

## RESEARCH NOTES

# The Forgotten Myrtle of the Alhambra Gardens of Granada: Restoring and Authenticating World Heritage

R. De la Herrán<sup>1</sup>, M. Casares<sup>2</sup>, F. Robles<sup>1</sup>, J. Tito<sup>3</sup>, R. Navajas-Pérez<sup>1</sup>, M. J. Molina-Luzón<sup>1</sup>, M. de los Reyes Gonzalez-Tejero<sup>2</sup>, P. J. Sola-Campoy<sup>1</sup>, A. Gutiérrez-Guerrero<sup>1</sup>, and J. C. Ruiz-Rejón<sup>1\*</sup>

## ABSTRACT

In the Alhambra (Granada, Spain), and in other Moorish locations, several individuals of the original variety of myrtle, the emblematic plant of their gardens, have been identified and genetically authenticated. After microsatellite analysis, we differentiated between the wild form (*Myrtus communis* L.) and two cultivated varieties: the one original to the Alhambra, the Moorish myrtle (subsp. *baetica*), and the variety introduced in more modern times (subsp. *tarentina*). The genetic and morphological differences between these two varieties confirm the taxonomic distinctness of the subsp. *baetica*. With very few individuals known, this Moorish myrtle is on the verge of extinction. The genetic identification offers the opportunity to restore a key element of this 14th-century garden and enhance the authenticity of a World Heritage site.

**Keywords:** Alhambra, Microsatellite, *Mirtus communis*, Subspecies, Taxon.

## INTRODUCTION

The Alhambra, one of the largest medieval complexes surviving in Europe, originally had a fortress, several palaces and an aristocratic quarter, surrounded by orchards and gardens. Today these gardens remain among the most extensive in Spain. Built from the 13<sup>th</sup> to the 15<sup>th</sup> centuries, the citadel served as the residence of the Nasrid Sultans, the royal family of the last Moorish territory in Europe. After the conquest of Granada by the Catholic Monarchs at the end of the 15<sup>th</sup> century, the Alhambra survived as the only Islamic palace complex that has been almost entirely conserved.

In 1943, the gardens of the Alhambra and the surrounding area of Generalife were designated as Historical Gardens, and in 1984 UNESCO declared them a World Heritage site. In fact, these gardens may be among the oldest in Europe and, since medieval times, the myrtle has been considered their emblematic plant (Casares-Porcel *et al.*, 2012). This is the identifying plant in the Courtyard of the Myrtles, where myrtle hedges flank the marble reflecting pond of the second palace of the Alhambra.

Myrtle (*Myrtus communis* L.), namesake of the family Myrtaceae, is an evergreen aromatic shrub both ornamental and wild (McVaugh, 1968) found throughout the Mediterranean Basin as well as in Asia. Well known to ancient cultures, it was

<sup>1</sup> Department of Genetics, Faculty of Sciences, University of Granada, Spain.

<sup>2</sup> Department of Botany, Faculty of Pharmacy, University of Granada, Spain.

<sup>3</sup> Botanical Garden of the University of Granada, Spain.

\* Corresponding author; e-mail: carmelo@ugr.es



mentioned in the Old Testament regarding the Feast of the Tabernacle (Moldenke and Moldenke, 1986), and ever since the ancient Greeks and Romans it has been associated with deities and their rituals. Over time, its use spread from ceremony to gardening and pharmacology, leading to the domestication of several varieties. The Roman naturalist Pliny the Elder (Pliny, 1960) first differentiated between the wild and cultivated varieties (Figure 1), describing for gardens a large-leaf form (*hexasticham*) and a small-leaf form (*tarentinam*).

Andalusian agricultural treatises from the 11<sup>th</sup> century (Ibn Bassal, 1995) to the 14<sup>th</sup> century (Ibn Luyun, 1988) discuss the myrtle as a widely cultivated plant. In the early 12<sup>th</sup> century, Abu I-Jayr (2004) in the Andalusian botanical codex, known as *Umdat al-Tabib*, described a myrtle variety in the Moorish kingdom of Granada matching Pliny's description of *hexasticham*.

In 1564, Flemish naturalist Carolus Clusius, the foremost botanist of his century,

concluded on a scientific journey through Spain and Portugal that the Alhambra myrtles differed from the varieties then known in the rest of Europe. He proposed the name *Myrtus baetica* (Moorish myrtle), as they were called in Granada, stating “*I have never seen this kind of myrtle in any place except in a monastery in Seville and in the splendid Moorish gardens of Granada, next to pools and lakes where all hedges are always made from this type of myrtle*” (Clusius, 1576). Direct testimony of the existence of the Moorish myrtle are the paintings of gardens in the 14<sup>th</sup>-century Hall of the Kings of the Alhambra, where this variety can be clearly identified, providing the oldest and clearest evidence of this variety in Al-Andalus (Figure 1).

This myrtle was widely used in the Alhambra until at least the 17<sup>th</sup> century (Mariutti, 1934) when it began to be gradually replaced by *Myrtus communis* subsp. *tarentina* for aesthetic and practical reasons (finer foliage, more compact growth), and the Moorish myrtle was



**Figure 1.** (A) Wild type *Myrtus communis*; (B) Cultivated variety *Myrtus communis* subsp. *tarentina*; (C) Cultivated variety *Myrtus communis* subsp. *baetica* (described by Pliny as *hexasticham* and named Moorish myrtle by Clusius); (D) *Myrtus communis* subsp. *Baetica*; (E) Detail of painting of Hall of the Kings of the Alhambra, where the morphological characteristic of Moorish myrtle can be clearly identified.

forgotten in the palace gardens.

In fact, today, the myrtle used for gardening in the Alhambra belongs to the subspecies *tarentina*. However, six unusual old myrtle shrubs were recently discovered in these gardens (Casares-Porcel *et al.*, 2012). These plants are unmistakable for their robustness (tree-like habit) and leaf (crowded large leaves, frequently arranged in trimerous whorls) (Casares-Porcel *et al.*, 2012), and they match one of the types morphologically described by Pliny (*hexasticham*) and by Clusius (*baetica*), differing markedly from the myrtle used today (*tarentina*).

This circumstantial evidence, which appears to identify the original myrtles of the Alhambra, requires molecular analyses for their authentication. Different genetic markers (AFLPs– Melito *et al.*, 2014; ISSRs– Melito *et al.*, 2013; RAPDs– Messaoud *et al.*, 2007) have been used to explore the genetic diversity of myrtle, and were successfully used to differentiate between cultivars and wild populations. In this study, we investigate the genetic variability of Moorish myrtles in relation to specimens from different locations and to other varieties in order to test for genetic differentiation. In other locations outside Alhambra, but in relation to Moorish culture, such as Toledo and the Alpujarras (central and southern Spain, respectively) and Fes (northern Morocco), we have found several specimens with similar morphological characteristics to those unusual specimens found in the Alhambra. The presence of different varieties in different regions and time periods poses the possibility of genetic divergence between specimens and the existence of variants typically associated with specific areas and cultures.

## MATERIALS AND METHODS

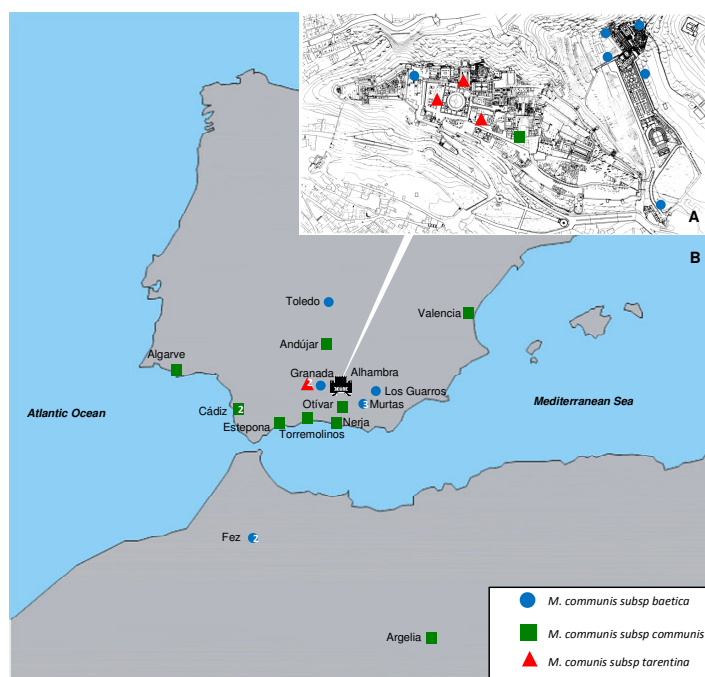
We analysed the six specimens having the Moorish morphology from the Alhambra, and eight from outside of Alhambra: One from

Toledo (Spain), four from Alpujarras (Granada province), one from the Botanical Garden of Granada (Spain), and two from Fes (Morocco). Also, we studied two other myrtle varieties: the wild form *Myrtus communis* subsp. *communis*: two plants from Alhambra and 10 from the Mediterranean area; and the cultivated form *Myrtus communis* subsp. *tarentina*: three plants from Alhambra and two from gardens of the city of Granada. Also, as reference, we included one cultivated plant of Jewish myrtle (Hadassah), and we used *Psidium guajava* (guava) as an out group species belonging to the family Myrtaceae (Table 1, Figure 2).

Genomic DNA isolation was carried out from leaves and using the Invisorb® Spin Plant Mini Kit (STRATEC Molecular GmbH, Berlin, Germany). The quality of genomic DNA was measured by Infinite® 200 PRO NanoQuant (Tecan, Switzerland) and confirmed by electrophoresis in a 1% agarose gel. Variability analyses were carried out using microsatellite markers described in Albadalejo *et al.*, 2010. PCR was performed in 20 µl of reaction containing 20 ng of genomic DNA, 1X of specific buffer (Bioline), 3.2 pmol of specific primers, 1 U of polymerase (MyTaq™ DNA Polymerase, Bioline) and ddH<sub>2</sub>O up to final volume and under the conditions described in Albadalejo *et al.* (2010) with some modifications. Polymorphism at each locus was screened using an ABI 3100 Avant sequencer (Applied Biosystems) and the alleles were designated according to the PCR product size, which was determined using Gene Scan™ 500 LIZ Size Standard (Applied Biosystems) as a reference marker for GeneMapper software (Applied Biosystems). For each marker, the variability values were determined using the program CERVUS 3.0 (Kalinowski *et al.*, 2006) and GENEPOP 4.2 (<http://genepop.curtin.edu.au/>) (Raymond and Rousset, 1995). Genetic distances (Ds, Nei) were calculated by Populations 1.2.31 software (<http://bioinformatics.org/~tryphon/population/>) (Langella, 1999) and those results were used to build a distance tree using the program MEGA 6 (Tamura *et al.*, 2013).

**Table 1.** Locations of the specimens studied.

Variety	Code	Location	Region	Country
<b>COMMUNIS</b>				
	OTIVAR-38C	Verde River, Otívar	Granada	SPAIN
	MALGA-39C	Torremolinos	Málaga	SPAIN
	ESTPO-40C	Mata Verdes, Estepona	Málaga	SPAIN
	NERJA-21C	Chillar River, Nerja	Málaga	SPAIN
	CADIZ-30C	Canuto del Risco Blanco, Los Barrios	Cádiz	SPAIN
	CADIZ-31C	Road Los Barrios to Facinas	Cádiz	SPAIN
	ANDUJAR-47C	Andújar	Jaen	SPAIN
	VALENC-46C	Albufera	Valencia	SPAIN
	ARGELIA-52C	Setif	Setif	ALGERIA
	ALGVE-35C	Valley do Lobo	Algarve	PORTUGAL
	ALHAMB-02C	Next to aqueduct (Alhambra)	Granada	SPAIN
	ALHAMB-05C	Court of the New Museums (Alhambra)	Granada	SPAIN
<b>TARENTINA</b>				
	GRANA-09T	Campus University of Granada gardens	Granada	SPAIN
	GRANA-20T	Granada town gardens	Granada	SPAIN
	ALHAMB-08T	Court of the Myrtles (Alhambra)	Granada	SPAIN
	ALHAMB-34T	Palace of Charles V (Alhambra)	Granada	SPAIN
	ALHAMB-01T	Next to Hall of the Abencerrajes (Alhambra)	Granada	SPAIN
<b>BAETICA</b>				
	BOTANIC-44B	Old Botanic Garden of the University of Granada	Granada	SPAIN
	MURTAS-15B	Farmhouse El Minchal, Murtas (Alpujarras)	Granada	SPAIN
	MURTAS-16B	Farmhouse Balauta, Murtas (Alpujarras)	Granada	SPAIN
	MURTAS-17B	Farmhouse Dietar, Murtas (Alpujarras)	Granada	SPAIN
	GUARRO-14B	Los Guarros (Alpujarras)	Almeria	SPAIN
	TOLEDO-51B	Real de S. Vicente	Toledo	SPAIN
	FEZ-22B	Dar Bhata	Fez	MOROCCO
	FEZ-50B	Borj Sud	Fez	MOROCCO
	ALHAMB-13B	Water ladder of the Generalife (Alhambra)	Granada	SPAIN
	ALHAMB-03B	New entrance to the Generalife (Alhambra)	Granada	SPAIN
	ALHAMB-06B	Adelfas way of the Generalife (Alhambra)	Granada	SPAIN
	ALHAMB-07B	Terrace of the Acequia of the Generalife (Alhambra)	Granada	SPAIN
	ALHAMB-11B	Square of Algibe (Alhambra)	Granada	SPAIN
	ALHAMB-33B	Medieval entrance to the Generalife (Alhambra)	Granada	SPAIN
<b>HADDAS</b>				
	ISRAEL-53	Be'er Ora	Arava	ISRAEL

**Figure 2.** Geographical distribution of genetically analysed specimens, in the Alhambra (A) and in other Mediterranean Regions (B). The number of samples studied at each location is indicated inside the symbol when there was more than one.

## RESULTS AND DISCUSSION

Nine of eleven polymorphic microsatellite loci described for myrtle in Albadalejo *et al.* (2010) were used (two loci were discarded for unsuccessful amplification reactions) in this study. Table 2 shows genotypes data (allele and frequency of each variety) for these nine microsatellite loci.

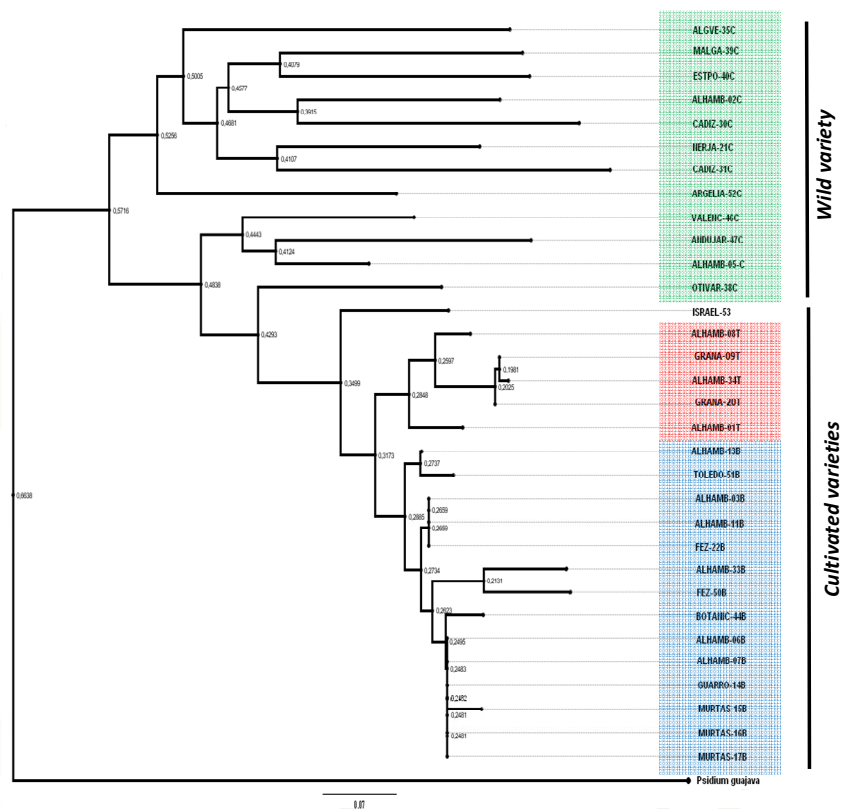
Their allelic combinations enabled us to calculate Nei's  $D_s$  distance (Nei, 1987) and to draw a neighbour-joining dendrogram

representing their genetic relationships (Figure 3).

The topology of the tree clearly distinguishes the wild and cultivated (Moorish, *tarentina*, and Hadassah) types, grouping the samples into the different myrtle varieties, regardless of their origin. Melito *et al.* (2013) reached the same result using ISSR profiling of Sardinian cultivars and wild populations of myrtle. In our study, all Moorish myrtle samples clustered into a single clade, close to the subsp. *tarentina*

**Table 2.** Allele and frequency of each variety for the nine microsatellite loci.

Allele				Allele			
Myrcom2		Frecuency		Myrcom7		Baetica	
Communis	Tarentina	Baetica	Myrcom7	Communis	Tarentina	Baetica	
171	0.0833	0	0	151	0.3333	0	0
175	0.0833	0	0	153	0.2500	0.5000	0.0357
180	0.0833	0	0	161	0.0417	0	0
187	0.0833	0	0	163	0.0417	0	0
191	0.5000	1.000	1.000	165	0.1667	0.5000	0.9286
201	0.1667	0	0	169	0.0833	0	0
Myrcom3				173	0.0833	0	0
151	0.3636	0.7000	1.000	177	0	0	0.0357
158	0.0455	0	0	Myrcom8			
160	0.0909	0	0	222	0.2727	0	0
162	0.3182	0.3000	0	224	0.0455	0	0
164	0.1818	0	0	226	0.2727	0	0
Myrcom4				228	0.0455	0	0
153	0.0833	0	0.0357	236	0.0455	0	0
156	0.0417	0	0.0357	252	0	1.000	0.6429
163	0.4583	0.6000	0.4286	257	0.0909	0	0
177	0	0	0.0714	260	0	0	0.2857
183	0.0417	0	0	266	0.0455	0	0.0714
185	0.1667	0	0	270	0.1364	0	0
187	0.1250	0.4000	0.4286	282	0.0455	0	0
193	0.0833	0	0	Myrcom9			
Myrcom5				166	0.9545	0.5000	0.5000
259	0.3750	0	0	170	0.0455	0.5000	0.5000
261	0.2083	0	0	Myrcom11			
265	0.0417	0	0	212	0.4091	0.5000	0.5000
267	0.1667	1.000	0.5000	214	0.0455	0	0
271	0.1667	0	0.5000	216	0.1364	0	0
273	0.0417	0	0	220	0.1364	0.4000	0
Myrcom6				222	0.0909	0	0
153	0.0417	0	0	224	0.0455	0.1000	0.5000
155	0.0417	0	0	234	0.0909	0	0
157	0.2083	1.000	1.000	244	0.0455	0	0
161	0.0417	0	0				
167	0.6667	0	0				



**Figure 3.** Neighbour-joining dendrogram by Nei's  $D_s$  distances (indicated in the nodes) calculated based in microsatellite genotypes. The topology distinguishes the wild (green block) and cultivated varieties, *tarentina* (red block), Moorish (blue block), and Hadassah (white) types. *Psidium guajava* was used as out group species

group and separate from the wild form, *M. communis* subsp. *communis*. The clear separation into these three clusters confirms subsp. *baetica* as a distinct taxon from the other varieties, in agreement with the classical descriptions.

This conclusion is also supported by the variability analysis. As expected, the diversity indexes (e.g. allele number, allelic richness, PIC, and genetic diversity) proved highest in the wild form (Table 3). As opposed to this fact, the analysis of Sardinian populations of myrtle suggested that diversity is higher in cultivars than in wild populations (Melito *et al.*, 2014). However, it might be the reflection of the fact that the authors deliberately chose for their analyses individuals highly variable at phenotypic level. In fact, in the same study, the smaller genetic diversity was found in a

population from a small island cut off from introgression (Melito *et al.*, 2013).

Together with the reduction of alleles number, the subspecies *baetica* and *tarentina* displayed a fixation of one allele in several markers, 3 loci in the first (Myrcom 2, 3, and 6) and 4 loci in the second (Myrcom 2, 5, 6, and 8), in contrast to the wild form, which had no fixed alleles for any loci (Table 1). Population genetic structure analyses indicate that myrtle populations exhibit high levels of genotypic diversity, and that gene flow between neighbouring populations is frequent (Melito *et al.*, 2013, 2014). The variability observed in the wild myrtle analysed in this study appears to be representative of the natural population given that the allele number found is similar to that of two populations

**Table 3.** Diversity statistic value within three varieties in *Myrtus*: C (*Communis*); T (*Tarentina*); B (*Baetica*). N (Number of samples); A (Allele number); AR (Allelic richness); PIC (Polymorphic information content); H (Genetic diversity).

Locus	N			A			AR			PIC			H																							
	C	T	B	C	T	B	C	T	B	C	T	B	C	T	B																					
Myrcom2	6	4	11	6	1	1	5	1	1	0.665	0	0	0.750	0	0																					
Myrcom3	11	5	14	5	2	1	4.321	2	1	0.675	0.332	0	0.764	0.450	0																					
Myrcom4	12	5	14	7	2	5	4.997	2	4.532	0.701	0.365	0.552	0.769	0.500	0.637																					
Myrcom5	12	5	14	6	1	2	4.647	1	2	0.721	0	0.375	0.788	0	0.500																					
Myrcom6	12	5	14	5	1	1	2.993	1	1	0.463	0	0	0.508	0	0																					
Myrcom7	12	5	14	7	2	3	4.746	2	2.571	0.751	0.375	0.131	0.799	0.500	0.140																					
Myrcom8	11	5	14	9	1	3	6.449	1	2.960	0.791	0	0.427	0.877	0	0.510																					
Myrcom9	11	5	14	2	2	2	1.545	2	2	0.083	0.375	0.375	0.091	0.500	0.500																					
Myrcom11	11	5	14	8	3	2	6.091	2.8	2	0.750	0.492	0.375	0.832	0.625	0.500																					
Mean	6.111±2.028			1.667±0.707			2.222±2.250			4.532±1.497			1.644±0.662			2.118±2.133			0.622±0.223			0.215±0.209			0.248±0.233			0.686±0.246			0.286±0.275			0.310±0.286		

genotyped in Albadalejo *et al.* (2010) for the same loci.

Additionally, despite the lower allele number found in the *baetica* and *tarentina*, the genetic analyses detected variety-specific alleles. We identified an allele that is exclusive (allele 252 of Myrcom 8) to both varieties and that has a high frequency (0.642 for *baetica* and 1 for *tarentina*), and three exclusive alleles for the subsp. *baetica* (177 for Myrcom 4, 177 for Myrcom 7 and 260 for Myrcom 8), though at a low frequency (0.071, 0.035 and 0.286, respectively) (Table 2).

The lower variability and the presence of fixed or quasi-fixed alleles in these two latter varieties are consistent with the fact that cultivars are generally less variable than wild forms. These differences in allelic composition for several loci and smaller genetic distances between the cultivated and wild form are presumably resulted from artificial selection to develop and maintain these two cultivars. Also, the presence of exclusive alleles in several loci and the different frequencies found for the remaining loci support the evidence of the genetic differentiation between the two cultivars. The genetic differences (in this study) and morphological ones (Casares-Porcel *et al.*, 2012) confirm the taxonomic distinctness of the subsp. *baetica*. The close genetic relationship that *baetica* specimens from the Alhambra share with those of the same morphology from Toledo, Alpujarras, and Fes implies a common origin a cultivar used in the Nasrid period, and thus the original ancient myrtle variety of the Alhambra, which today remains forgotten in a few remnant specimens in the palace gardens.

It bears emphasizing that the specimens of Moorish myrtle analysed in this work are among the few that have been identified. This reflects the urgency of preserving this disappearing plant, but also underscores the value of preserving and restoring the botanical world heritage. The Alhambra, aside from being one of the world's most visited monuments, also represents the historical crossroads between different continents and cultures (Europe, Asia, Africa; Judaism, Christianity, Islam), and thus its authenticity,



both architectural as well as horticultural requires the most rigorous attention. This study may serve as a model for preserving botanical world heritage and guaranteeing its authenticity.

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گیاه مورد فراموش شده باغ های الحمیرای گرانادا: بازسازی و اعتبار میراث جهانی

ر. دلاهران، م. کاسارس، ف. روبلس، ج. تیتو، ر. ناواجاس-پرز، م. ج. مولینا-لوزون، م. دلوس ریس گونزالس-تجرو، پ. ج. سولا-کمپوی، ا. گوتیرز-گوررو، و ج. س. رویز-رجون

### چکیده

در الحمرا (گرانادا، اسپانیا)، و در دیگر مکان های شمالی آفریقا، چندین رقم اصلی گیاه مورد (myrtle)، گیاهی سمبلیک در باغ هایشان، شناسایی شد و از لحاظ ژنتیکی مورد تایید قرار گرفت. بعد بررسی ریز ماهواره، بین فرم وحشی (*Myrtus communis* L.) و دو رقم کشت شده: یکی از الحمیرا (Alhambra)، مورد شمال آفریقا (Moorish) (*subsp. baetica*)، و رقم معرفی شده در دوران مدرن تر (*subsp. tarentina*) تمایز قائل شدیم. تفاوت های ژنتیکی و مورفولوژیکی بین این دو رقم، تمایز طبقه بندی *subsp. baetica*، را تایید می کند. مورد Moorish با تعداد افراد شناخته شده کم، در معرض خطر انقراض می باشد. شناسایی ژنتیکی این فرصت را برای بازگرداندن عنصر اصلی و کلیدی این باغ با عمر ۱۴ قرن فراهم میکند و اعتبار یک میراث جهانی را افزایش می دهد.