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Analysis of genetic diversity and population structure of the endangered *Origanum compactum* from Morocco, using SSR markers: Implication for conservation

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ABSTRACT

Origanum compactum L. (*Lamiaceae*) is one of the most popular medicinal and culinary herbs with a long history of traditional use. This species, endemic to Morocco and Southern Spain, is considered as threatened, due mainly to over-harvesting and habitat destruction. Assessment of genetic polymorphism of threatened species constitutes a prerequisite for germplasm conservation. To support and monitor future conservation and management measures, we used 15 microsatellite loci to genotype 670 individuals of *O. compactum* distributed over 59 localities covering the entire geographic distribution of the species in Morocco. In contrast to what was argued in most previous studies, the genetic diversity of the Moroccan oregano was greater mostly on isolated and declined populations compared to less fragmented accessions. Accordingly, our study identifies the most relevant management units for conservation purposes on the basis of their genetic diversity level. Factors such as the perennity of the species, somatic mutations, or multiple founder events could explain this unexpected level of genetic diversity at the species level in this endangered plant. The population genetic results reflect a strong genetic structure and identify three major clusters among *O. compactum* populations: a Northern cluster composed of populations near the strait of Gibraltar (Tangier-Tetouan and Chefchaouen regions as well as some adjacent populations from Taounate), and two other clusters with significant mixed lineage. This result supports the hypothesis that the strait of Gibraltar could be a migration route for plants between Southern Spain and Northern Morocco. The high differentiation of populations (Fst = 0.22) and the low rate of gene flow between them (Nm = 0.88) might been caused by recent isolation of the populations through habitat disturbances. Historical demographic patterns inferred with a generalized stepwise mutation model (SSM) and two-phase model (TPM) using maximum likelihood showed significant past demographic changes in 39 of the 59 sampled populations. Implications of these results to develop appropriate conservation strategies of this endangered species are proposed.

1. Introduction

The Mediterranean region is considered one of the World's biodiversity hotspots (Myers et al., 2000). Actually it consists of two separate areas of very high diversity: the Eastern Mediterranean (mainly Turkey) and the Western Mediterranean (NW Africa and Iberian Peninsula) (Medail and Quezel, 1999; Medail and Diadema, 2009; Schmitt, 2007). Morocco is the second most biologically diverse country in the Mediterranean basin after Turkey, with about 3913 vascular plants, including 1298 subspecies in 981 genera and 155 families (Fennane and

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Ibn Tattou, 2012). The Moroccan flora is characterized by a high percentage of endemic species (22%), comprising 878 endemic taxa (Fennane and Ibn Tattou, 2012; Rankou et al., 2013). However, the endemic Moroccan flora is declining to critically low levels, with about 2193 endangered species, 1288 (53%) being highly vulnerable (Fennane and Ibn Tattou, 1999), because of many threats, mainly anthropogenic activities, habitat disturbance, and global climatic change (Slapcinsky et al., 2010).

In the last decades, the conservation of endemic and threatened species has received more attention throughout the world. Studies on the genetic diversity of endangered species using molecular markers have increased in recent years due to their central importance in planning both in-situ and ex-situ conservation efforts (De Joode and Wendel, 1992; Francisco-Ortega et al., 2000; Batista et al., 2001; Bouza et al., 2002). Fragmented populations may be subjected to extinction mainly due to the increased probability of the phenomena of genetic drift as a result of inbreeding and restricted gene flow (Murawski et al., 1994). The loss of genetic diversity within populations decreases their response to selection and stochasticity and increases extinction risk (Lande, 1988). Sufficient genetic variability is essential to ensure the persistence of species and increase the adaptative potential to face environmental changes and avoid inbreeding depression (Barrett and Kohn, 1991; Kim et al., 2005; Booy et al., 2000; Jones, 2003). Consequently, determining the genetic diversity of isolated and threatened species is of great importance when planning a suitable conservation strategy because it can help in the selection of appropriate populations and management strategies that will maintain that variation. For example, highly diverse populations could be targeted for protection while small and depauperate populations might be directed for management actions to restore diversity (Godt et al., 1996; Petit et al., 1998), such as for example, enhancement or reinforcement plans that implies the addition of individuals into existing populations or reintroduction that refers to the re-establishment of a species within its natural habitat, where it had become extinct (IUCN, 1998).

The spatial distribution of genetic diversity in plants is primarily determined by their population history and reproductive traits (Schaal et al., 1998). Plant reproductive traits affect the population genetic structure via the influence of the species mating system (Hamrick and Godt, 1996; Schoen et al., 1996). Compared with autogamous species, outcrossing species are generally expected to have higher genetic diversity and heterozygosity within populations and lower genetic differentiation among populations (Hamrick and Godt, 1996). Therefore, genetic diversity within and among populations can reflect, to a certain extent, the relative rates of inbreeding and outcrossing in a given species. Population history, including the evolutionary and biogeographic history of species as well as fluctuations in the population's size, may also play a crucial role in the constitution of its current genetic composition (Schaal et al., 1998). Thus, the genetic structure of a species can elucidate its evolutionary history (Bauert et al., 1998).

Oregano is one of the high-valued culinary and medicinal herbs that have traditionally been of human use for centuries. The genus *Origanum* L. (*Lamiaceae*) comprises 43 species and 18 hybrids (Carlström, 1984; Danin, 1990; Danin and Künne, 1996; Duman et al., 1995; Duman et al., 1998; Ietswaart, 1980), most of which are locally distributed around the Mediterranean region. The taxonomy of the genus is complex and complicated by the noticeable extent of the morphological variability between and within species. The occurrence of a great number of natural hybrids even between distantly related species further complicates the classification which is still under investigation (Azizi et al., 2009; Bariotakis et al., 2016; Helsen et al., 2013; Lukas et al., 2013).

O. compactum is a perennial plant species endemic to Morocco and Southern Spain (Ietswaart, 1980). Among *Origanum* species, it is the most widely distributed species throughout Morocco. It occurs mainly in the Occidental and Central Rif as well as the Middle Atlas regions, under diverse ecological and environmental conditions (Benabid, 2000). Considering the high economic interest and wide ranging culinary as well as therapeutic properties of this species, natural populations are extensively exploited from the wild. The severe anthropogenic pressures (over-harvesting through up-rooting, habitat alteration, etc.), combined with other environmental stresses (drought, climate change, irregular precipitations etc.) have caused a significant decline of natural populations. Our recent field investigation indicated that, due to the extreme pressure of human disturbance, a great number of populations have been destroyed and thus the species is at a highly endangered status (Aboukhalid et al., 2016). Currently, the species occurs mainly in fragmented and scattered populations with disjunct distribution, decreasing progressively in size. Thus, measuring levels of population's genetic diversity among *O. compactum* populations is a critical step for implementing appropriate conservation strategies.

The present study aimed to evaluate the intra- and inter-population genetic diversity and spatial structural patterns of *O. compactum* populations sampled across the remaining natural habitat of the species in Morocco using SSR markers. Specifically, the following questions were addressed: (i) What is the level of genetic diversity and spatial genetic structure within and among *O. compactum* populations? (ii) What is the level of inbreeding and the degree of population differentiation in this species? (iii) What are the evolutionary factors that may affect the observed population structure? (iiii) What is the implication of the detected genetic variation for the conservation of genetic resources of *O. compactum*?. Providing the first extensive genetic data of this endangered species, we attempt to assess the implications of our findings to implement an efficient conservation and management strategies.

2. Materials and methods

2.1. Study species

O. compactum (*Lamiaceae*) is a perennial plant species that grows spontaneously mainly in Central and North-Eastern Morocco on dry hills and low mountains, sometimes between trees and shrubs, in mesophytic forests. The species grows in rocky or calcareous soils under humid, sub-humid and semi-arid bioclimates, at altitudes usually below 700 m (Benabid, 2000). Stems are hirsute (hairs 2 mm long), erect or ascending, up to 75 cm long. The flowering period extends from June to August. The foliage is dotted with small glands containing the essential oils that gives to the plant its aroma and flavour (Simon et al., 1984). The individual flowers have two lips, the upper ones two-lobed and the lower three-lobed. Although there are no reported studies on its pollination biology, or on the rate of cross-pollination, the flower structure of *O. compactum* (i.e., white flowers with a strong scent and longer stamens than petals, filaments up to 4.5–6 mm, styles up to 16 mm long) (Ietswaart, 1980), and frequent visits by pollinator insects are good indicators of a high rate of cross-pollination. In general, outcrossing mating system and insect pollination are common in *Lamiaceae* (Judd et al., 1999). The seeds are ellipsoidal, approximately 0.4 mm in length, and shows no morphological adaptation to long distance dispersal.

2.2. Sampling and DNA extraction

To analyze the genetic diversity and population structure of *O. compactum* plants, 59 populations consisting of 670 individuals were sampled across the whole distribution area of the species in Morocco. The 59 *O. compactum* populations were sampled over 2013/2014 (between April and June). The distance between sampled individuals within a population exceeded 20 m, to avoid the collection of clonal material. The several populations confront some barriers to gene flow, such as valleys, the Rif and Middle Atlas mountains, etc. Each location was recorded using a Global Positioning System (GPS) receiver and spatial distribution of studied populations was depicted on a GIS (Geographic Information System) map using ArcGis 10.1 software (Fig. 1). Leaves were collected from each individual and immediately