Biochemical Systematics and Ecology 61 (2015) 312–318

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Chloroplast and nuclear DNA studies in Iberian Peninsula endemic *Silene scabriflora* subspecies using cpSSR and ISSR markers: Genetic diversity and phylogenetic relationships

Vanessa Ferreira [a, *], Isaura Castro [b, c], João Rocha [d], António L. Crespí [e], Olinda Pinto-Carnide [a], Francisco Amich [f], Rubim Almeida [d], Valdemar Carnide [b, c]

[a] University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
[b] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
[c] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
[d] Department of Biology, CIBIO – Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal
[e] Department of Biology and Environment – CITAB, Botanical Garden and Herbarium, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal
[f] Evolution, Taxonomy and Conservation Group (ECOMED), Department of Botany, University of Salamanca, E-37008 Salamanca, Spain

ARTICLE INFO

Article history:
Received 16 December 2014
Received in revised form 18 June 2015
Accepted 27 June 2015
Available online 8 July 2015

Keywords:
Silene scabriflora
Molecular markers
Genetic differentiation
Geographic variation
Refugia

ABSTRACT

The Iberian Peninsula is considered to be a center of natural distribution and diversity for several species of flora. In this study, the genetic diversity and phylogenetic relationships of *Silene scabriflora* from the Iberian Peninsula were analyzed using ISSR and cpSSR markers. A total of 161 ISSR markers were produced, with a percentage of polymorphic loci of 99.4%. A high level of genetic differentiation among *S. scabriflora* subspecies (*S. scabriflora* spp. *scabriflora*, *S. scabriflora* spp. *megacalycina*, *S. scabriflora* spp. *gallaecica* and *S. scabriflora* spp. *tuberculata*) was observed (Gst = 0.3685), which was illustrated by UPGMA dendrogram. Molecular results are in agreement with subspecies morphological characterization, particularly supporting the morphological similarities between *S. scabriflora* spp. *scabriflora* and *S. scabriflora* spp. *megacalycina*. Three of the five cpSSR loci analyzed were polymorphic and the two different alleles found in each polymorphic locus were combined in two different haplotypes. The results obtained in this study provide evidences for a long distance dispersion theory, Southern of Spain as the biodiversity center of *S. scabriflora*; for speciation, Southern of Spain as a refugia-within-refugia considering the speciation that occurs in *S. scabriflora* spp. *scabriflora* found in this region and the possibility of *S. scabriflora* spp. *scabriflora* as being ancestral of the species.

1. Introduction

The genus *Silene* belongs to the Caryophyllaceae family and has been recognized since the early days of evolutionary biology as an important model system in ecology and evolution with remarkably interesting features for studying sexual and

* Corresponding author.
E-mail address: vanessa.cm.ferreira29@gmail.com (V. Ferreira).

http://dx.doi.org/10.1016/j.bse.2015.06.029
0305-1978/

mating systems (Bernasconi et al., 2009). This genus comprises about 23 sections and 700 species worldwide, 194 of which are reported to be present in Europe (Bratteler et al., 2006).

The variety of ecological habitats of *Silene* distribution in the Mediterranean region of Eastern Europe and their strong differentiation makes this region a natural global centre of this genus and a site for diversity.

The Iberian Peninsula is described as one of the most important Pleistocene glacial refugia in the European subcontinent (Hewitt, 2000, 1999) and the high level of endemism in Iberian plants and animals (Doadrio, 1988; Garcia-Barros et al., 2002; Gómez-Campo et al., 1984; Moreno Saiz et al., 1998; Ribera, 2000) indirectly suggest *in situ* long-term survival, differentiation and speciation in this area.

Silene scabriflora Brot. is an endemic species of the Iberian Peninsula and includes four subspecies, *Silene scabriflora* spp. *scabriflora*, *Silene scabriflora* spp. *tuberculata* (Ball) Talavera, *Silene scabriflora* spp. *megacalycina* Talavera and *Silene scabriflora* spp. *gallaecica* Talavera.

S. scabriflora spp. *scabriflora* is the most widespread subspecies throughout the Iberian Peninsula, although absent in the northwestern (NW) region. The remaining three subspecies are narrowly endemic, with more restricted distribution. *S. scabriflora* spp. *megacalycina* is confined to the Spanish provinces of León, Lugo and Ourense in the NW Iberian Peninsula. *S. scabriflora* spp. *gallaecica* is also endemic to the NW of Iberian Peninsula and is restricted to the Atlantic coast (La Coruña and Pontevedra provinces). *S. scabriflora* spp. *tuberculata* mainly appears in the southern part of the Iberian Peninsula, in the Portuguese Algarve and in the Spanish provinces of Cádiz, Granada, Malaga and Seville and with sporadic occurrences in NW Morocco.

These four subspecies are currently recognized as independent subspecies by the Spanish Flora book (Talavera, 1991). However, this classification is based only on morphological characters which are very similar between some of them: *S. scabriflora* spp. *scabriflora*, *S. scabriflora* spp. *megacalycina* and *S. scabriflora* spp. *tuberculata*, differ only in a few morphological characters. To date, no systematic studies are available and more detailed studies are important for providing new insights into the phylogenetic relationship between these *S. scabriflora* subspecies.

Different types of molecular markers have been successfully used in phylogenetics, systematic, evolutionary and conservation biology and molecular ecology. Nuclear inter-simple sequence repeats (ISSR) are a powerful tool for investigating genetic variation within species (Coutinho et al., 2014; Mao and Fang, 2014; Zietkiewicz et al., 1994). However, these markers have limitations due to their biparental inheritance. In contrast, uniparental inherited organelle DNA markers, such as chloroplast simple sequence repeats (cpSSRs), allow overcoming this limitation and complement information given by nuclear markers. An important advantage of ISSR and cpSSR marker systems is that no knowledge of the target species' genome sequence is required for analyses (Wang et al., 2008).

The Iberian Peninsula has been described as a biodiversity hotspot (Arroyo, 1997; Medail and Quezel, 1997), and as a melting pot for plant biodiversity (Rodríguez-Sánchez et al., 2008). Although some reports on endemic *Silene* species are available, none of them include the Iberian Peninsula *S. scabriflora* subspecies. In the present study the *S. scabriflora* subspecies were analyzed by ISSRs and cpSSRs in order to for the first time, assess the genetic diversity of *S. scabriflora* (1), estimate the genetic differentiation and phylogenetic relationships among *S. scabriflora* subspecies (2) and understand possible glacial refugia of *S. scabriflora* subspecies on the Iberian Peninsula (3).

2. Material and methods

2.1. Plant material and sampling

Young intact leaves of 4–8 individual plants, sometimes the total number of plants found, of each *S. scabriflora* subspecies – *Silene scabriflora* spp. *scabriflora*, *Silene scabriflora* spp. *megacalycina*, *Silene scabriflora* spp. *tuberculata* and *Silene scabriflora* spp. *gallaecica* were collected in their natural habitat in the Iberian Peninsula, then immediately dried in silica gel and stored at −80 °C until DNA extraction. The distance between the plants sampled was at least 1 m in order to avoid collecting leaves from the same individual. *S. scabriflora* spp. *scabriflora* was collected in Southern Spain and Central Portugal (HVR 19519, HVR 20901); the remaining three subspecies have a more restricted distribution and so were collected only in the provinces where their specimens exist: *S. scabriflora* spp. *tubeculata* in Southern Spain (HVR 19578), *S. scabriflora* spp. *megacalycina* in NW Spain (HVR 20902; HVR20903) and *S. scabriflora* spp. *gallaecica* in NW Spain (HVR 19534, HVR 19565; HVR20904; HVR20905) Atlantic coast (Fig. 1). All these specimens are included in the HVR herbarium (http://sweetgum.nybg.org/ih/herbarium.php?irn=126327).

2.2. DNA isolation

Total DNA was extracted from 100 mg of frozen leaf tissue following the protocol supplied in the DNeasy® Plant Mini Kit (QIAGEN, Düren, Germany). Extracted DNA was quantified by UV spectrometer (Nanodrop® ND-1000, Thermo Fisher Scientific, Waltham, MA, USA) followed by a quality check in 1.0% agarose gel electrophoresis and necessary dilutions were done (about 10 ng/μL).