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Genetic diversity in two Italian almond collections

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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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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
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

Table 2
Population genetic parameters for the Sardinian, Apulian and commercial almond accessions. Italicized values indicate the most important values.

SSR locus	Allele number						Allele richness (Rs)			H_o	H_e	HW eq. P	H_o	H_e	HW eq. p	H_o	H_e	HW eq. p	FST [a]
	Tot	(Priv)	Tot	(Priv)	Tot	(Priv)	Sardinian	Apulian	Comm.	Sardinian			Apulian			Comm.			
	Sardinian		Apulian		Comm.														
UDP 96003 [16]	13	(4)	8		5	(1)	7.2	4.7	5	0.77	0.84	*0.0009*	0.67	0.64	0.35	0.80	0.82	0.66	0.108
UDP 96005 [16]	13	(2)	17	(6)	6	(1)	8.4	9.7	5.7	0.72	0.88	*0.0005*	0.69	0.91	*<0.0001*	0.40	0.73	*0.007*	0.051
UDP 96013 [16]	15	(5)	11	(1)	7	(1)	7.8	7.5	6.5	0.74	0.84	*<0.0001*	0.77	0.82	0.14	0.60	0.68	0.78	0.135
UDP 98024 [16]	13	(5)	9	(2)	6		8.5	6.7	5.8	0.88	0.89	0.26	0.65	0.85	*0.0007*	0.90	0.81	0.90	0.065
UDP 98409 [16]	16	(8)	9	(1)	5		9.3	5.5	4.8	0.42	0.91	*<0.0001*	0.23	0.63	*<0.0001*	0.40	0.51	0.24	0.175
BPPCT 004 [17]	18	(5)	12		7		9.2	7.8	6.6	0.65	0.86	*0.0004*	0.74	0.82	0.0137	0.90	0.79	0.85	0.104
BPPCT 007 [17]	18	(9)	13	(4)	4		9.7	8	3.9	0.72	0.91	*<0.0001*	0.70	0.85	*0.0032*	0.70	0.66	0.24	0.107
BPPCT 039 [17]	23	(6)	16	(2)	7		11.7	9	6.7	0.72	0.94	*<0.0001*	0.71	0.88	*0.0005*	0.70	0.82	0.13	0.058
BPPCT 025 [17]	12	(2)	11	(1)	4		7.7	6.6	4	0.79	0.86	*0.0018*	0.76	0.81	0.0738	0.20	0.61	*0.0003*	0.098
BPPCT 028 [17]	10	(1)	11	(2)	5		7.3	7.9	5	0.51	0.84	*<0.0001*	0.52	0.88	*<0.0001*	0.44	0.56	0.27	0.112
CPPCT 033 [18]	9	(1)	14	(5)	6		7.2	8.1	5.6	0.83	0.86	0.64	0.84	0.85	0.18	0.90	0.68	0.07	0.085
Mean	14.5		11.9		5.6		8.5	7.4	5.4	0.71	0.88		0.66	0.81		0.63	0.70		0.099
SD	0.3		2.88		11.2		1.37	1.45	0.98	0.14	0.03		0.16	0.16		0.24	0.11		

[ ] references of SSR loci assayed in this study.
[a] With a 99% interval confidence from bootstrapping over loci (10,000 replications).

58°C/45 s, the extension step was 72°C/2 min and the final extension step was 72°C/4 min. The resulting amplicons were separated using an ABI® PRISM 310 Genetic Analyser (Applied Biosystems, Foster City, California, USA) device. The Genemapper 4.0 software (Applied Biosystems) was used to estimate fragment lengths based on the migration of GeneScan™–500 Liz™ size standards.

2.3. Data analysis

The observed (H_o) and expected (H_e) heterozygosities, F_{st} and the *p* value associated with the Hardy–Weinberg equilibrium test were computed using the TFPGA software [21]; allelic richness (Rs) was assessed by FSTAT v2.9.3.2. [22]. Genetic relationships among the accessions were derived by both STRUCTURE v2.2 analysis [23], and by a comparison of pairwise genetic distances using the TREECON software [24]. For the former, the admixed model was adopted, and for each value of *K*, 20 runs (100,000 burn-in generations and 200,000 Markov chain generations) were carried out. The most likely *K* value was determined using the Evanno procedure [25]. Assignment of an accession to a cluster was based on a Q value threshold of 0.65. The cluster analysis was based on the Nei and Li similarity matrix [26], and the genetic distances were calculated according to the proportion of shared alleles. A dendrogram was obtained using the UPGMA method.

3. Results and discussion

3.1. SSR polymorphism

All 11 primer pairs were informative across the germplasm set. The range in fragment size for each SSR amplicon is given in Table S1. Considering the germplasm in the form of the three groups (Sardinian and Apulian accessions, along with commercial cultivars) F_{st}, a measure of genetic diversity between groups, was on average 0.099, ranging from 0.051 (UDP 96005) to 0.175 (UDP 96409). The Sardinian germplasm was highly polymorphic, and harboured the greatest number of private alleles (Table 2). The mean number of alleles per locus detected was 14.5, while Rs ranged from 7.2 (UDP 96003 and CPPCT 033) to 11.7 (BPPCT 039) (mean 8.5). The lowest H_o was 0.42 (UDP 98-409) and the highest 0.88 (UDP 96-024), with a mean of 0.71. There was a significant deviation ($P < 0.01$) from Hardy–Weinberg equilibrium at nine of the eleven loci. Among the Apulian accessions, the mean number of alleles per locus was 11.9, and Rs varied from 4.7 (UDP 96003) to 9.7 (UDP 96005) (mean 7.4). The range in H_o was 0.23 (UDP 98409) to 0.84 (CPPCT 033) (mean 0.66), and there was a significant deviation ($P < 0.01$) from Hardy–Weinberg equilibrium at six of the loci. Across the commercial cultivars, the mean number of alleles per locus was 5.6, while mean Rs was 5.4, ranging from 3.9 (BPPCT 007) to 6.7 (BPPCT 039). The mean H_o value was lowest for BPPCT 025 (0.20) and highest for UDP 98024, BPPCT 004 and CPPCT 033 (0.90). Only two loci deviated significantly from Hardy–Weinberg equilibrium. A comparable study of diversity in a core collection of 21 almond accessions carried out by Sánchez-Pérez et al. [27] revealed a mean of 10.7 alleles per SSR locus and a mean H_o of 0.86, while a panel of 57 Iranian local varieties harboured 8.8 alleles per SSR locus and a mean H_o of 0.67 [28]. Distefano et al. [13] reported an average of 18 alleles per locus and a mean H_o of 0.71 in a collection of almond cultivars mainly constituted of Sicilian accessions. The extent of the variation present in the Italian material contrasts with its rather limited extent among the commercial varieties. Some 80% of the world's almond production is based in the USA [29], and relies heavily on just six varieties (36% from “Nonpareil” and a further 40% from “Monterey”, “Carmel”, “Butte” “Mission” and “Fritz”); the first three of the latter were bred from a cross between “Nonpareil” and “Mission” [11].

3.2. Genetic structure

The most likely value for the STRUCTURE parameter *K* was 2 (Fig. 1b). Of the two groups defined at this *K*, C1 (red bars) contained all the commercial varieties, plus all of the Sardinian accessions except for “Farrau”; C2 (blue bars) contained all of the Apulian material except for “Pizzuta d'Avola”. The genetic distance-based clustering (Fig. 2) mirrored the STRUCTURE groupings. Two major groups — I, including the Sardinian materials plus the commercial varieties, and II (Apulian accessions) — were readily distinguished, and these were largely congruent with C1 and C2. The exceptions were the Apulian entry “Fra Giuglio” (Group I and C2), “Picantili”, and “Niedda I” and “Troito A” (Group II and C1) (Fig. 1a). Group I contained two recognizable sub-groups (I_1 and I_2). “Pizzuta d'Avola” did not group with any of the Apulian accessions, either on the basis of the STRUCTURE or the distance-based analyses, presumably reflecting its Sicilian origin. The Sardinian entry “Farrau” mapped to the C2 cluster (Fig. 1a); however, in the phylogenetic analysis it was separated from all the other accessions. A survey of self-incompatibility in the Sardinian collection has highlighted the peculiarity of this accession, in that it carries a self-incompatibility allele apparently derived from peach (data not shown).

The analysis of genetic structure revealed that the Sardinian germplasm is quite different from the Apulian germplasm. The Sardinian types appeared to be more similar to the commercial varieties, although still distinct from them. A possible explanation for this surprising result is that Sardinia has a strong historical connection

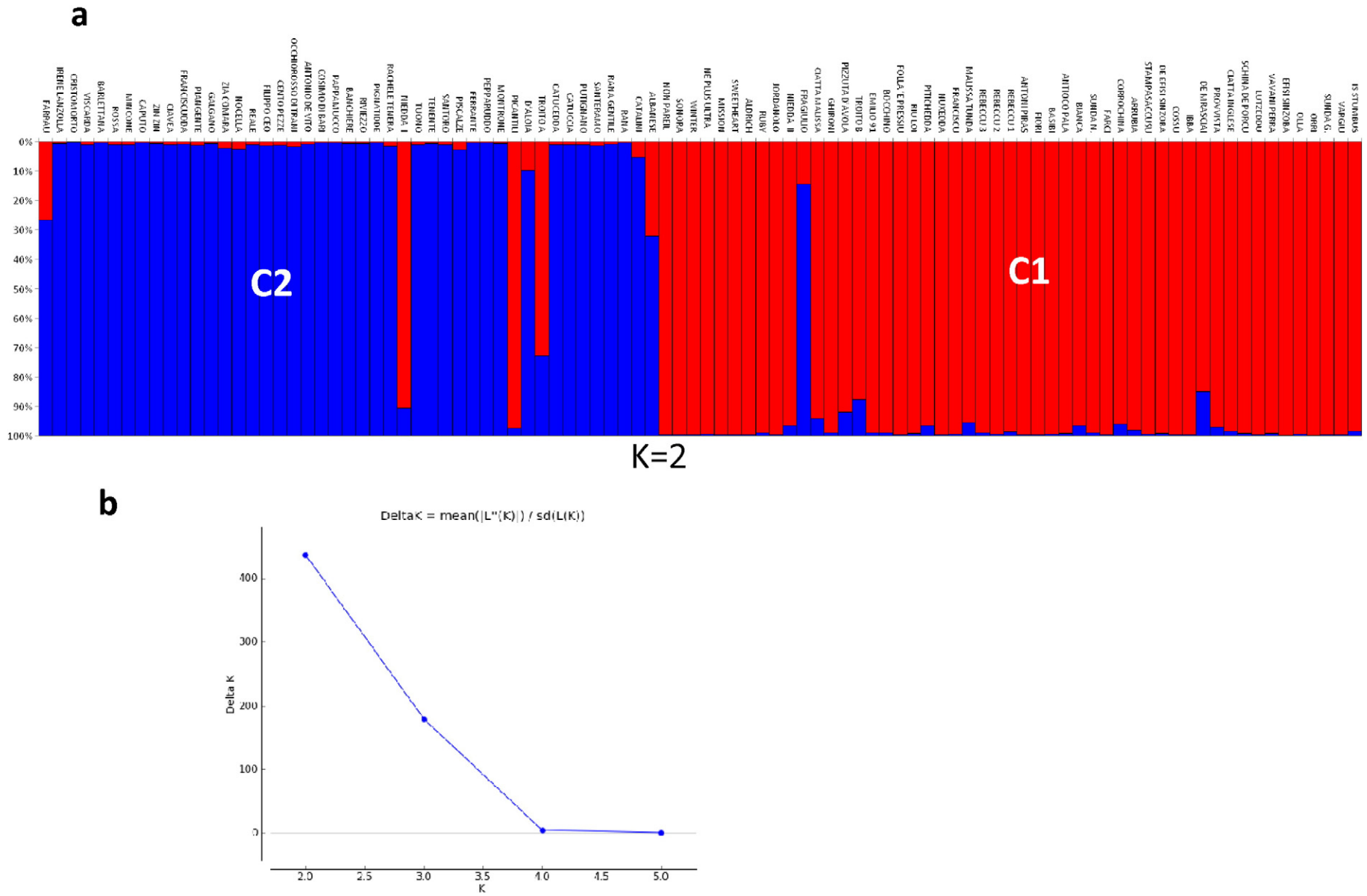

Fig. 1. Genetic architecture of the almond germplasm as revealed by STRUCTURE analysis. (a) K = 2: the two cluster C1 (red bars) C2 (blue bars). Q coefficients for each cluster are given; (b) most likely *K* value determined by Evanno method.

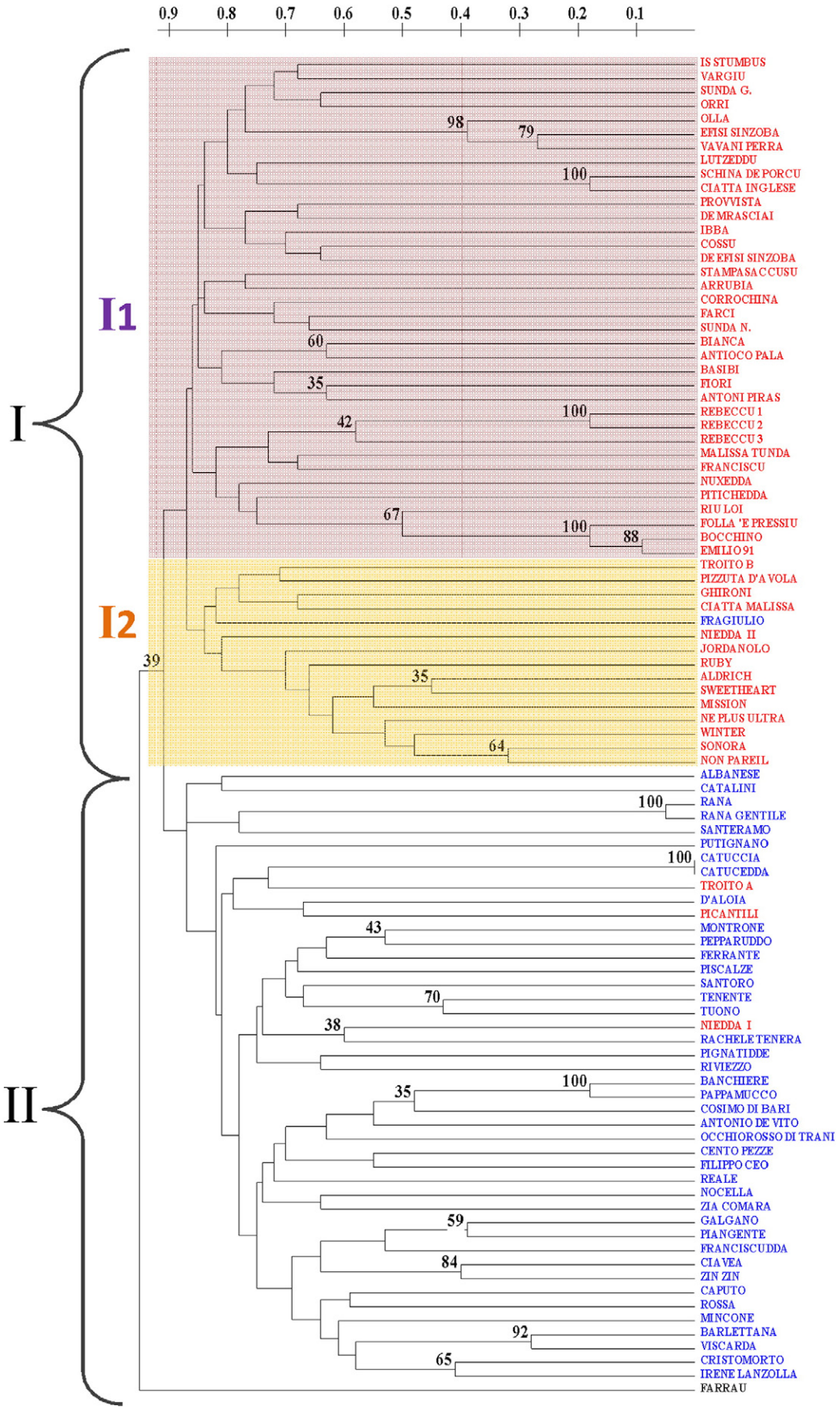

Fig. 2. UPGMA dendrogram based on similarity matrix. Accession names are coloured according to the K = 2 model shown in Fig. 1b. Subgroup I_1 is shaded in pink and I_2 in orange.

with Spain, and Spanish settlers would have likely carried the same germplasm to both Sardinia and California. Alternatively, it may be that there has been introgression from the commercial gene pool in Sardinia, but not in Apulia. Almond cultivation in Sardinia is almost entirely small-scale, and it is claimed that most of the trees in traditional almond orchards have been raised from seed [30]. Therefore the occurrence of gene flow from commercial germplasm into the local gene pool appears to be a possible scenario. Note that Fathi et al. [28] also observed that some registered Iranian almond cultivars appeared to be genetically related to Spanish and US commercial varieties, and concluded that this probably reflected gene flow between commercial varieties and local materials. Accordingly, Delplancke et al. [31] have recently reported that the Italian almond population show the largest level of mixed ancestry among western areas of Mediterranean basin. These authors proposed that this finding would be consistent with human driven migration and with reconstructed maps of ancestral trade routes.

Whatever the origin of this shared genetic variation, it is clear that no assumption should be made that traditional almond varieties are completely unrelated to commercial varieties, a realization which is important in the context of establishing germplasm collections.

The present study represents a contribution to the preservation and management of almond germplasm, revealing local Italian material as a valuable source of genetic diversity. The identification of local types and the explanation of phylogenetic relationships among Sardinian, Apulian and reference accessions are of interest to ongoing breeding efforts to improve the adaptation and quality of the almond crop. Moreover the adoption of highly reproducible SSR loci already used in other studies [9,11,27,32], will facilitate dataset integrations. An integrated molecular fingerprint dataset of almond genotypes will be a helpful instrument for planning conservation and valorisation policies both at international and local level.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejbt.2014.11.006.

References

[1] FAOSTAT. [cited January 14, 2014]. Available from Internet: http://faostat.fao.org/site/339/default.aspx; 2011.
[2] Martínez-Gómez P, Sánchez-Pérez R, Dicenta F, Howad W, Arús P, Gradziel TM. Almond. In: Kole C, editor. Genome mapping and molecular breeding in plants. Fruits and nutsBerlin Heidelberg: Springer-Verlag; 2007. p. 229–42.
[3] Xu Y, Ma RC, Xie H, Liu JT, Cao MQ. Development of SSR markers for the phylogenetic analysis of almond trees from China and the Mediterranean region. Genome 2004; 47:1091–104. http://dx.doi.org/10.1139/G04-058.
[4] Rieger M. Almond. Introduction to fruit crops. NewYork–London–Oxford: Food Products Press, an Imprint of the Haworth Press, Inc.; 2006. p. 37–46.
[5] Gradziel TM. Almond breeding. In: Jain SM, Priyadarshan PM, editors. Breeding plantation tree crops; 2009. p. 1–31.
[6] Avanzato D, Vassallo I. Following almond footprints (*Amygdalus communis* L.): Across Sicily cultivation and culture, folk and history, traditions and uses. Scr Hortic 2006;165 [cited December 9, 2013]. Available from Internet: http://www.actahort.org/chronica/pdf/sh_4.pdf.
[7] ISTAT. Data on production of main crops. [cited January 14, 2014]. Available from Internet: http://dati.istat.it/Index.aspx?DataSetCode=DCSP_COLTIVAZ⟪ 2011.
[8] Aranzana M, Pineda A, Cosson P, Dirlewanger E, Ascasibar J, Cipriani G, et al. A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor Appl Genet 2003;106:819–25. http://dx.doi.org/10.1007/s00122-002-1094-y.
[9] Amirbakhtiar N, Shiran B, Moradi H, Sayed-Tabatabei BE. Molecular characterization of almond cultivars using microsatellite markers. Proc. IVth is on pistachios and almonds, 726. ISHS acta hortic; 2006 [cited December 9, 2013. Available from Internet: http://www.actahort.org/books/726/726_5.htm].
[10] Shiran B, Amirbakhtiar N, Kiani S, Mohammadi SH, Sayed-Tabatabei BE, Moradi H. Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. Sci Hortic 2007;111:280–92. http://dx.doi.org/10.1016/j.scienta.2006.10.024.
[11] Dangl GS, Yang J, Golino DA, Gradziel T. A practical method for almond cultivar identification and parental analysis using simple sequence repeat markers. Euphytica 2009;168:41–8. http://dx.doi.org/10.1007/s10681-008-9877-0.
[12] Fernández Marti A, Alonso JM, Espiau MT, Rubio-Cabetas MJ, Socias i Company R. Genetic diversity in Spanish and foreign almond germplasm assessed by molecular characterization with simple sequence repeats. J Am Soc Hortic Sci 2009;134: 535–42 [cited December 9, 2013]. Available from Internet: http://journal.ashspublications.org/content/134/5/535.full#T2.
[13] Distefano G, Caruso M, La Malfa S, Ferrante T, Del Signore B, Gentile A, et al. Genetic diversity and relationships among Italian and foreign almond germplasm as revealed by microsatellite markers. Sci Hortic 2013;162:305–12. http://dx.doi.org/10.1016/j.scienta.2013.08.030.
[14] El Hamzaoui H, Oukabli A, Charafi J, Moumni M. Moroccan almond is a distinct gene pool as revealed by SSR. Sci Hortic 2013;154:37–44. http://dx.doi.org/10.1016/j.scienta.2013.02.022.
[15] De Giorgio D, Leo L, Zacheo G, Lamascese N. Evaluation of 52 almond (*Prunus amygdalus* Batsch) cultivars from the Apulia region in Southern Italy. J Hortic Sci Biotechnol 2007;82:541–6.
[16] Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R. AC/GT and AG/CT microsatellite repeats in peach (*Prunus persica* (L) Batsch): Isolation, characterisation and cross-species amplification in *Prunus*. Theor Appl Genet 1999;99:65–72. http://dx.doi.org/10.1007/s001220051209.
[17] Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, et al. Development of microsatellite markers in peach (*Prunus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor Appl Genet 2002;105:127–38. http://dx.doi.org/10.1007/s00122-002-0867-7.
[18] Aranzana MJ, Garcia-Mas J, Carbó J, Arús P. Development and variability of microsatellite markers in peach. Plant Breed 2002;121:87–92. http://dx.doi.org/10.1046/j.1439-0523.2002.00656.x.
[19] Lopes MS, Sefc KM, Laimer M, Da Camara Machado A. Identification of microsatellite loci in apricot. Mol Ecol Notes 2002;2:24–6. http://dx.doi.org/10.1046/j.1471-8286.2002.00132.x.
[20] Yamamoto T, Mochida K, Imai T, Shi YZ, Ogiwara I, Hayashi T. Microsatellite markers in peach (*Prunus persica* (L.) Batsch) derived from an enriched genomic and cDNA libraries. Mol Ecol Notes 2002;2:298–301. http://dx.doi.org/10.1046/j.1471-8286.2002.00242.x.
[21] Miller MP. Tools for population genetic analyses (TFPGA) version 1.3: A Windows® program for the analysis of allozyme and molecular population genetic data. [cited December 9, 2013]. Available from Internet: http://www.ccg.unam.mx/~vinuesa/tlem09/docs/TFPGADOC.PDF; 1997.
[22] Goudet J. FSTAT (vers 2.9.3.2) updated from Goudet J., FSTAT (vers. 1.2): A computer program to calculate F-statistics. [cited December 9, 2013]. Available from Internet: http://www2.unil.ch/popgen/softwares/fstat.htm; 2002.
[23] Pritchard JK, Wen X, Falush D. Documentation for STRUCTURE software, version 2.2. 2007. [cited December 9, 2013]. Available from Internet: http://pritchardlab.stanford.edu/software/structure22/readme.pdf.
[24] Van De Peer Y, De Wachter R. TREECON for Windows: A software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Appl Biosci 1994;10:569–70.
[25] Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software structure: A simulation study. Mol Ecol 2005;14:2611–20. http://dx.doi.org/10.1111/j.1365-294X.2005.02553.x.
[26] Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci U S A 1979;76:5269–73. http://dx.doi.org/10.1073/pnas.76.10.5269.
[27] Sánchez-Pérez R, Ballester J, Dicenta F, Arús P, Martínez-Gómez P. 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. Sci Hortic 2006;108:310–6. http://dx.doi.org/10.1016/j.scienta.2006.02.004.
[28] Fathi A, Ghareyazi B, Haghinazari A, Ghaffari MR, Pirseyedi SM, Kadkhodaei S, et al. Assessment of the genetic diversity of almond (*Prunus dulcis*) using microsatellite markers and morphological traits. Iran J Biotechnol 2008;6:98–106.
[29] Almond Board of California. [cited January 14, 2014]. Available from Internet: http://www.almondboard.com; 2013.
[30] Agabbio M, Frau AM, Chessa I. Remarks on a five year survey based on ninety-two almond selections of the Sardinian patrimony variety. Option Mediterraneennes Serie Etudes II; 1984 39–50.
[31] Delplancke M, Alvarez N, Benoit L, Espíndola A, Joly HI, Neuenschwander S, et al. Evolutionary history of almond tree domestication in the Mediterranean basin. Mol Ecol 2013;22:1092–104. http://dx.doi.org/10.1111/mec.12129.
[32] Zeinalabedini M, Khayam-Nekoui M, Grigorian V, Gradziel TM, Martínez-Gómez P. The origin and dissemination of the cultivated almond as determined by nuclear and chloroplast SSR marker analysis. Sci Hortic 2010;125:593–601. http://dx.doi.org/10.1016/j.scienta.2010.05.007.