identical individuals are critical for biodiversity conservation. Phenotypic classification is simple, but the number of traits which are informative is limited. Genotypic methods are more flexible and, unlike most phenotypic ones, are unaffected by the plants' growing environment. Among the various marker systems to hand, simple sequence repeat (SSR) assays have proven to be highly polymorphic and simple to implement in both almond and peach [8,9,10,11,12,13,14]. Only few reports have described the diversity of Italian almond cultivars. De Giorgio et al. [15] reported a first phenotypic evaluation of 52 almond cultivars from the Apulian region. More recently, Distefano et al. [13] compared the level of genetic diversity of Italian almond accession with that of foreign cultivars from Mediterranean, American and Australian areas. Distance and model-based analysis revealed a high level of genetic variability both within and among Italian accessions. Based on this data, these authors suggested that germplasm collection of locally adapted cultivars represent a valuable source of genetic variability

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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2014.11.006

Table 1
Accessions of almonds and their provenance.

Accession	Collection site/putative origin	Flowering date	Self-compatibility
ANTIOCO PALA	Sardinia	Intermediate	Unknown
ANTONI PIRAS	Sardinia	Intermediate	Unknown
ARRUBIA	Sardinia	Intermediate	Unknown
BASIBI	Sardinia	Early-intermediate	Unknown
BIANCA	Sardinia	Intermediate	Unknown
BOCCHINO	Sardinia	Early-intermediate	Unknown
CIATTA INGLESE	Sardinia	Intermediate	Unknown
CIATTA MALISSA	Sardinia	Intermediate	Unknown
CORROCHINA	Sardinia	Early-intermediate	Unknown
COSSU	Sardinia	Intermediate	Unknown
DE EFISI SINZOBA	Sardinia	Intermediate	Unknown
DE MRASCIAI	Sardinia	Intermediate	Unknown
EFISI SINZOBA	Sardinia	Early-intermediate	Unknown
EMILIO 91	Sardinia	Early-intermediate	Unknown
FARCI	Sardinia	Intermediate	Unknown
FARRAU	Sardinia	Intermediate	Unknown
FIORI	Sardinia	Early-intermediate	Unknown
FOLLA 'E PRESSIU	Sardinia	Early-intermediate	Unknown
FRANCISCU	Sardinia	Intermediate	Unknown
GHIRONI	Sardinia	Intermediate	Unknown
IBBA	Sardinia	Early-intermediate	Unknown
IS STUMBUS	Sardinia	Early-intermediate	Unknown
LUTZEDDU	Sardinia	Early-intermediate	Unknown
MALISSA TUNDA	Sardinia	Intermediate	Unknown
NIEDDA I	Sardinia	Intermediate	Unknown
NIEDDA II	Sardinia	Intermediate	Unknown
NUXEDDA	Sardinia	Intermediate	Unknown
OLLA	Sardinia	Early-intermediate	Unknown
ORRI	Sardinia	Early-intermediate	Unknown
PITICHEDDA	Sardinia	Early-intermediate	Unknown
PROVVISTA	Sardinia	Early-intermediate	Unknown
REBECCU 1	Sardinia	Intermediate	Unknown
REBECCU 2	Sardinia	Intermediate	Unknown
REBECCU 3	Sardinia	Early-intermediate	Unknown
RIU LOI	Sardinia	Early-intermediate	Unknown
SCHINA DE PORCU	Sardinia	Intermediate	Unknown
STAMPASACCUSU	Sardinia	Early-intermediate	Unknown
SUNDA G.	Sardinia	Intermediate	Unknown
SUNDA N.	Sardinia	Intermediate-late	Unknown
TROITO A	Sardinia/Unknown	Intermediate-late	Unknown
TROITO B	Sardinia/Unknown	Intermediate	Unknown
VARGIU	Sardinia	Intermediate	Unknown
VAVANI PERRA	Sardinia	Early-intermediate	Unknown
ALBANESE	Apulia	Early	Unknown
ANTONIO DE VITO	Apulia	Early	Self-compatible
BANCHIERE	Apulia	Intermediate	Unknown
BARLETTANA	Apulia	Early-intermediate	Unknown
CAPUTO	Apulia	Intermediate	Unknown
CATALINI	Apulia	Intermediate	Unknown
CATUCCIA	Apulia	Early-intermediate	Self-incompatible
CATUCEDDA	Apulia	Early-intermediate	Unknown
CENTOPEZZE	Apulia	Early	Unknown
CIAVEA	Apulia	Early	Unknown
COSIMO DI BARI	Apulia	Late	Unknown
CRISTOMORTO	Apulia	Intermediate	Self-incompatible
D'ALOIA	Apulia	Early-intermediate	Unknown
FERRANTE	Apulia	Intermediate	Self-compatible
FILIPPO CEO	Apulia	Intermediate	Self-compatible
FRAGIULIO	Apulia	Intermediate	Unknown
FRANCISCUDDA	Apulia	Intermediate	Unknown
GALGANO	Apulia	Late	Self-incompatible
IRENE LANZOLLA	Apulia	Early-intermediate	Self-compatible
MINCONE	Apulia	Intermediate	Unknown
MONTRONE	Apulia	Early-intermediate	Self-incompatible
NOCELLA	Apulia	Early	Unknown
OCCHIOROSSO DI TRANI	Apulia	Early	Unknown
PAPPAMUCCO	Apulia	Intermediate	Unknown
PEPPARUDDA	Apulia	Intermediate	Self-compatible
PIANGENTE	Apulia	Intermediate	Unknown
PIGNATIDDE	Apulia	Late	Unknown
PISCALZE	Apulia	Early	Self-compatible
PIZZUTA D'AVOLA	Apulia/Sicily	Early	Self-incompatible

Table 1 (*continued*)

Accession	Collection site/putative origin	Flowering date	Self-compatibility
PUTIGNANO	Apulia	Early	Unknown
RACHELE TENERA	Apulia	Late	Unknown
RANA	Apulia	Late	Unknown
RANA GENTILE	Apulia	Late	Self-incompatible
REALE	Apulia	Intermediate	Unknown
RIVIEZZO	Apulia	Intermediate	Unknown
ROSSA	Apulia	Early-intermediate	Unknown
SANTERAMO	Apulia	Intermediate	Self-compatible
SANTORO	Apulia	Intermediate	Self-incompatible
TENENTE	Apulia	Early	Unknown
TUONO	Apulia	Late	Self-compatible
VISCARDA	Apulia	Early-intermediate	Unknown
ZIA COMARA	Apulia	Early-intermediate	Unknown
ZIN ZIN	Apulia	Unknown	Unknown
JORDANOLO	Sardinia/USA	Intermediate	Self-incompatible
NE PLUS ULTRA	Sardinia/USA	Early	Self-incompatible
NONPAREIL	Sardinia/USA	Intermediate	Self-incompatible
PICANTILI	Sardinia/Ukraine	Intermediate-late	Self-incompatible
ALDRICH	USA	Intermediate	Self-incompatible
MISSION	USA	Late	Self-incompatible
RUBY	USA	Late	Self-incompatible
SONORA	USA	Early-intermediate	Self-incompatible
SWEETHEART	USA	Intermediate	Partially self-incompatible
WINTER	USA	Intermediate	Partially self-incompatible

to be applied in breeding programmes. To date, little information is available on Sardinian almond genotypes. This paper reports the results of an analysis of the genetic diversity present in Sardinian and Apulian local varieties, based on SSR genotyping.

2. Materials and methods

2.1. Plant material and DNA extraction

The germplasm set consisted of 96 accessions (Table 1). Of these, 47 were represented by trees maintained by AGRIS Sardegna; these consisted of 40 sweet and three bitter entries and a set of outgroup varieties (three from USA and one from Ukraine). The Apulian germplasm was in the form of DNA extracted at CRA (Council for Research and experimentation in Agriculture) Bari, from 43 accessions. Finally, six USA commercial varieties were represented as leaf samples.

Overall therefore, the germplasm set comprised 43 Sardinian and 43 Apulian accessions, (the latter including “Pizzuta d'Avola” originated from Sicily as shown in Table 1), along with ten commercial cultivars. Total genomic DNA was extracted from powdered leaf samples using a GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich).

2.2. SSR marker genotyping

Among 21 SSRs assayed [16,17,18,19,20], 10 were excluded from further analysis based on chromosome position and amplification quality (see Table S1 for further details). Additional information on the performance of the 11 selected primers are reported in Table 2.

Each 25 μL PCR contained 1× PCR buffer (Invitrogen, Carlsbad, CA, USA), 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 μM of each primer (the forward primer was labeled with 6-FAM), 60 ng genomic DNA and 0.5 U recombinant Taq polymerase (Invitrogen, Carlsbad, CA, USA). The cycling regime for the UDP and CPPCT SSRs comprised an initial denaturation step (95°C/5 min), followed by 35 cycles of 94°C/45 s, T_a/45 s (annealing temperatures given in Table 2), 72°C/45 s, finishing with an extension step of 72°C/8 min; for the BPPCT microsatellites, the initial denaturation was 94°C/60 s, the annealing step was