Journal of Horticultural Science & Biotechnology (2012) **87** (6) 647–653

Microsatellite marker-based identification of mother plants for the reliable propagation of olive (*Olea europaea* L.) cultivars in Australia

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SUMMARY
Olive production in Australia has continued to increase in recent years, however there remains a high degree of confusion on the genetic identities of the cultivars being grown. In the present study, seven microsatellite (simple sequence repeat; SSR) loci were used to identify a set of 53 olive tree samples from different sources. The microsatellite DNA profiles of all 53 tree samples, including seven unknown trees, were compared with the SSR profiles of 14 reference olive cultivars. A total of 60 fragments (alleles), averaging 8.57 alleles per microsatellite locus, were amplified. High average values were found for the observed heterozygosity, the expected heterozygosity, and the polymorphic information content (0.73, 0.74, and 0.72, respectively). While all seven microsatellite markers proved useful for characterisation and identification purposes, a combination of three SSR primer pairs (DCA9, DCA18, and EM030) was sufficient to distinguish all 53 olive samples. The microsatellite allelic profiles allowed the 53 tree samples to be grouped into 23 genotypes. The allelic profiles of 14 of these genotypes matched with their reference cultivars, while the genetic identities of the remaining nine genotypes could not be confirmed. Some of these unknown genotypes may have been derived from feral olive trees, or were due to mislabelling and/or planting errors among Australian olive cultivars. Our results confirm the usefulness of microsatellite markers as a tool for cultivar differentiation and identification, and indicate the need for reliable identification of mother plants for commercial propagation.

Olive (*Olea europaea* L.) is increasingly being recognised as a crop of significant economic and health importance, worldwide. In recent years, the average consumption of olives in Australia has risen to more than 1.0 kg per person per year (Fabbri *et al*., 2004) with imports increasing from 28,500 metric tonnes (MT) in 2005, to 33,000 MT in 2011. Concurrently, olive oil exports have also risen from approx. 1,600 MT in 2005, to 8,000 MT in 2011 (http://www.internationaloliveoil.org), reflecting the rapid expansion of the olive industry in Australia. Many of the leading olive cultivars around the World, including those propagated on a commercial scale, were introduced into Australia in the 19th century at different experimental farms, including the collection based at Wagga Wagga, New South Wales (Ayton *et al*, 2001).

However there is confusion about the identity of some of the olive cultivars presently grown in Australia, which then incurs costs for olive nurseries who provide incorrect cultivars to growers. This problem was probably caused by mis-identification and/or mis-labelling of the original stocks that were used as the source of genetic material for both growers and nurserymen. In some cases, the plant material used as mother trees was sourced from various abandoned groves or collections where pedigree records were incomplete, unreliable, or non-existent (Burr, 1998; Mekuria *et al*., 1999). It is therefore becoming increasingly necessary to discriminate the olive cultivars grown in Australia at the genetic level, including mother trees prior to their commercial propagation, as the cost of vegetative propagation represents a major investment for olive sales outlets and growers. Furthermore, the variety of Australian environmental conditions can alter the oil characteristics of individual olive cultivars from those obtained at their purported site of origin (Mailer and Ayton, 2010; Montealegre *et al*., 2010). The expanding Australian olive industry, and worldwide recognition of its high quality, extra virgin olive oils (EVOO), has prompted an urgent need to safeguard human health, oil quality, and consumer interests against the intentional labelling of cheap, adulterated oils as authentic EVOO. The use of microsatellite (simple sequence repeat; SSR) markers, to verify the true identity of Australian olive cultivars, and compositional markers unique to the Australian environment to identify Australian olive oils with special characteristics, are becoming increasingly important.

The current classification of olive cultivars is complicated due to the richness of the germplasm, coupled with a lack of reference cultivars and errors in cultivar denomination (Bracci *et al*., 2011). Various

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molecular markers systems such as Randomly Amplified Polymorphic DNA (RAPD) and microsatellite markers have been used internationally to characterise olive cultivars (Belaj *et al*., 2001; 2003a; Bandelj *et al*., 2007; Bracci *et al*., 2009; Mekuria *et al*., 1999; Guerin *et al*., 2002; Mailer and May., 2002). In recent years, microsatellites have been recognised as the markers of choice for the discrimination of cultivars and the assignment of cultivars to their geographic origin, as well as providing data for various other molecular analyses (Sarri *et al*., 2006; Noormohammadi *et al*., 2007; Poljuha *et al*., 2008; Muzzalupo *et al*., 2009). Microsatellite markers are particularly suited to these analyses as they are highly polymorphic, amenable to detecting heterozygosity, and highly reproducible (Belaj *et al*., 2003a; Baldoni *et al*., 2009). However, their use has been restricted to paternity tests and cultivar-compatibility in Australian olive groves (Mokerjee *et al*., 2005).

In this study, we used microsatellite (SSR) markers to characterise 53 olive trees, which included plant material from different sources in Australia. To the best of our knowledge, this is the first attempt to characterise key Australian olive cultivars by means of microsatellite markers. The screening of microsatellite marker (alleles) in these important olive cultivars would be useful for: (i) studies on the diversity existing in olive germplasm; (ii) detecting cases of homonymy and synonymy; and (iii) exposing mis-labelling or planting errors in mother trees. Finally the results of this study could generate a database for varietal identification and future olive breeding programmes in Australia.

MATERIALS AND METHODS

Plant material and DNA extraction

Leaf samples from 53 individual olive trees (*Olea europaea* L.) were obtained from the collections of the Australian Olive Association, the Charles Sturt University Olive Grove, private nurseries, and olive growers (Supplementary Table I; available on-line at www.hortscib.com).

DNA was isolated using the standard phenol-chloroform method (Davis *et al*., 1989) with minor modifications, which included re-suspending the precipitated DNA in 500 µl of 10 mM Tris-HCl pH 8.0, 100 mM EDTA buffer and re-extracting twice with an equal volume of 1:1 (v/v) phenol-chloroform to eliminate leaf phenolics. DNA was also extracted from leaf samples of 14 reference olive cultivars obtained from three different sources. The cultivars 'Arbequina', 'Coratina', 'Frantoio', 'Koroneiki', 'Leccino', 'Manzanilla de Sevilla', 'Pendolino', 'Picual', 'Hojiblanca', 'Verdale', 'Lechin de Sevilla' and 'Cornicabra' were acquired from the World Olive Germplasm Bank, Cordoba, Spain. 'Kalamata' was from the Olea Nursery, Western Australia, and 'Hardy's Mammoth' was from the Charles Sturt University Olive Grove.

PCR and microsatellite analysis

Seven microsatellite primer-pairs [DCA3, DCA4, DCA9, DCA16, and DCA18 (Sefc *et al*., 2000) and EM090 and EM030 (De La Rosa *et al*., 2002)] that have been used successfully for olive genotyping (Baldoni *et al*., 2009) were applied to characterise the 53 Australian olive genotypes. The 5' end of each forward primer was tagged with the generic 19-mer M13 sequence (CACgACgTTgTAAAACgAC), as previously described (Raman *et al*., 2005).

Each PCR was performed in a 12 µl reaction volume containing 80 ng DNA, 10 ng forward primer, 30 ng M13 core sequence labelled with one of three fluorescent dyes (D2, D3, or D4; Beckman Coulter Inc., Fullerton, CA, USA), 20 ng unlabelled reverse primer, 25 µM dNTPs, 2 mM $MgCl_2$ and 1.0 Unit of Immolase DNA Polymerase, and 1.2 µl of 10X reaction buffer (Bioline Pty. Ltd., Alexandria, NSW, Australia). PCR amplification was performed using a GeneAmp 2700 thermal cycler (Applied Biosystem, Foster City, CA, USA). Thermal profiles included an initial denaturation step of 95ºC for 4 min, touch-down of ten cycles (1ºC per cycle) of 94ºC for 30s, 65ºC for 30s, and 72ºC for 80s, followed by 35 cycles of 94ºC for 15s, 55ºC for 30s, and 72ºC for 45s, and a final extension at 72°C for 10 min. Fragment sizing of the PCR amplified fragments was achieved using denaturing capillary gel electrophoresis in a CEQ 8000-Genetic Analysis System with associated software (Beckman Coulter Inc.). Each sample contained an internal standard in the size range of 60 – 400 bp. Final allele sizes were estimated by excluding the 19-mer M13 sequence. In addition to comparisons with the 14 reference olive DNA samples, the sizes of the microsatellite loci were also compared with those in previous studies (Bandelj and Javornik., 2002; 2007; Diaz *et al*., 2007; Bracci *et al*., 2009; Reale *et al*., 2006; Khadari *et al*., 2007; Baldoni *et al*., 2009) including the FAO (Food and Agriculture Organisation of the United Nations) and the olive germplasm database (www.oleadb.it).

Polymorphic information contents (PIC), the numbers of alleles, allele frequencies, probabilities of identity (PI), and observed (H_o) and expected heterozygosity (H_e) values were calculated for each microsatellite locus using Cervus software (Marshall *et al*., 1998). Genetic similarities among the 53 genotypes were calculated on the basis of Dice coefficients and used for a hierarchical cluster analysis. A dendrogram was constructed using the weighted pair group method with arithmetic averages (complete linkage method) in the Primer 6 software package (Clarke and Gorley, 2006).

RESULTS AND DISCUSSION

Microsatellite polymorphism

Sixty alleles were identified among the 53 individual olive tree samples, with an average of 8.57 alleles per SSR locus, with no less than 50% of samples being heterozygous. A minimum number of seven alleles (at the SSR loci *DCA16*, *EM090*, and *EM030*) and a maximum of 12 alleles at the *DCA9* locus were observed. Of the seven microsatellite markers, DCA9 gave the maximum polymorphism (12 alleles) with the ability to distinguish 11 genotypes, whereas DCA16, EM090, and EM030 revealed the minimum polymorphism (Table I). H_o values among the 53 samples ranged from 0.49 – 0.98, with a mean value of 0.73, while H_e values ranged from 0.48 – 0.84, with an average value of 0.74. These values indicated a high level of genetic variability among the 53 olive samples. Although only two loci (*DCA18* and *EMO30*) showed comparable H_o and H_e values, the

TABLE I
Microsatellite (SSR) marker polymorphism and genetic information parameters in 53 individual Australian olive tree DNA samples

SSR marker name	No. of alleles	No. of unique alleles	Unique allelic pattern	H_o[‡]	H_e	R	PIC	PI
DCA9	12	3	11	0.98	0.830	-0.092	0.803	0.0524
DCA3	10	4	9	0.68	0.840	0.1058	0.813	0.0489
DCA16	7	1	8	0.94	0.830	-0.07	0.804	0.0525
DCA18	8	1	7	0.79	0.820	0.0021	0.792	0.0560
DCA5	9	5	5	0.49	0.480	-0.04	0.467	0.2830
EMO90	7	1	4	0.56	0.690	0.0884	0.647	0.1389
EMO30	7	1	7	0.72	0.750	0.0256	0.705	0.1059
Average	8.57	2.28	7.28	0.73	0.740	0.0024	0.719	3×10^{-8}
Total Number	60	16	51					

[‡]H_o, observed heterozygosity; H_e, expected heterozygosity; R, probability of null alleles; PIC, polymorphic information content; PI, probability of identity.

differences between low H_o and H_e values at the other five loci (*DCA3*, *DCA5*, *DCA9*, *DCA16*, and *EM090*), were non-significant. The most informative marker (DCA9) had high H_e (0.83), H_o (0.98), and PIC (0.80) values, while the DCA5 locus was the least informative with H_e, H_o, and PIC values of 0.49, 0.48, and 0.46, respectively. These findings are comparable to those estimated by Baldoni *et al* (2009).

The combined probablility of identity (PI) value that expresses the likelihood of finding two individuals with the same genotype per locus was analysed for each microsatellite locus. PI values varied from as low as 0.04 – 0.28, with a combined estimate of 3×10^{-8}, indicating a negligible chance of finding two identical individuals in the test population. The high levels of genetic diversity observed in this study are in agreement with previous findings in olive (Baldoni *et al*., 2009; Sarri *et al*., 2006; Bracci *et al*., 2009).

Despite the high reproducibility of SSR markers, small (1 - 2 bp) differences in the lengths of amplicons were observed between runs. Comparisons with published microsatellite profiles, using the same olive cultivars, showed differences of 1 – 4 bp (Bandelj and Javornik., 2002; 2007; Diaz *et al*., 2007; Bracci *et al*., 2009). Such discrepancies in the lengths of PCR amplification products, and heterozygous *vs.* homozygous misreading, have previously been found between different laboratories (Baldoni *et al*., 2009; Doveri *et al*., 2008). Our results, and those from earlier studies, suggest that comparisons between analyses using the same make and model of analysis system, including DNA polymerase, thermocycler, fluorescent dye, size standards, analysis software and, more importantly, use of a reference cultivar with the same registration number, ensures greater reproducibility of results between laboratories.

Cultivar identification

Based on their allelic polymorphism at the seven microsatellite marker loci, the 53 individual olive trees could be grouped into 23 genotypes (Supplementary Table I available on-line at www.jhortscib.com; Table II). The genetic profiles of 14 of these genotypes matched those of the 14 reference cultivars, while the genetic identity of the remaining nine genotypes could not be confirmed. The allelic profiles of only three highly polymorphic microsatellite loci (DCA9, EM030, and DCA18) allowed all 53 samples to be assigned to each of the 23 genotypes (Table II), suggesting that these microsatellite markers can be used as diagnostic markers. These results reconfirmed the discriminatory capacity of microsatellite markers for characterising different olive cultivars, as reported previously (Belaj *et al*., 2003a; Noormohammadi *et al*., 2007; Bracci *et al*., 2009). At the individual level, 20 out of the 53 tree

TABLE II
Amplified microsatellite (SSR) fragments (in bp) of 53 individual Australian olive trees assembled into 23 genotypes using the SSR markers DCA9, EM030, and DCA18

			SSR marker					
Genotype no.	Genotype name	Primers or combination of primers	DCA9		DCA18		EMO30	
1	'Hardy's Mammoth'	EMO30					**190**	**196**
2	'Unknown'	EMO30					**186**	**188**
3	'Unknown'	DCA9; EMO30	**163**[‡]	**195**			**188**	**192**
4	'Pendolino'	DCA9+DCA18+EMO30	163	207	176	178	190	190
5	'Unknown'	DCA18			**168**	**168**		
6	'Lechin de Sevilla'	DCA18			**168**	**176**		
7	'Manzanilla de Sevilla'	DCA9+DCA18+EMO30	163	207	172	180	192	192
8	'Leccino'	DCA9+DCA18+EMO30	163	207	176	176	190	192
9	'Unknown'	DCA9	**164**	**211**				
10	'Unknown'	DCA9+DCA18+EMO30	167	195	172	180	188	190
11	'Kalamata'	DCA18			**184**	**184**		
12	'Unknown'	DCA9; EMO30	**175**	**207**			**183**	**192**
13	'Coratina'	DCA9; EMO30	**183**	**195**			**192**	**198**
14	'Koroneiki'	DCA18			**172**	**174**		
15	'Unknown'	DCA9+DCA18+EMO30	183	207	176	176	190	192
16	'Frantoio'	DCA9+DCA18+EMO30	183	207	176	178	192	192
17	'Picual'	DCA9	**185**	**193**				
18	'Cornicabra'	DCA9	**185**	**195**				
19	'Arbequina'	DCA9; DCA18; EMO30	**185**	**207**	**168**	**178**	**183**	**188**
20	'Unknown'	DCA9; DCA18; EMO30	**195**	**195**	**170**	**180**		
21	'Unknown'	DCA9; DCA18; EMO30	**195**	**205**	**176**	**184**		
22	'Hoji Blanca'	DCA9	**195**	**207**				
23	'Unknown'	DCA9; EMO30	**195**	**213**			**188**	**188**

[‡]Marker sizes (bp) in bold font represent a unique allelic pattern per locus. Marker sizes (bp) in bold font and underlined represent unique alleles.

samples (37.7%) correctly matched their presumed cultivar identity. Ten tree samples were found to match cultivars different from those they were supposed to be, four samples matched known cultivars, and 19 samples did not match any of the reference cultivars (Supplementary Table I; available on-line at www.jhortscib.com).

The fact that all test samples representing the cultivars 'Hardy's Mammoth', 'Pendolino', 'Kalamata', 'Coratina', 'Picual', and 'Arbequina' matched their reference cultivar assures the genetic purity of these important commercially-grown cultivars in Australia. Similarly, the three samples of 'Nevadillo blanco' shared the same alleles at all loci. These results were consistent with previous studies which showed that samples of 'Nevadillo blanco', sourced from different nurseries in Australia displayed the same RAPD profiles (Sweeney, 2005). The microsatellite profiles of the samples labelled FS-17-1 and FS-17-2 matched the maternal alleles of 'Frantoio', confirming their authenticity as true cultivars of 'FS17'(Fontanazza *et al*., 1998). However, in order to ascertain the true identity of 'Nevadillo blanco' and 'FS17', the allelic profiles of 'Nevadillo blanco' and 'FS17' references will be needed.

Large differences in the sizes of microsatellite alleles were found between some samples that represented other important commercially-grown cultivars such as 'Leccino', 'Hojiblanca', 'Picholine', 'Frantoio', 'Corregiola', and 'Manzanillo'. In the case of 'Leccino', only two (Leccino-1 and Leccino-4) of the four samples analysed matched the SSR profile of the 'Leccino' reference. 'Hojiblanca-1' had the complete microsatellite allelic profile of 'Pendolino'. While Picholine-1, 'Picholine-2', and 'CSU Manzanillo-3' matched the reference cultivars 'Frantoio', 'Lechin de Sevilla', and 'Hojiblanca', respectively.

'Mission-1', 'Black Italian-1', and 'Unknown-3, matched the samples 'Verdale-1' and 'Verdale-2'. However, 'Verdale-1' and 'Verdale-2' differed significantly from the reference 'Verdale' SSR profile. Similar observations, using RAPD fingerprinting analysis, were reported by Sweeney (2005) in which the genetic fingerprints of 'Verdale' from the USA matched those of 'Black Italian' and 'Californian Mission', but did not match the reference 'Verdale' from France. In addition to the above cases of mismatches, 'Unknown-2', 'Picholine-1', 'CSU Corregiola-1', and 'Corregiola-2' showed complete genetic similarity to the reference cultivar, 'Frantoio'.

Previous studies on Australian olive cultivars using RAPD markers have reported that 'Paragon', 'Frantoja', and 'Correggiola' could be synonyms of 'Frantoio' (Archer, 1999; Mekuria *et al*., 1999). Recently, several samples of the French olive varieties 'Boutellion' and 'Leccure', were identified as synonyms of the Italian cultivar 'Frantoio' (data not shown). The genetic profiles of 'Arecuzzo-1' and 'Azapa-1' matched 'Arbequina' and 'Cornicabra', respectively; whereas 'Arecuzzo-2' did not match any of the reference samples in this study.

Four of the seven, initially unknown, samples showed microsatellite profiles corresponding to 'Frantoio', 'Kalamata', or 'Manzanilla de Sevilla' (Supplementary Table I; available on-line at www.jhortscib.com). Attempts to match the microsatellite profiles of the remaining 19 accessions with those cited in several published articles were unsuccessful [Bandelj and Javornik, 2002; 2007; Diaz *et al*., 2007; Bracci *et al*., 2009; Reale *et al*., 2006; Khadari *et al*., 2007; Baldoni *et al*., 2009; FAO Olive Germplasm Database (www.oleadb.it) validated on 31/02/2012].

The discrepancies observed between some of the 53 samples and their presumed reference cultivar could be attributed to the improper management of trees in olive collections and nurseries from which the materials were obtained. They may also reflect different provenances and/or errors in labelling or propagation of olive plant material. Our results reconfirm the importance of the use of molecular markers for the correct management of olive collections, as shown previously (Belaj *et al*., 2003a,b; Noormohammadi *et al*., 2007). However, the most striking result here, compared with previous work on olive cultivar identification, was the use of a set of microsatellite markers to characterise mother trees that had been used extensively for the commercial propagation of olive varieties in Australia. Nurseries should be encouraged to use molecular markers routinely for cultivar identification in view of these results, and the few previous efforts to identify nursery plantings (Belaj *et al*., 1999; Rubio and Arús, 1997; Cavagnaro and Rouselli, 2002).

Clustering of olive cultivars

Cluster analysis revealed that the 53 samples grouped into six major clusters based on genetic similarities and heterogeneity within and between samples (Figure 1). The first cluster (Cluster I) consisted of the cultivars 'Hardy's Mammoth', 'Manzanilla de Sevilla', and an unknown genotype. Due to their similarity, 'Hardy's Mammoth' and 'Manzanilla de Sevilla' were placed close to each other. Cluster II contained unknown samples initially labelled as 'Verdale-1', 'Verdale-2', 'Mission-1', 'Black Italian-1', 'Unknown-3', and 'Unknown-53'. All olive cultivars of Spanish origin, except 'Manzanilla de Sevilla', grouped in Cluster III and Cluster IV, including some unknown samples tentatively identified (in the absence of any reference DNA) as cultivars of Spanish origin. The two Greek cultivars, 'Kalamata' and 'Koroneiki', clustered with olive cultivars of Italian origin in Cluster V and Cluster VI, respectively, and were well separated from cultivars of Spanish origin (Clusters III and IV).

The lack of association of several of the unknown samples with their reference cultivar suggested that these may well have been selections from feral populations chosen for their desirable agronomic characteristics. Similarly, the close clustering of 'Hardy's Mammoth' with the 'Manzanilla de Sevilla' samples supported its feral origin *via* a cross between 'Manzanilla de Sevilla' and an unknown donor parent. In Australia, feral olive trees usually arise from seed that escaped from cultivated trees of crosses between existing cultivars and may show good adaptation under their edapho-climatic growing conditions (Sedgley, 2000). The selection of such genotypes having superior oil quality and fruit attributes would certainly advance the Australian olive industry. Future studies comparing the genetic profiles of these unknown genotypes with Australian feral populations may shed more light on these aspects.

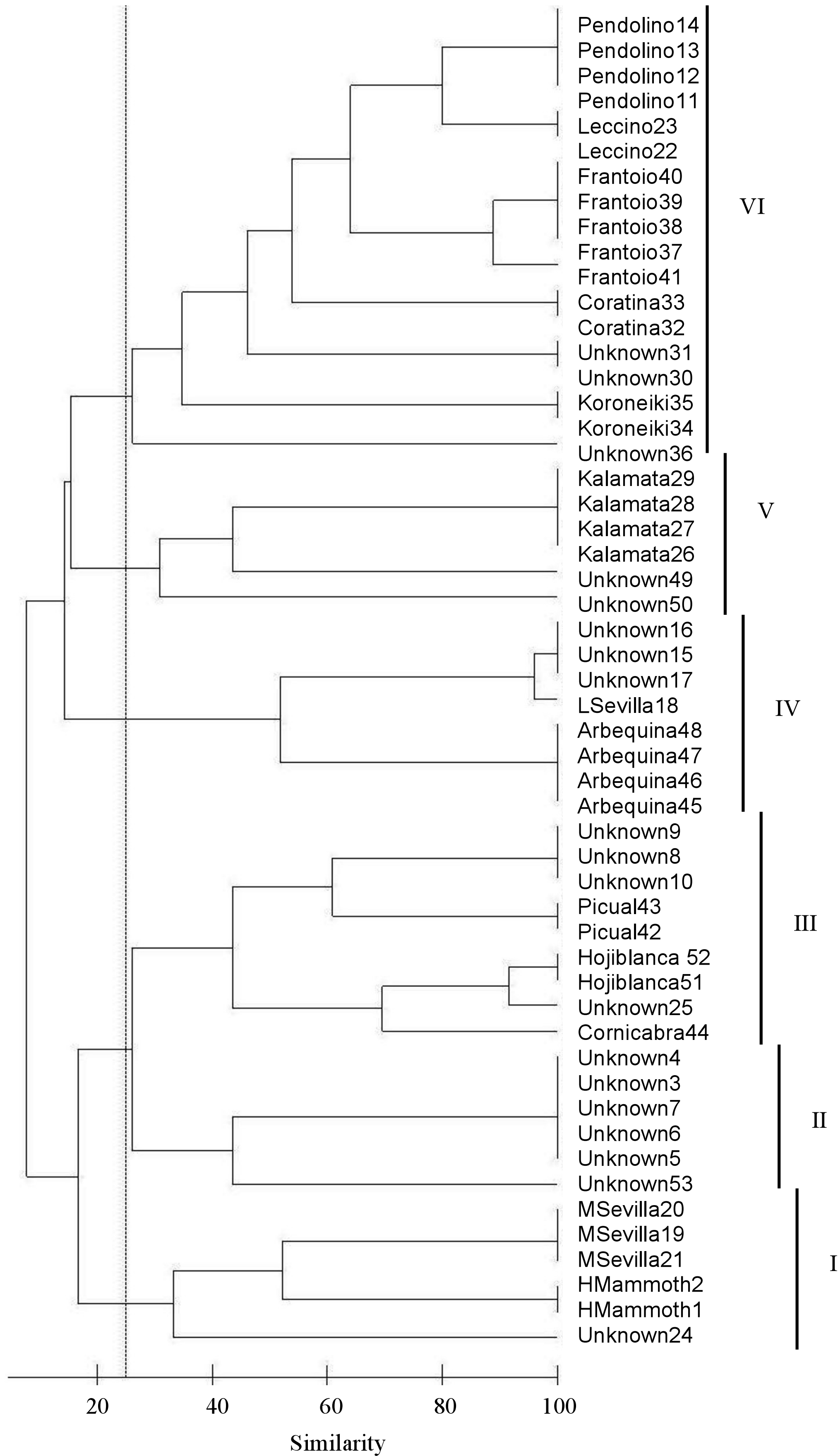

FIG. 1

Dendogram of 53 individual Australian olive tree DNA samples based on Dice's similarity coefficients and UPGMA cluster analysis arranged in six Clusters (I – VI). Codes are: LSevilla, 'Lechin de Sevilla'; MSevilla, 'Manzanilla de Sevilla'; HMammoth, 'Hardy's Mammoth'. Numbers 1 – 53 refer to accessions (see SUPPLEMENTARY TABLE I, available on-line at www.jhortscib.com). Dotted vertical line at 25% similarity refers to the six distinct Clusters

CONCLUSIONS

The microsatellite (SSR) marker data reported here provide, for the first time, a detailed characterisation and identification of many common and commercially important olive cultivars in Australia. The results confirmed the utility of microsatellite markers for the correct identification of trees planted in olive groves, including mother trees, prior to their use for commercial propagation. In addition, our results confirmed the need for an Australian database of olive germplasm based on descriptors, as well as to enrich the international olive database. The creation of a comprehensive database including both genotypic and phenotypic information will provide a valuable resource for the Australian olive industry and for future olive breeding programmes.

The authors thank Ms. Kerry Graham for technical assistance and Dr. Ben Stodart and Dr. Livinus Emberi for critically reviewing the manuscript. Dr. Belaj acknowledges a post-doctoral INIA contract (Subprograma DOC-INIA) from the National Institute of Agricultural Research (INIA), Ministry of Education and Culture, Spain. Thanks are also due to Dr. Carmen del Río for providing reference cultivars from the World Olive Germplasm Bank of Cordoba, Spain.

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Supplementary Table I

Allelic profiles of 53 individual Austrilian olive tree DNA samples and 14 reference olive cultivars based on seven microsatellite (SSR) markers

Sample No.	Expected Genotype	Observed Genotype	SSR marker													
			DCA9		DCA3		DCA16		DCA18		DCA5		EM090		EMO30	
		Hardy's Mammoth Reference (CSOG)	163	167	244	248	**175**	**175**	172	176	205	205	187	193	**190**	**196**
1	Hardy's Mammoth-1 (AOA)[†]	Hardy's Mammoth	163	167	244	248	**175**	**175**	172	176	205	205	187	193	**190**	**196**
2	Hardy's Mammoth-2 (G)	Hardy's Mammoth	163	167	244	248	**175**	**175**	172	176	205	205	187	193	**190**	**196**
		Verdale Reference (WOGB R. 76)	163	172	238	252	127	127	178	180	194	205	187	194	MD	MD
3	Verdale-1 (CSOG)	Unknown	163	167	238	238	151	155	176	180	200	205	189	189	**186**	**188**
4	Verdale-2 (CSOG)	Unknown	163	167	238	238	151	155	176	180	**200**	**205**	189	189	**186**	**188**
5	Mission-1** (CSOG)	Unknown	163	167	238	238	151	155	176	180	**200**	**205**	189	189	**186**	**188**
6	Unknown-3 (G)	Unknown	163	167	238	238	151	155	176	180	**200**	**205**	189	189	**186**	**188**
7	Black Italian-1** (AOA)	Unknown	163	167	238	238	151	155	176	180	**200**	**205**	189	189	**186**	**188**
8	UC1346-1** (CSOG)	Unknown	**163**	**195**	**248**	**248**	**127**	**151**	170	176	205	205	187	187	**188**	**192**
9	UC1346-2** (AOA)	Unknown	**163**	**195**	**248**	**248**	**127**	**151**	170	176	205	205	187	187	**188**	**192**
10	Leccino-2 (AOA)	Unknown	**163**	**195**	**248**	**248**	**127**	**151**	170	176	205	205	187	187	**188**	**192**
		Pendolino Reference (WOGB R. 87)	163	207	242	252	151	173	176	178	205	205	187	193	190	190
11	Pendolino-1 (AOA)	Pendolino	163	207	242	252	151	173	176	178	205	205	187	193	190	190
12	Pendolino-2 (CSOG)	Pendolino	163	207	242	252	151	173	176	178	205	205	187	193	190	190
13	Pendolino-3 (CSOG)	Pendolino	163	207	242	252	151	173	176	178	205	205	187	193	190	190
14	*Hoji Blanca-1* (AOA)	Pendolino	163	207	242	252	151	173	176	178	205	205	187	193	190	190
15	Nevadillo Blanco-1** (AOA)	Unknown	163	207	244	248	127	147	**168**	**168**	202	205	187	187	188	190
16	CSU Nevadillo Blanco-2** (CSOG)	Unknown	163	207	244	248	127	147	**168**	**168**	202	205	187	187	188	190
17	Nevadillo Blanco-3** (CSOG)	Unknown	163	207	244	248	127	147	**168**	**168**	202	205	187	187	188	190
		Lechin de Sevilla Reference(WOGB R.5)	163	207	244	248	127	147	**168**	**176**	202	205	187	187	188	190
18	*Picholine-2* (CSOG)[‡]	Lechin de Sevilla	163	207	244	248	127	147	**168**	**176**	202	205	187	187	188	190
		Manzanilla de Sevilla Reference (WOGB R. 127)	163	207	244	252	155	175	172	180	205	205	187	187	192	192
19	Unknown-5 (G)	Manzanilla de Sevilla	163	207	**244**	**252**	**155**	**175**	172	180	205	205	187	187	192	192
20	*Manzanillo-1* (AOA)	Manzanilla de Sevilla	163	207	**244**	**252**	**155**	**175**	172	180	205	205	187	187	192	192
21	*Manzanillo-2* (N)	Manzanilla de Sevilla	163	207	**244**	**252**	**155**	**175**	172	180	205	205	187	187	192	192
		Leccino Reference (WOGB R.82)	163	207	242	252	151	175	176	176	198	205	187	193	190	192
22	Leccino-1 (AOA)	Leccino	163	207	242	252	151	175	176	176	198	205	187	193	190	192
23	Leccino-4 (CSOG)	Leccino	163	207	242	252	151	175	176	176	198	205	187	193	190	192
24	Delmoroco-1** (AOA)	Unknown	**164**	**211**	**240**	**250**	151	175	172	180	205	205	189	189	186	190
25	Leccino-3 (G)	Unknown	167	195	238	248	127	155	172	180	205	205	189	189	188	190
		Kalamata reference (ONWA)	167	195	252	252	124	127	**184**	**184**	205	205	**187**	**197**	190	192
26	Kalamata-1 (CSOG)	Kalamata	167	195	252	252	124	127	**184**	**184**	205	205	**187**	**197**	190	192
27	Kalamata-2 (CSOG)	Kalamata	167	195	252	252	124	127	**184**	**184**	205	205	**187**	**197**	190	192
28	Unknown-4 (G)	Kalamata	167	195	252	252	124	127	**184**	**184**	205	205	**187**	**197**	190	192
29	Unknown-6 (G)	Kalamata	167	195	252	252	124	127	**184**	**184**	205	205	**187**	**197**	190	192
30	FS17-1** (AOA)	Unknown	**175**	**207**	**242**	**242**	151	155	172	176	205	205	**189**	**193**	**183**	**192**
31	FS17-2** (CSOG)	Unknown	**175**	**207**	**242**	**242**	151	155	172	176	205	205	**189**	**193**	**183**	**192**
		Coratina Reference (WOGB R. 79)	183	**195**	**238**	**242**	151	173	176	180	198	205	187	193	**192**	**198**
32	Coratina-1 (AOA)	Coratina	183	**195**	**238**	**242**	151	173	176	180	198	205	187	193	**192**	**198**
33	Coratina-2 (CSOG)	Coratina	183	**195**	**238**	**242**	151	173	176	180	198	205	187	193	**192**	**198**
		Koroneiki Reference (WOGB R. 218)	183	207	238	238	**147**	**151**	**172**	**174**	**194**	**194**	187	193	192	192
34	Koroneiki-1 (CSOG)	Koroneiki	183	207	238	238	**147**	**151**	**172**	**174**	**194**	**194**	187	193	192	192
35	Koroneiki-2 (G)	Koroneiki	183	207	238	238	**147**	**151**	**172**	**174**	**194**	**194**	187	193	192	192
36	Frantoio-1 (AOA)	Unknown	183	207	**236**	**242**	124	127	176	176	**196**	**205**	186	192	190	192
		Frantoio Reference (WOGB R. 80)	183	207	**236**	**242**	151	155	176	178	198	205	187	193	192	192
37	Frantoio-2 (CSOG)	Frantoio	183	207	**236**	**242**	151	155	176	178	198	205	187	193	192	192
38	*Picholine-1* (CSOG)	Frantoio	183	207	**236**	**242**	151	155	176	178	198	205	187	193	192	192
39	*CSU Corregiola-1* (CSOG)	Frantoio	183	207	**236**	**242**	151	155	176	178	198	205	187	193	192	192
40	Unknown-2 (G)	Frantoio	183	207	**236**	**242**	151	155	176	178	198	205	187	193	192	192
41	*Corregiola-2* (CSU)	Frantoio	183	207	**236**	**242**	151	155	176	178	198	205	187	193	188	190
		Picual Reference (WOGB R. 9)	185	193	238	248	127	155	170	176	205	205	187	187	188	190
42	Picual-1 (AOA)	Picual	185	193	238	248	127	155	170	176	205	205	187	187	188	190
43	Picual-2 (CSOG)	Picual	185	193	238	248	127	155	170	176	205	205	187	187	188	190
		Cornicabra Reference (WOGB R .10)	**185**	**195**	238	248	124	127	172	180	205	205	187	187	190	190
44	*Azapa-1* (AOA)	Cornicabra	**185**	**195**	238	248	124	127	172	180	205	205	187	187	190	190
		Arbequina Reference (WOGB R. 231)	**185**	**207**	230	242	124	147	**168**	**178**	202	205	187	193	**183**	**188**
45	Arbequina-1 (AOA)	Arbequina	**185**	**207**	**230**	**242**	**124**	**147**	**168**	**178**	202	205	187	193	**183**	**188**
46	Arbequina-2 (N)	Arbequina	**185**	**207**	**230**	**242**	**124**	**147**	**168**	**178**	202	205	187	193	**183**	**188**
47	Arbequina-3 (CSOG)	Arbequina	**185**	**207**	**230**	**242**	**124**	**147**	**168**	**178**	202	205	187	193	**183**	**188**
48	*Arecuzzo-2*** (AOA)	Arbequina	**185**	**207**	**230**	**242**	**124**	**147**	**168**	**178**	202	205	187	193	**183**	**188**
49	Unknown-7 (G)	Unknown	**195**	**195**	252	252	**147**	**175**	**170**	**180**	**201**	205	186	192	190	192
50	Unknown-1 (G)	Unknown	**195**	**205**	**234**	**244**	**147**	**155**	**176**	**184**	**207**	**213**	**184**	197	190	192
		Hoji Blanca Reference (WOGB R. 2)	**195**	**207**	238	248	127	155	172	180	205	205	189	189	188	190
51	Hoji Blanca-2 (CSOG)	Hoji Blanca	**195**	**207**	238	248	127	155	172	180	205	205	189	189	188	190
52	*CSU Manzanillo-3* (CSOG)	Hoji Blanca	**195**	**207**	238	248	127	155	172	180	205	205	189	189	188	190
53	Arecuzzo-1** (AOA)	Unknown	**195**	**213**	**236**	**252**	**127**	**151**	176	178	205	205	**193**	**193**	**188**	**188**

[†]Codes or numbers in parentheses refer to its registration number or source of the accession: WOGB, World Olive Germplasm Bank; CSOG, Charles Sturt University Olive Grove; AOA, Australian Olive Association; ONWA, Olea Nursery Western Australia; N, private nursery; G, olive grower.
[‡]Sample/accessions in italics means the original sample matched a different cultivar; MD, missing data.
Marker sizes (bp) in bold font represent a unique allelic pattern per locus. Marker sizes (bp) in bold font and underlined represent unique alleles.