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Genetic diversity in *Mentha cervina* based on morphological traits, essential oils profile and ISSRs markers

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ABSTRACT

Morphological, phytochemical and genetic differences were studied to evaluate the level and distribution of diversity in twelve populations of the Portuguese endangered medicinal plant *Mentha cervina* L. Morphological variation was correlated with ecological conditions at the site of origin. Pulegone was the major essential oils compound in all of the populations collected at full flowering (68–83%), in different growing conditions (51–82%), and for all the developmental stages studied (47–82%). Although clusters were defined, the analysis revealed a high chemical correlation among all populations ($S_{corr} \geq 0.95$%). Inter-simple sequence repeats markers were used to assess the population structure and genetic variation. Populations exhibited a relatively low genetic diversity (PPB = 14.3–64.6%, H_e = 0.051–0.222, I = 0.076–0.332), with high structuring between them (G_{ST} = 0.51). However, the genetic diversity at species level was relatively high (PPB = 97.7%; H_e = 0.320). The levels and patterns of genetic diversity were assumed to result largely from a combination of evolutionary history and its unique biological traits, such as breeding system, clonal growth, low capacity of dispersion and habitat fragmentation. The relatively low genetic diversity in the populations analyzed indicates that the maintenance of their evolutionary potential is at risk if population sizes are maintained and if there is no protection of the habitats.

1. Introduction

Genetic variation is fundamentally involved in the ability of a species to adapt to biotic and abiotic changes and in its evolution. Recognition of the levels and distribution of genetic variation within and among populations of a species is the base for development and selection of plant genotypes in breeding programs and increases the understanding of the historical

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processes underlying the genetic diversity providing information for the management and preservation of endangered and geographically restricted species (Escudero et al., 2003; Shah et al., 2008).

Plants belonging to the genus *Mentha* L. (Lamiaceae) have evolved in nature through natural hybridization and selection, showing substantial variation in terms of their natural habitats, growth characteristics, and aromas (Franco, 1984; Tutin et al., 1972). They have a substantial importance in the botanical economy and to the pharmaceutical industry, mainly because of the essential oils produced and their antimicrobial properties, used since ancient times for the treatment of many digestive tract diseases and in culinary (İşcan et al., 2002).

Mentha cervina L. (commonly known as hart's pennyroyal) is an aromatic plant that is traditionally used in Portugal to flavour food dishes and for its medicinal properties, preventing different gastric disorders and inflammations of the respiratory tract (Monteiro et al., 2007; Póvoa et al., 2006; Rodrigues et al., 2008). It has a western steno-Mediterranean distribution, found in France, Portugal, Spain, Morocco, Algeria and it is presumed extinct in Italy (Rhazi and Grillas, 2010). In Portugal it occurs, mainly in river banks and other damp and wet places, which require a longer flooded period that is characteristic of the priority habitat Mediterranean temporary ponds (3170) (Silva et al., 2009). The growth of commercial demands in recent years, the excessive harvesting from the wild, overgrazing and the unfavourable conservation status of their habitats, has shrunk the natural resource of *M. cervina* to a narrow distribution (Póvoa et al., 2006). Nowadays, it is considered to be decreasing in number and classified as Near Threatened in the IUCN Red List of Threatened Species (Rhazi and Grillas, 2010).

Morphological, molecular and biochemical markers are complementary in determining the genetic similarity of inter- and intra-species and the relationship between the populations (Chahal and Gosal, 2002; Kohler and Friedt, 1999). Given the well-known genus chemical variability, the essential oil composition from cultivated *M. cervina* populations cultivated (Alentejo Region, Portugal) was recently examined (Rodrigues et al., 2008). This study showed no essential oil chemical polymorphism despite the cultivated population's different provenance. A low genetic diversity associated with high differentiation among populations was also observed when *M. cervina* genetic diversity was assessed by inter-simple sequence repeats (ISSRs) (Rodrigues et al., 2013c).

Given the medicinal and aromatic potential of this species and its current threatened situation, the present study aims at assessing *M. cervina* genetic diversity level in Portugal based on the combination of molecular, phytochemical and morphological traits and also to provide guidelines for the conservation and sustainable use of this medicinal species.

2. Materials and methods

2.1. Plant material

A total of 12 populations of *M. cervina* with different geographic origins were included in the analysis. Geographic distances between populations vary from 9 km (between Mc32 and Mc33) to 450 km (between Mc33 and Mc43). Vouchers for each population were deposited in the LISI Herbarium (Table 1).

2.2. Morphological study

In this study, the 12 populations of *M. cervina* were kept in the same culture conditions, in the essay field at the Elvas Agrarian School (Alentejo), Portugal (Table 1). For each population, 24 plants were employed, in three lanes 1 m apart. The soil was soft and well drained. Dripping wings for irrigation and fertilization were placed among the lanes throughout their length. The cultural operations, until harvesting, consisted of manual elimination of weeds. For each population, 15 plants

Table 1
Data on collection site and sample type of *Mentha cervina* populations studied.

Populations	Sample type	Specific sample collection sites					Voucher
		Location	Altitude (m)	Latitude	Longitude	Hydrographic basin	
Mc10	M/G/W/C/DS	Ouguela, Campo Maior	207	39°4′54.96″N	7°0′4.33″W	Guadiana	532/2005
Mc32	M/G/W	Vilar Seco, Miranda do Douro	725	41°31′25.48″N	6°24′5.56″W	Douro	759/2008
Mc33	M/G/W	Póvoa, Miranda do Douro	750	41°34′22.71″N	6°19′17.53″W	Douro	760/2008
Mc34	M/G/W	Bagaúste, Peso da Régua	50	41°9′0.41″N	7°45′2.24″W	Douro	761/2008
Mc35	M/G/W	Escarigo, Figueira de Castelo Rodrigo	560	40°50′34.73″N	6°49′33.62″W	Douro	762/2008
Mc36	M/G/W/C/DS	Segura, Idanha-a-Nova	235	39°49′11.06″N	6°58′52.99″W	Tejo	763/2008
Mc39	M/G/W/C/DS	Oledo, Idanha-a-Nova	335	39°58′10.64″N	7°18′27.85″W	–	764/2008
Mc41	M/G/W/C/DS	Valência de Alcântara	313	39°28′1.17″N	7°12′24.16″W	Tejo	766/2008
Mc42	M/G/W	Torrão, Alcácer do Sal	50	38°17′0.32″N	8°13′57.81″W	Sado	767/2008
Mc43	M/G/W	Entradas, Castro Verde	154	37°44′36.51″N	7°58′44.60″W	Guadiana	768/2008
Mc44	M/G/W/C/DS	La Codosera	298	39°16′48.08″N	6°52′20.89″W	Guadiana	769/2008
Mc45	M/G/W/C/DS	Alburquerque	234	39°11′0.69″N	7°1′59.03″W	Guadiana	770/2008

Morphological study (M); genetic study (G); and phytochemical study from wild grown plants (W), from cultivated *vs* wild growing conditions (C), and at developmental stages (DS).