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The genetic diversity of Sardinian myrtle (*Myrtus communis* L.) populations

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Abstract

Background: The myrtle (*Myrtus communis*) is a common shrub widespread in the Mediterranean Basin. Its fruit and leaves exhibit antioxidant, antibacterial and antifungal properties, and are used for their content of essential oils and for their medicinal properties, but most commonly as an ingredient in locally made liquor. The uncontrolled exploitation of natural stands has reduced both the species' geographical coverage and the size of individual populations. The selection of genotypes for controlled cultivation requires a characterization of the genetic diversity present both within and between populations.
Results: Genotypic variation was evaluated using ISSR profiling and genetic diversity characterized using standard population genetics approaches. Two major clusters were identified: one capturing all the candidate cultivars selected from various Sardinian localities, and the other wild individuals collected from Asinara, Corsica and Surigheddu. A moderate level of gene flow between the Sardinian and Corsican populations was identified. Discriminant analysis of principal components revealed a level of separation among the wild populations, confirming the population structure identified by the clustering methods.
Conclusions: The wild accessions were well differentiated from the candidate cultivars. The level of genetic variability was high. The genetic data were compatible with the notion that myrtle has a mixed pollination system, including both out-pollination by insects and self-pollination. The candidate cultivars are suggested to represent an appropriate basis for directed breeding.

Keywords: domestication; genetic structure; germplasm; ISSR genotyping.

INTRODUCTION

The myrtle (*Myrtus communis*) is a typical shrub of the Mediterranean Basin, and Sardinia is recognized as one of its main local centres of diversity (Médail and Quézel, 1999).

Its young leaves and mature fruits provide the raw material for the extraction of essential oils and the production of local liquors (Gardeli et al. 2008; Mulas and Melis, 2011; Mulas, 2012). However, the economic value of the species reflects in addition both the proven antibacterial and anti-inflammatory properties of certain extracts (Feibt et al. 2005; Rossi et al. 2009) and the use of leaves and berries in various processed food and cosmetic formulations (Bonjar, 2004). In Sardinia alone, the annual production of myrtle based liquor is > 4 ML (Mulas, 2012). The plant's biomass is used in either fresh or dry form as a food additive, and its leaf can substitute for hops in beer manufacture (Barboni et al. 2010).

To date, the source of both myrtle fruit and leaf material has relied on exploiting wild stands (Mulas and Cani, 1999; Tuberoso et al. 2007). The harvesting of biomass, along with increasing urbanization, natural fires, grazing by livestock and wild herbivores, and the cutting of firewood are all putting pressure on the size of wild populations, and therefore inevitably on the genetic diversity retained by the species. These same pressures have encouraged our attempt to identify candidate cultivars with a view to establishing managed stands of the species for commercial cultivation (Mulas et al. 2002; Mulas, 2012). In recent years, over 200 ha of myrtle plantations have been established.

Three major considerations have prompted the decision to genetically characterize myrtle. Firstly, it is important that the end products derived from a cultivated plantation maintain the quality of those produced from wild stands. Secondly, the diversity captured by cultivars needs to be defined in order to inform future breeding strategy, and thirdly, the evaluation of genetic diversity is a major component of any *ex situ* conservation programme (Pressey et al. 2007). A deal of information has been assembled with respect to the biology of the myrtle plant and its chemical composition (Mulas and Fadda, 2004; Fadda and Mulas, 2010; Messaoud and Boussaid, 2011; Mulas et al. 2013). A comparison of myrtle populations present in Sardinia and Corsica has suggested that they are rather homogeneous, suggesting a significant amount of gene flow between these neighbouring Mediterranean islands (Migliore et al. 2012). Wild populations are thought to be genetically quite variable (Bruna et al. 2007; Migliore et al. 2012), but over-exploitation and domestication will inevitably exert a negative pressure on the level of diversity retained. The development of DNA-based marker technology has facilitated the assessment of genetic diversity in myrtle, as in so many other plant species. For example, AFLP genotyping has indicated that Italy represents a genetic transition zone between the western and eastern ends of the Mediterranean Basin (Bruna et al. 2007; Şerçe et al. 2008; Albaladejo et al. 2010).

Here, we report the characterization of the diversity captured by a number of Sardinian candidate cultivars of myrtle and some wild populations, evaluated using ISSR (inter simple sequence repeat) markers.

MATERIALS AND METHODS

Plant materials

Leaf tissue from 80 wild accessions of myrtle was sampled, along with tissue harvested from 36 candidate cultivars, originally selected from various Sardinian localities (Figure 1). The latter sets of plants are maintained by the Experimental Station of the University of Sassari in Oristano, on the west coast of the island (39º53'N, 8º37'E, 11 m above sea level, 10 km from the sea). These individuals have emerged from a mass selection program initiated in 1995. The wild material was sourced from either an area no longer used for cropping near the locality of Surigheddu, or from the Asinara Island National Park (Table 1; Figure 1). A small sample (nine accessions) of wild Corsican material (Table 1) was included, originating from an area close to the southern coast and hence in a location where gene flow between Sardinia and Corsica - if it occurs - is possible. The var. *tarentina* clone SAS1 represented a control. Because wild myrtle propagates both sexually and vegetatively (González-Varo et al. 2009), no plants growing < 20 m from one another were sampled, to avoid including clones of any specific genotype.

ISSR genotyping

Genomic DNA was extracted from young leaves following Lodhi et al. (1994) protocol. Out of a set of 17 primers obtained from the University of British Columbia Biotechnology Laboratory (Goldman, 2008), 12 proved to be informative and able to generate robust profiles. Eleven of the primer sequences harboured di-nucleotide repeats (nine were anchored at their 3' end and the other two at their 5' end); the remaining primer was based on a tetra-nucleotide repeat (Table 2). Each 20 µl PCR contained 50 ng genomic DNA, 8 µl 2.5 X Hot Master Mix (Eppendorf), 0.2 µM of one of the 12 primers. The reactions were initially denatured (94ºC/2 min), then cycled 30 times as follows: 94ºC/1 min, annealing temperature (Table 2) / 1 min, 65ºC/1 min, and completed with an extension of 65ºC/7 min. The resulting amplicons were electrophoretically separated through 6% denaturing polyacrylamide gels, and visualized using silver staining, following Bassam et al. (1991) protocol. Profiles were scored by recording the presence/absence of each fragment. Each template/primer combination was amplified in three independent reactions to identify the reproducible fragments.

Table 1. The set of accessions studied and their geographical origin.

CODE	Name	Location	Latitude	Longitude
COR	COR4	Coti-Chiavari	41° 48' 09.65''	8° 46' 38.58''
	COR7	Tareu	41° 45' 04.91''	9° 24' 00.56''
	COR8	Aleria	42° 06' 22.76''	9° 29' 27.46''
	COR9	Poggio Mezzana	42° 23' 46.17''	9° 29' 18.97''
	COR10	Brando	42° 46' 42.00''	9° 27' 28.12''
	COR11	Santa Lucia	42° 42' 42.35''	9° 25' 55.73''
	COR13	Calvì	42° 33' 46.54''	8° 44' 03.35''
	COR14	Isolella	41° 51' 30.28''	8° 48' 23.93''
	COR15	Propriano	41° 40' 16.54''	8° 54' 28.90''
ASI	ASI1	Fornelli	40° 59' 7.91''	8° 14' 23.3''
	ASI4	Santa Maria	40° 59' 1.16''	8° 15' 30.6''
	ASI5	Tumbarino	41° 02' 2.41''	8° 14' 51.2''
	ASI6	Cala d'orata	41° 01' 1.17''	8° 14' 43.6''
	ASI7	Cala Sant'Andrea	41° 01' 0.87''	8° 14' 40.3''
	ASI9	Sant'Andrea	41° 00' 8.40''	8° 14' 43.5''
	ASI11	Sant'Andrea	41° 01' 0.87''	8° 14' 40.9''
SUR	SUR6	Surigheddu	40° 35' 8.41''	8° 23' 0.40''
	SUR10	Surigheddu	40° 35' 8.36''	8° 23' 0.35''
	SUR11	Surigheddu	40° 35' 8.36''	8° 23' 0.33''
	SUR15	Surigheddu	40° 35' 8.33''	8° 23' 0.22''
	SUR22	Surigheddu	40° 35' 8.25''	8° 23' 0.32''
	SUR23	Surigheddu	40° 35' 8.25''	8° 23' 0.30''
	SUR30	Surigheddu	40° 35' 8.23''	8° 23' 0.48''
	SUR35	Surigheddu	40° 35' 8.16''	8° 23' 0.40''
	SUR38	Surigheddu	40° 35' 8.18''	8° 23' 0.33''
	SUR42	Surigheddu	40° 35' 8.40''	8° 23' 0.29''
	SUR46	Surigheddu	40° 35' 8.20''	8° 23' 0.20''
	SUR51	Surigheddu	40° 35' 8.28''	8° 23' 0.14''
	SUR57	Surigheddu	40° 35' 8.11''	8° 23' 0.08''
	SUR64	Surigheddu	40° 35' 8.09''	8° 23' 0.16''
	SUR74	Surigheddu	40° 35' 8.06''	8° 23' 0.57''
	SUR81	Surigheddu	40° 35' 8.02''	8° 23' 0.91''
	SUR90	Surigheddu	40° 35' 8.10''	8° 23' 0.63''
	SUR93	Surigheddu	40° 35' 7.94''	8° 23' 0.52''
	SUR99	Surigheddu	40° 35' 7.91''	8° 23' 0.48''
	SUR103	Surigheddu	40° 35' 7.94''	8° 23' 0.46''
	SUR109	Surigheddu	40° 35' 7.91''	8° 23' 0.40''
	SUR112	Surigheddu	40° 35' 7.90''	8° 23' 0.42''
	SUR118	Surigheddu	40° 35' 79.3''	8° 23' 030''
	SUR125	Surigheddu	40° 35' 80.0''	8° 23' 024''
	SUR127	Surigheddu	40° 35' 79.3''	8° 23' 018''
	SUR131	Surigheddu	40° 35' 80.3''	8° 23' 011''
	SUR135	Surigheddu	40° 35' 79.3''	8° 23' 014''
SAR	BUD1	Budoni	40° 42' 11''	9° 42' 35''
SAR	BOS1	Bosa	40° 20' 06''	8° 22' 51''
	BOS2	Bosa	40° 20' 06''	8° 22' 51''

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SAR	CPT3	Uta-Monte Arcosu	39° 11' 20"	8° 56' 08"
	CPT4	Uta-Monte Arcosu	39° 11' 20"	8° 56' 08"
	CPT5	Uta-Monte Arcosu	39° 11' 20"	8° 56' 08"
	CPT6	Uta-Monte Arcosu	39° 11' 20"	8° 56' 08"
SAR	ISL3	Isili	39° 44' 23"	9° 06' 61"
	ISL1	Isili	39° 44' 23"	9° 06' 61"
SAR	LAC1	Laconi	39° 51' 07"	9° 03' 09"
	LAC10	Laconi	39° 51' 07"	9° 03' 09"
	LAC11	Laconi	39° 51' 07"	9° 03' 09"
	LAC3	Laconi	39° 51' 07"	9° 03' 09"
	LAC31	Laconi	39° 51' 07"	9° 03' 09"
SAR	MON2	Monti	40° 48' 04"	9° 19' 29"
	MON4	Monti	40° 48' 04"	9° 19' 29"
	MON5	Monti	40° 48' 04"	9° 19' 29"
SAR	ORO2	Orosei	40° 22' 21"	9° 41' 50"
	ORS1	Orosei	40° 22' 21"	9° 41' 50"
	ORS2	Orosei	40° 22' 21"	9° 41' 50"
	ORS3	Orosei	40° 22' 21"	9° 41' 50"
SAR	PSF1	Parco dei sette fratelli	39° 20' 29"	9° 13' 04"
	PSF4	Parco dei sette fratelli	39° 20' 29"	9° 13' 04"
SAR	RUB	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM12	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM13	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM14	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM15	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM20	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM3	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM4	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM4B	Rumanedda	40° 40' 56"	8° 21' 38"
	RUM6	Rumanedda	40° 40' 56"	8° 21' 38"
SAR	SBD1	Olia Speciosa	39° 16' 38"	9° 31' 36"
SAR	SIN2	Siniscola	40° 34' 39"	9° 41' 23"
SAR	TEL2	Telti	40° 52' 47"	9° 21' 20"

Assessment of population structure and genetic diversity

The genetic structure of the populations was explored using the Bayesian clustering model implemented in Structure v 2.3.3 (Pritchard et al. 2000). The program was run 20 times, applying a *K* value (the number of clusters) varying from one to seven. For each value of *K*, 20 replicated sets of 10,000 Monte Carlo Markov chain interactions with a burn-in of 10,000 steps were calculated. The maximum likelihood value of *K* was estimated as described by Evanno et al. (2005).

Within and between populations diversity was estimated using Popgene v1.31 software (Yeh and Boyle, 1997), assuming the existence of a Hardy-Weinberg equilibrium.

The proportion of polymorphic loci (P%), the observed mean number of alleles per locus (n_o), the effective mean number of alleles per locus (n_e), Shannon's information index I_s and Nei's gene diversity index H_e (Nei, 1973) were calculated within each population. Genetic diversity between the populations was estimated using the Nei (1973) parameters, namely the coefficient of genetic differentiation G_{ST}, the between population genetic diversity coefficient D_{ST}, the total population genetic diversity

coefficient H_T and the within population genetic diversity coefficient H_S. The average level of gene flow among populations N_m was calculated from $(1- G_{ST})/2G_{ST}$.

A dendrogram based on the Nei and Li (1979) pairwise distance matrix was derived using the UPGMA method (Nei, 1973). The confidence level attached to each node was calculated from 1,000 bootstrap replicates implemented in Winboot software (www.irri.org/science/software/winboot.asp). A discriminant analysis was used to reveal the genetic relationship between the various populations. As the number of markers was much larger than the number of observations, a standard discriminant analysis could not be performed, so a multivariate analysis procedure termed "discriminant analysis of principal components" (DAPC) was employed (Jombart et al. 2010). Computations were performed using the Syn-tax 2000 program package (Podani, 2001). A Euclidean geographical distance matrix was generated using latitude and longitude coordinates. A Mantel test was performed to assess potential association between the genetic and geographical distance matrices using Ntsys-pc v2.1 software (Rohlf, 2000), applying 1,000 random permutations.

Table 2. The twelve informative ISSR primers used to explore the genetic diversity of myrtle.

Primer	Sequence (5'-3')	T (°C)	Total number of bands	Number of polymorphic bands detected
808	(AG)8C	46	20	14
810	(GA)8T	46	19	16
814	(CT)8A	48	14	11
818	(CA)8G	45	16	12
827	(AC)8G	45	19	19
840	(GA)8YT	45	18	11
841	(GA)8YC	45	25	17
855	(AC)8YT	48	17	17
857	(AC)8YG	45	23	20
873	(GACA)4	40	20	16
888	DBD(AC)7	52	14	12
890	VHV(GT)7	54	16	14
Total			221	179
Average			18.42	14.92

T: annealing temperature used in the PCR.

RESULTS

Population genetic structure and diversity

The variation in the number of individual plants within each population reflected the prior mass selection exercise. The candidate cultivar group was assembled on the principle of maintaining as high a level of phenotypic variability as possible. Several accessions originated from an identical sampling location: for instance, the Rumanedda accessions included multiple selections of white berry types. The wild Surigheddu samples (SUR) consisted of 27 individuals randomly selected, while at both Asinara (ASI) and Corsica (COR), some preliminary screening was performed to cover the range of phenotypes present. The 12 informative ISSR primers amplified 221 reproducible fragments (18.4 fragments per primer, Table 2). The number of polymorphic within population fragments ranged from 123 to 179 (mean 151.5, Table 3), which satisfied the threshold number required to provide a reliable estimate of genetic similarity (Nybom, 2004; Kafkas et al. 2006).

Table 3. Measures of genetic diversity.

Cluster	n	P%	n_o	n_e	I_s	H_e
Cluster 1	179	81.42	1.99	1.59	0.52	0.35
Cluster 2	142	74.00	1.78	1.48	0.41	0.28
Mean	151.50	75.86	1.84	1.51	0.44	0.30
S.D			0.14	0.08	0.07	0.05

n: number of polymorphic loci per population; P%: proportion of polymorphic loci; n_o: observed number of alleles per locus; n_e: effective number of alleles per locus; I_s; Shannon's information index; H_e: Nei's gene diversity. SD: standard deviation.

The maximum likelihood *K* value was 2 (ΔK = 25.95) (Figure 2a). Cluster #1 harboured all the Sardinian candidate cultivars (SAR), while all the wild materials (ASI, COR and SUR) fell into Cluster #2 (Figure 2b). Cluster #1 was divisible into two subgroups (#1A and #1B). The Laconi, Monti and Orosei accessions clearly belonged to Cluster #1B, but about 42% of the Cluster #1 accessions showed no definite affinity to either of the sub-clusters (Q < 0.7). In Cluster #2, it was also possible recognize two subgroups (#2A and #2B) (Figure 2c). The ASI, COR and SUR accessions were not separated by subgrouping (Figure 2d), and the coefficient of membership of most individuals was low, as also obtained for the subgroups of Cluster #1.

Across all accessions, P% was 75.9, n_o was 1.83, n_e was 1.51, I_s was 0.44 and H_e was 0.29 (Table 3). The members of Cluster #1 were more variable than those of Cluster #2 (P% was, respectively, 81.4% and 74.0%, n_o respectively 1.99 and 1.78, n_e respectively 1.59 and 1.48, I_s respectively 0.52 and 0.41 and H_e respectively 0.35 and 0.28). Within the Cluster #2 subgroups, the lowest H_e (0.24) was associated with the ASI population (data not shown). The value of H_T was 0.34, partitioned into a D_{ST} of 0.04 and an H_S of 0.30 (Table 4), showing that the within population diversity far outweighed the between population component. A strong influence on this partitioning was exercised by the Cluster #1 members, collected at different Sardinian sites. The G_{ST} value across all loci was 0.13, while the N_m was calculated to be 3.42 (Table 4).

Table 4. Measures of genetic differentiation between populations.

Populations	H_T	H_S	D_{ST}	G_{ST}	N_m
Mean value of loci	0.34	0.30	0.04	0.13	3.42
SD	0.02	0.02	0.03	0.10	

H_T: total genetic diversity; H_S: within-population diversity; D_{ST}: between-population diversity; G_{ST}: coefficient of gene differentiation; N_m: gene flow between populations. SD: standard deviation.

Relationships between the accessions

The relationships among the 80 accessions were subjected to a cluster analysis based on the simple matching coefficient (SM) and the UPGMA algorithm (Figure 3). The UPGMA r of 0.87 indicated that the clustering gave an accurate representation of the genotypic data. The accessions were separated into two major clades (SM = 0.66); the first clade was populated almost entirely by the candidate cultivars, while the second included a mixture of wild (ASI, COR and SUR) material, plus the candidate cultivar BOS1. The phylogeny was fairly consistent with the outcomes of both the Structure analysis and the UPGMA cluster analysis. The control SAS1, a var. *tarentina* clone, did not cluster with any of

the 79 accessions. The DAPC analysis is illustrated in Figure 4. The data for this procedure was reduced to a set of 59 individuals (33 candidate cultivars, 12 SUR accessions, and seven accessions each of the COR and ASI sets) scored for 179 ISSR loci. A centred (*i.e*., covariance-based) principal components analysis produced 58 components, with the first five accounting for, respectively, 9.5%, 7.0%, 5.0%, 4.0% and 3.8% of the genetic variance. The first 45 components (cumulatively explaining 95% of the variance) were adopted for the DAPC, which generated a three dimensional ordination in which only the first two axes proved to be of importance: the first explained 85% and the second 13% of the between group variation (Figure 4). In the ordination of individuals, components (*i.e.*, input variables for DA) are superimposed as arrows to allow interpretation of explanatory variables. Confidence circles, drawn around the centroids of groups, contain the population means with a probability of 95%, provided that sampling was random and multivariate normality was satisfied. The resulting separation of the four populations was reasonably clear even in two dimensional canonical spaces. Axis 1 coincided with principal component 1, and separated the candidate cultivars from the other three populations. SUR and COR, though they mapped closely to one another, were distinct. The implication is that 85% of the between population variance is explained by the three wild populations. Axis 2 was most closely associated with principal component 5, and explained the separation between the ASI population and the other three populations. In the Structure analysis, the candidate cultivars all mapped within Cluster #1 (Figure 3), but DAPC was able to separate ASI, SUR and COR.

There was no convincing evidence of correlation between genotype and geographical origin, since the Mantel test revealed a non-significant overall association ($r = 0.065$, $P = 0.55$) (data not shown).

DISCUSSION

A major object of the present study was to explore the genetic diversity present in a set of candidate cultivars compared to what was retained in wild populations. The genotypic platform ISSR proved to be as effective for detecting variation as AFLPs have been reported to be (Bruna et al. 2005; Agrimonti et al. 2007). The standard measures of diversity P% and H_e suggested that diversity was higher in the candidate cultivars than in the wild materials, and in concert with this, both the average number of observed and effective alleles were higher in the candidate cultivars than in the wild accessions. The strong implication is that phenotypic selection can be effective in maximizing the genetic variation of wild populations, at least during the initial stages of domestication. Once domesticated, there is still the possibility of enhancing genetic diversity by deliberate introgression from wild material - so, for example, microsatellite analysis in raspberry has demonstrated a higher level of heterozygosity in cultivated germplasm than in wild accessions (Dossett et al. 2010).

The ASI group of accessions showed the least genetic diversity of the three wild populations. The origin of these materials is from a relatively small island, cutting the population off from introgression; as a result it may have become quite vulnerable to genetic erosion. The structure analysis established a clear separation between the candidate cultivars and the three wild populations ASI, COR and SUR. Within the SUR and COR populations (although not the ASI one), there was no evidence for any correlation between genotype and geographical origin, which suggests that gene flow between natural stands of the species must be commonplace.

Although the three analytical approaches adopted (Structure, UPGMA and DAPC) are quite distinct from one another, nevertheless, their outcomes were in good agreement. The candidate cultivars regularly formed a discrete clade. A possible reason why the three wild populations failed to be distinguished from one another by the UPGMA analysis may be that the use of a simple matching coefficient emphasizes the effect of correlated markers, whereas the DAPC approach is based on the use of derived variables which are by definition orthogonal to one another. Both DAPC and structure generated a clear separation between the candidate cultivars and the wild populations; and at the same time provided evidence for gene flow between Corsican and Sardinian populations (Figure 4).

The moderate level of differentiation between the Corsican and Sardinian germplasm may reflect one or all of (a) the sharing of a common ancestral genetic pool, (b) the absence of any differential selective pressure, and (c) a limited generational separation between the two populations. Fossil records confirm that *Myrtus* genus enjoyed a wide distribution in the Mediterranean Basin during the late Miocene era

(Migliore et al. 2012). The myrtle populations able to survive the climatic fluctuations during the Pleistocene era probably relied on refugia, where the plant still grows today. Thus the rather close genetic relationship between the Corsican and Sardinian populations might simply reflect a rather recent dispersion of the species. The species reproduces via both self- and cross-fertilization (Mulas and Fadda, 2004; González-Varo et al. 2009). Birds are thought to be important agents of myrtle seed dispersal, and would certainly have the range to enable the dissemination of material between these two neighbouring islands. The lack of any significant correlation between genotype and geographical origin is a diagnostic of populations enjoying a high rate of gene flow. However, according to both Messaoud et al. (2006) and Agrimonti et al. (2007), such a correlation was recognizable.

About 87% of the overall genetic diversity was contributed by the within population component. A major influence on this result was the high variability contributed by the candidate cultivars, which were selected from wild populations growing in several distinct environments across Sardinia (Table 1). An additional positive factor relates to the significant degree of self-pollination of the species (Mulas and Fadda, 2004; González-Varo et al. 2010), since it has been established that this mating system tends to conserve within population genetic diversity more effectively than does cross-pollination (Barrett, 2013; Cowling, 2013). Both the present ISSR genotyping and the AFLP-based system used by Agrimonti et al. (2007) uphold the idea that within population gene flow is much more prevalent than that occurring between populations. Apart from the additional contribution of seed dispersal by birds, within population genetic variation can also be acted on by mutation, genetic drift, selection and geographic range (Aguilar et al. 2008; Rauf et al. 2010; Pickup et al. 2012).

The domesticated collection of candidate cultivars has succeeded in capturing a wide range of genetic variation, which could form the basis of a myrtle breeding programme. More broadly, such characterization of the genetic diversity present in wild plant populations can help to identify the major factors influencing the level of genetic variation retained in diversity hot spots. The rather low level of diversity uncovered in the ASI population emphasizes the vulnerability of small, isolated populations faced with environmental fragmentation. Our ongoing exploration of gene flow between domesticated and wild myrtle populations is focused on a set of hybrids established in an effort to enhance the agronomic performance of myrtle.

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Figures

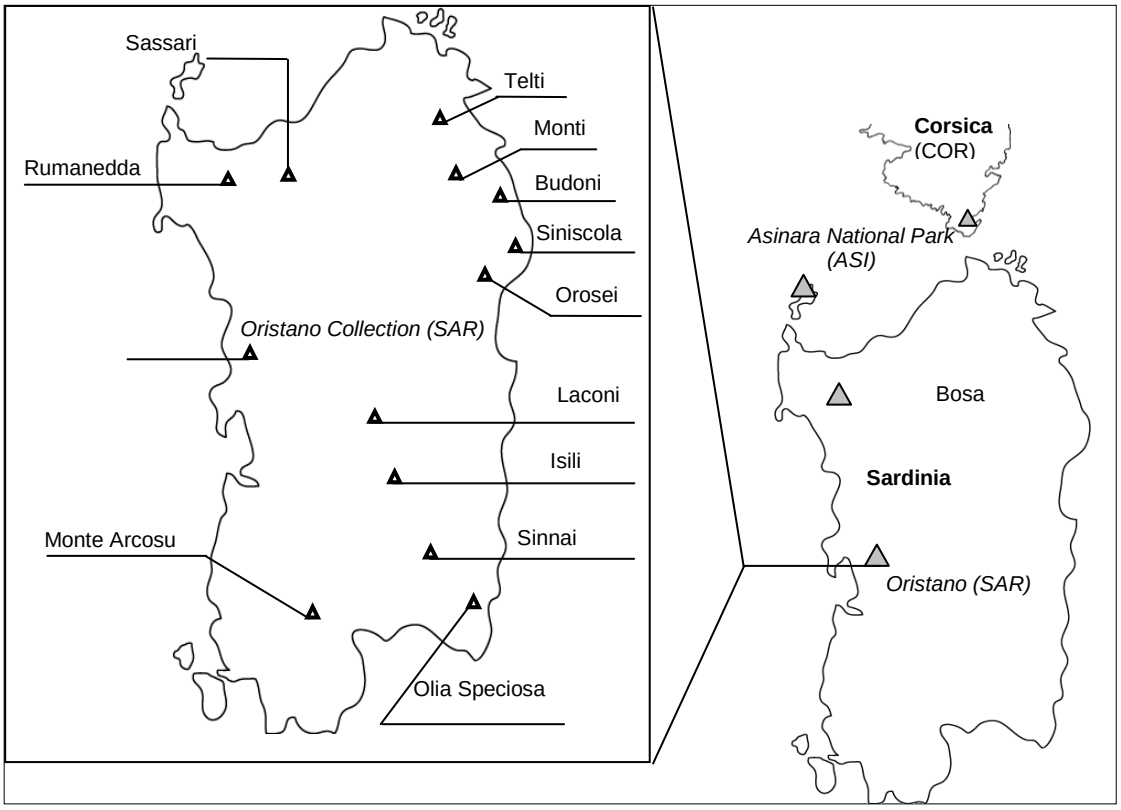

Fig. 1 Myrtus communis collection sites. Four different populations were chosen: Corsica, Asinara, Surigheddu and the collection field of Oristano. In the right site of the figure is reported the origin of the wild Sardinian myrtle accessions selected as candidate cultivar selection and held in the experimental field of Oristano.

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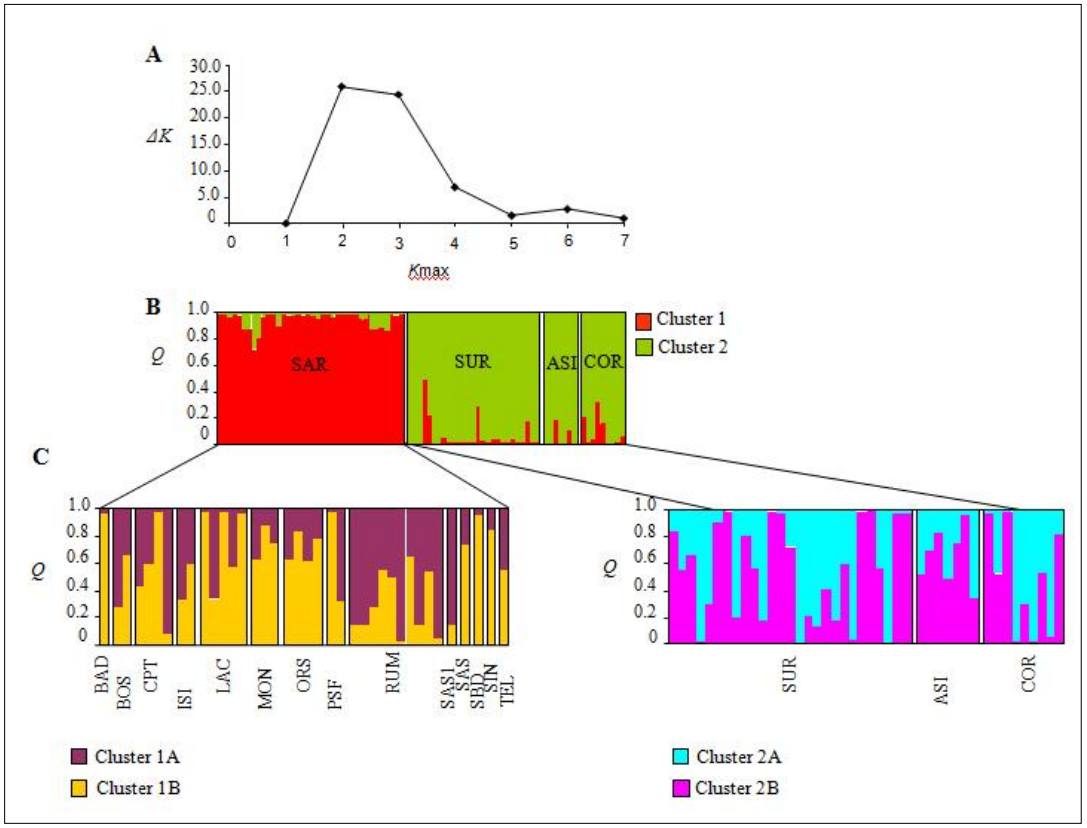

Fig. 2 Population genetic structure analysis. Admixture proportions (*K* = 2) of 80 *Myrtus communis* individuals corresponding to 4 populations. Bayesian clustering analysis was performed using program Structure. A: results for a run with the highest likelihood identified using Δ*K*; B: the population genetic structure division; C and D: analysis of population genetic structure performed on Clusters 1 and 2.

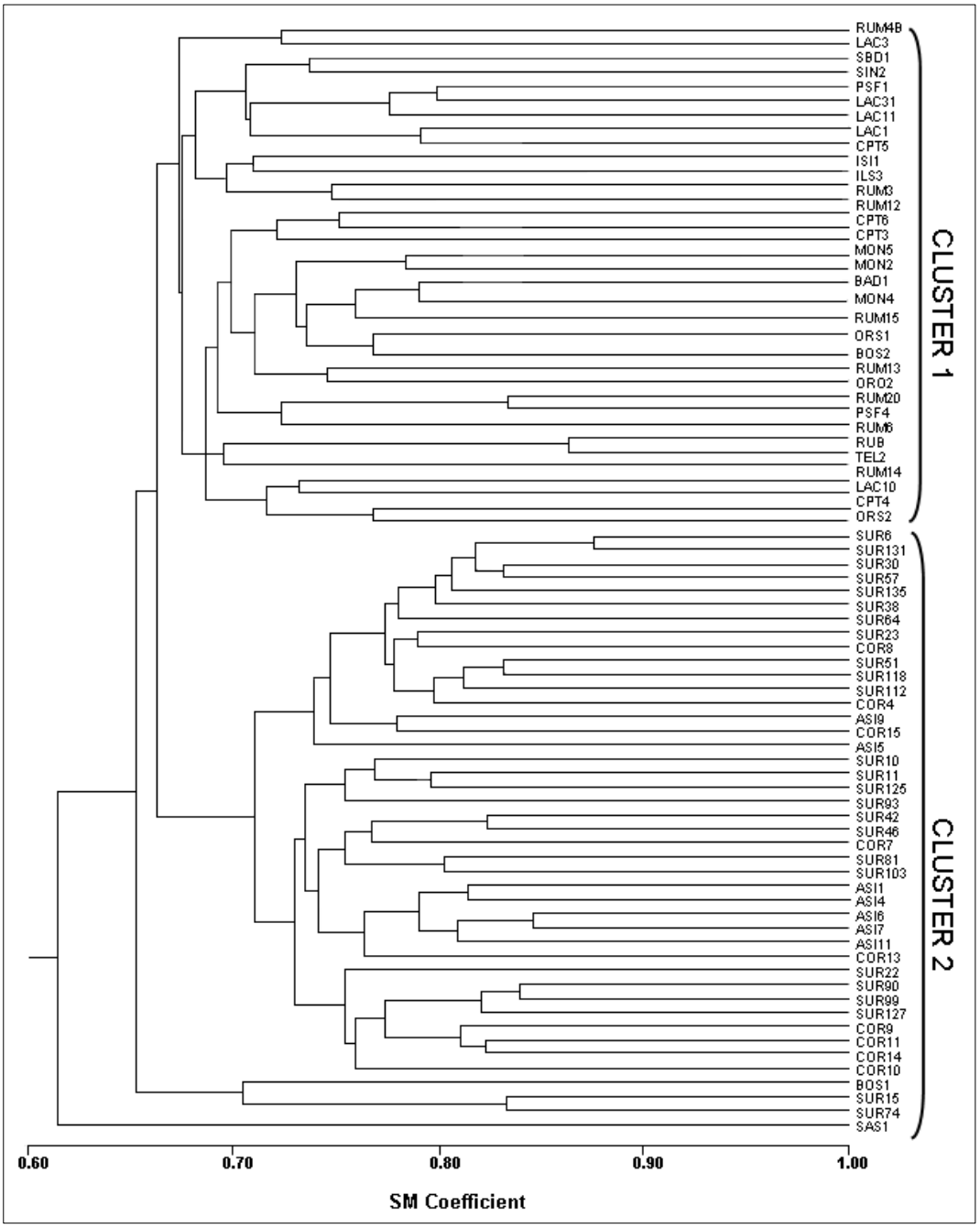

Fig. 3 Dendogram of 80 myrtle genotypes based on UPGMA clustering from the matrix of SM coefficient. On the right side of the dendrogram are reported the clusters 1 and 2 as identified by structure.

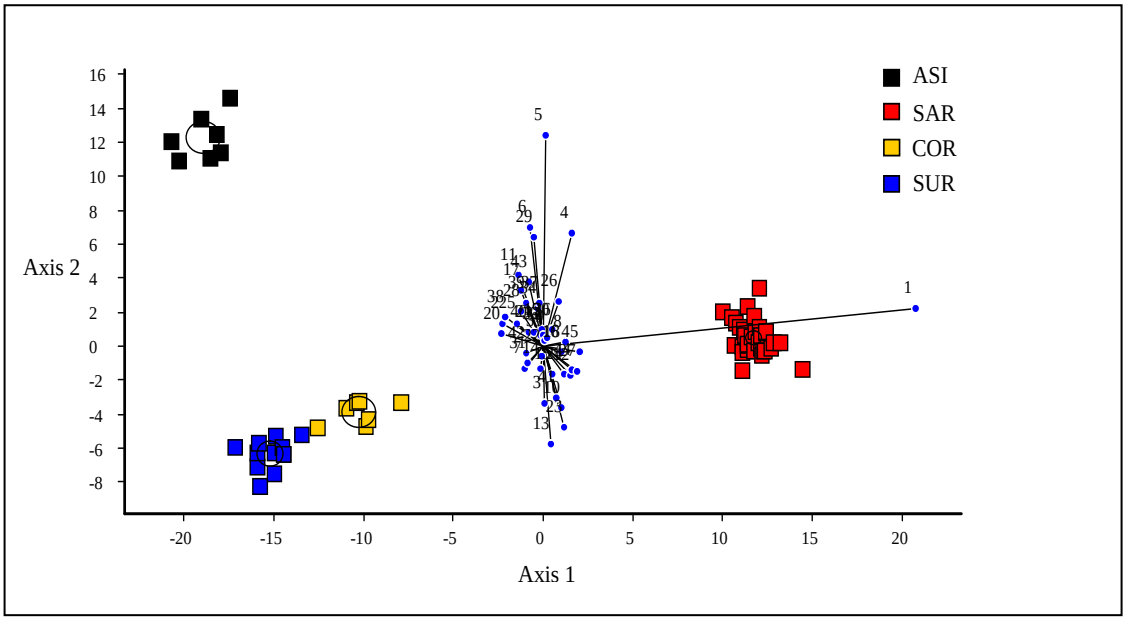

Fig. 4 DAPC ordination of 59 Myrtus individuals, with confidence circles around the centroids. Components are superimposed over the ordination as arrows showing that DA axis 1 coincides with component 1, while DA axis 2 is most highly correlated with component 5. Other components do not play significant role in group separation