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Hybridization in *Capparis spinosa* L.: Molecular and morphological evidence from a Mediterranean island complex

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ABSTRACT

Inter-Simple Sequence Repeat (ISSR) molecular markers and morphological analysis were used in order to characterize wild populations and cultivated forms of orphan crop species *Capparis spinosa* L. in a Mediterranean island complex. Nineteen wild populations belonging to two different subspecies, *C. spinosa* subsp. *spinosa* and subsp. *rupestris*, were sampled in different environments in Sicily and the surrounding islets Lampedusa, Pantelleria and Salina. Different biotypes cultivated in Pantelleria and Salina were analysed. Six ISSR primers were selected for genetic characterization, and all clear and reproducible bands were scored and analysed. Among the 47 ISSR bands obtained, 97.5% were polymorphic. Results of AMOVA and STRUCTURE analysis suggested a clear genetic distinctness between subspecies at the regional level and suggested the existence of two taxonomic groups among wild populations, with different ecological preferences and distinctive morphological characters. Cultivated forms showed genetic affinity to subsp. *rupestris*. ISSR analysis not only provided specific molecular markers to discriminate the taxa, but also proved useful in supporting the hypothesis of a hybrid origin of the intermediate phenotypes found in overlapping distribution areas. The identified molecular markers provided a basic tool for the DNA fingerprinting of wild and commercial capers in the Mediterranean region and nearby territory.

Introduction

The genus *Capparis* L. includes about 250 species and subspecies (Fici, 2001), many of which showing medicinal and alimentary interest (Akgül and Özcan, 1999; Bhoyar et al., 2012; Bobrov, 1970; Rivera et al., 2003; Saadaoui et al., 2011; Sozzi, 2001; Tlili et al., 2011). The most important products are immature flower buds—the commercial capers—from different taxa, that are brined or pickled and used as a condiment in the Mediterranean and Near East traditional cuisine (Lentini and Venza, 2007; Pasta et al., 2011; Sozzi and Vicente, 2006). Capers and caperberries, whose principal producers are Spain, Italy, Morocco, Turkey and Greece, are collected from wild and cultivated plants mainly referred to *Capparis spinosa* L. s. str. (Inocencio et al., 2005). This species is widely distributed in the Mediterranean and Near East Regions and is considered a polymorphic group (species complex) (Bobrov, 1970; Fici, 2001; Higton and Akeroyd, 1991; Inocencio et al., 2006; Zohary, 1960). In this area *C. spinosa* shows a considerable pattern of morphological and ecological variation that led some authors to differentiate several species and intraspecific taxa (Danin, 2010; Inocencio et al., 2006; Zohary, 1960), although the pure morphological approach often resulted in dubious classification or misidentification (Bobrov, 1970; Moubasher et al., 2011).

From a morphological perspective, the main discriminating features usually are stipules' thorniness, shoots ramification and habit, petiole type, life form, presence/absence of indumentum, leaf shape, colour, pubescence and texture, the latter two strongly influenced by the habitat and the vegetative period. Taxonomical distinction among taxa mainly based on these morphological traits is rather complex due to the presence of intermediate phenotypes (hereinafter "IP"), that often exhibit mixed morphological characters. These individuals are often found in overlapping distribution areas in the Mediterranean and Near East region (Eisikowitch et al., 1986; Fici, unpublished data; Inocencio et al., 2006; Jiménez, 1987; Özbek and Kara, 2013; Saadaoui et al., 2011; Zohary, 1960), where intra- and inter-specific hybridization is mostly inferred from morphological observations, but no genetic evidences or straightforward ecological data of this phenomenon are available (Inocencio et al., 2006; Özbek and Kara, 2013).

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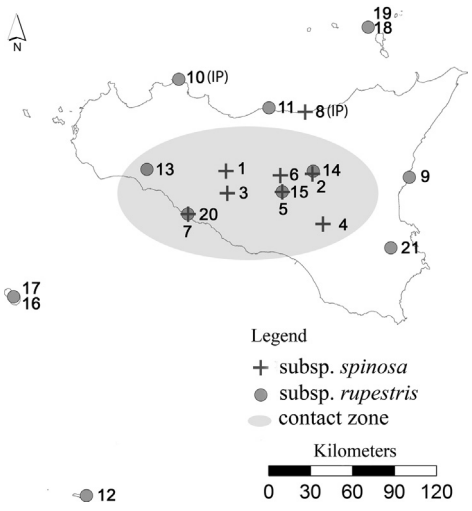

Fig. 1. Studied Sicilian populations of *Capparis spinosa* subsp. *spinosa* (+) and *Capparis spinosa* subsp. *rupestris* (●); grey zone represents the contact zone between the two subspecies; IP in brackets indicates wild populations with intermediate phenotypes outside the contact zone (see Table 1 for legends).

In Sicily and in some surrounding islets such as Lampedusa, Pantelleria and Salina (the latter two are the most important producers in Italy of commercial capers) *C. spinosa* is widespread both in natural habitats and under cultivation. The species is locally represented by two ecologically and morphologically differentiated intra-specific taxa: *C. spinosa* subsp. *spinosa* and *C. spinosa* subsp. *rupestris* (Sm.) Nyman (Higton and Akeroyd, 1991) (hereinafter subsp. *spinosa* and *rupestris*, respectively). Subsp. *spinosa* shows an extended areal being widespread in the Mediterranean Region, Middle East and Irano- Turanian Regions (Inocencio et al., 2006); subsp. *rupestris* has a more restricted distribution, including the Mediterranean Region and North Africa (Inocencio et al., 2006). Subsp. *spinosa* is a thorny, generally hairy, hemicryptophyte/chamaephyte with prostrate/procumbent habit and a winter-desiccating shoot system with many secondary ramifications (Fici, 2001). This taxon colonizes clayey soils in markedly xeric areas of southern and central Sicily (Fici and Gianguzzi, 1997), and shows a wide range of morphological variation (Fici, 2001; Saadaoui et al., 2011) and ecological plasticity, occupying also secondary habitats such as roadsides. Subsp. *rupestris* is a spineless chamaephyte/phanerophyte with unramified or few-ramified branches and is more uniform from a morphological point of view; it is mainly distributed along the coastal area on calcareous and volcanic cliffs, with scattered populations in calcareous and chalky outcrops of the inland (Fici and Gianguzzi, 1997). In the inner area of Sicily, in the contact zone between limestone/chalk outcrops and clayey substrates (Fig. 1), the two taxa are parapatric, while in ecotone zones between halo-nitrophilous dry grassland/shrub communities and chasmophytic vegetation, plants with intermediate phenotypes are usually found (Fici, 2001). IP individuals often colonize anthropogenic habitats such as stonewalls and roadsides, and are also found within isolated populations of both subspecies, in secondary habitats outside the contact zone (Gristina, 2011). Based on morphological data, these morphotypes have been usually referred to as hybrids (Fici, 2001; Zohary, 1960). In addition, cultivated forms, mainly propagated through cuttings at Salina and sexually at Pantelleria, most often are referred to subsp. *rupestris*, the only wild taxon growing on the volcanic rocks of both islands. Nevertheless some spiny biotypes, more common in Salina and today rather rare in Pantelleria (Barbera et al., 1991), are still found under cultivation. Their taxonomical treatment is quite unclear, as some authors (*e.g.* Rivera et al., 2003) attributed it to subsp. *spinosa*, while others (*e.g.* Fici and Gianguzzi, 1997) to subsp. *rupestris*.

While morphological approaches have been widely used to discriminate intra- and inter-specific taxa (Danin, 2010; Fici, 2001; Higton and Akeroyd, 1991; Inocencio et al., 2006; Saadaoui et al., 2009, 2011; Zohary, 1960), at present molecular data to ascertain the taxonomical identity of *Capparis* are rather scarce (Özbek and Kara, 2013). Systematic identification has been carried out through molecular markers (AFLP, RAPD and ISSR) on accessions from East Azerbaijan (Nosrati et al., 2012), Egypt (Moubasher et al., 2011), Morocco (Inocencio et al., 2005; Saifi et al., 2011), Spain, Syria (Inocencio et al., 2005), Tunisia (Khouildi et al., 2000) and Turkey (Özbek and Kara, 2013), but no genetic data are available so far on populations from the Mediterranean islands. Systematic research dealing with both molecular and morphological data is of great importance to reveal phylogenetic relationships, evolutionary trends and clarify the taxonomic treatment of systematic groups (El-Bakatoushi et al., 2013; Migdałek et al., 2013; Rakić et al., 2012; Soltis and Soltis, 2000). The cross analysis of genotype and phenotype can provide evidence for convergent evolution and/or hybridization and introgression phenomena.

In order to investigate the genetic relationships between the two subspecies of *C. spinosa* and clarify the taxonomic treatment of wild, cultivated and IP forms within a Mediterranean island complex, including Sicily and the surrounding islets of Lampedusa, Pantelleria and Salina, a combined analysis was carried out at population level through ISSR molecular markers (for background on ISSR technique see Pradeep Reddy et al., 2002; Wolfe et al., 1989) coupled to morphological and ecological traits analysis. The main objectives were: (i) to provide the first genetic characterization of Sicilian populations including intra-specific taxa discrimination, identification of private bands, estimation of overall level of genetic variation, (ii) to assess any correlation between genetic and morphological differentiation, (iii) to test the hypothesis of a hybrid origin of IP individuals and (iv) to check the taxonomic treatment of cultivated forms.

Materials and methods

Population selection and sampling

We selected19 Sicilian wild populations of the two subspecies from different substrates, both isolated and in contact zones, with IP (Table 1 and Fig. 1). We also included different biotypes cultivated in Pantelleria and Salina (Barbera, 1993). Leaf material for genetic analysis was collected from about 10 labelled individuals per population directly in the field.

DNA extraction, ISSR-PCR, data scoring

A total of 222 DNA samples were obtained with CTAB extraction protocol modified by Doyle and Doyle (1987). Six ISSR primers were used in single-primer reactions (Table 2). Optimization reactions were run on a 1 °C temperature gradient (50 – 60 °C) with a matrix of different $MgCl_2$ and Taq polymerase concentrations. After optimization, standard reaction conditions were 0.5 μM primer, 1× Taq polymerase buffer (Quiagen), 0.2 μM dNTPs, 1 U Taq polymerase (Recombinant, Quiagen), 2 mM $MgCl_2$, and 5 ng of DNA in a 25 μL volume. We used a GeneAmp®PCR System 9700 (Applied Biosystem, Foster City, California) with the thermocycler programme set at 4 min at 94 °C; 36 cycles of 30 s at 94 °C, 45 s at 50 °C to 58 °C depending on the primer, 2 min at 72 °C; final extension of 7 min at 72 °C. Replicate accessions were tested in separate experiments to verify repeatability of results. Negative controls, with genomic DNA omitted, were run with every PCR to check for DNA contamination.