C. R. Biologies xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Molecular biology and genetics/Biologie et génétique moléculaires

Genetic diversity and population structure of six species of *Capparis* in Tunisia using AFLP markers

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ARTICLE INFO

Article history:
Received 22 June 2016
Accepted after revision 4 September 2016
Available online xxx

Keywords:
Capparis
AFLP
Genetic distance
Phylogeny
Population structure

ABSTRACT

In order to study the genetic diversity, the phylogeographic pattern and hybridization between six Tunisian *Capparis* species, 213 accessions of Caper were genotyped with three primer combinations of amplified fragment length polymorphism (AFLP) markers. Out of 750 fragments generated, 636 were polymorphic and 407 of them were restricted to a single species. STRUCTURE and PCoA analyses clearly separated morphologically different populations into six distinct genetic ones. The UPGMA analysis grouped the species into three main clusters: G1 grouped *C. spinosa* subsp. *spinosa* var. *spinosa* and *C. sicula* subsp. *sicula*; G2 grouped *C. ovata* subsp. *ovata* and *C. orientalis* and G3 clustered *C. zoharyi* and *C. aegyptia*. Populations from G1, G2 and G3 were mainly distributed in arid, subhumid, and semi-arid bioclimates, respectively. Additional genetic studies on *Capparis* could help to identify genes underlying speciation events and local adaptation to geographic areas leading to the development of breeding programs.

1. Introduction

Capparis spinosa L. (the thorny caper, *Capparaceae*) is a xerophyte plant mainly exploited as a condiment to flavor foods [1–3]. It has a wide geographic distribution in the Mediterranean area and in Central Asia [4,5], and is adapted to several bioclimatic environments, from humid to Saharan [4,6–8] and to different soil types [4,8–10]. It has a high phenotypic diversity and several morphological distinct intraspecific taxa and intermediate forms were reported making its classification very ambiguous [10–13]. In addition, morphological markers are influenced by the environmental conditions and developmental stages, which make their use limited in diversity studies. It has become obvious these days that morphological characterization of the species of *Capparis* is not sufficient to make definitive discrimination among the species, subspecies, and varieties [14]. More recently, alternate approaches, including application of appropriate molecular markers, have been increasingly adopted to address the problems in *Capparis* taxonomy. Analyses on genetic diversity and relationship among the species of *Capparis* could also provide useful information for the conservation of genetic resources and the establishment of a *Capparis* breeding program. In fact, several recent works assessed the biological activities of caper extracts (whole plant, floral roots, sheets, buds, flowers and fruits) on bacteria [15–20], fungi [19,21], nematodes [22], pests

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http://dx.doi.org/10.1016/j.crvi.2016.09.001
1631-0691/

[23] and adventitious [24], and suggested that *C. spinosa* is a potential source of natural antioxidant molecules [3,25–27]. Genetic diversity studies could give a general guide for choosing parental lines to make suitable cross combinations for the selection of valuable traits with large possible applications in agriculture, food industry and medicine.

The inter-simple sequence repeats (ISSRs) and random amplified polymorphic DNAs (RAPDs) analysis have been the most commonly used techniques in wild and cultivated forms of *Capparis* species, revealing genetic variation among genotypes/species and/or collected sites in Iran [28], Trans-Himalayan region [29], Syria [30], Turkey [31], Egypt [32], Italy [33] and Morocco [34]. In Syria, ISSR combined with simple sequence repeat (IRAP) revealed genetic variation among 47 samples of three *Capparis* species genotypes collected from 21 locations and divided the samples into three genetic groups: *Capparis sicula*, *C. aegyptia* and *C. spinosa* [30]. In Morocco, ISSR markers identified 20 genetic groups among 90 accessions of *Capparis* spp. [34]. In Italy, Gristina et al. [33] characterized 90 wild populations and cultivated forms using ISSR markers and suggested a clear genetic distinctness between two different subspecies of C. *spinosa* at the regional level, C. *spinosa* subsp. *spinosa* and subsp. *rupestris*. However, another recent study on eight Egyptian taxa of *Capparis* and related genera (*Capparaceae*) conducted by Moubasher et al. [32] using three primers in randomly amplified polymorphic DNA (RAPD) analyses revealed four varieties of *Capparis spinosa*: *C. spinosa* var. *deserti*, *C. spinosa* var. *canescens*, *C. spinosa* var. *spinosa* and *C. spinosa* var. *inermis*. Using RAPD technique, Özbek and Kara [31] also differentiate five different varieties, *Capparis spinosa* L. var. *spinosa*, var. *aegyptia* and var. *canescens* and *C. ovata* Desf. var. *palaestina*, and var. *herbacea*, and intermediate forms between 15 Turkish natural *Capparis* populations. In the other hand, among the different molecular tools, the Amplified Fragment Length Polymorphism (AFLP) method has been extensively used for a wide range of species, including medicinal plants [35–37], to investigate the population genetic structure and assess the genetic differentiation among species [38,39]. This technique is a powerful DNA fingerprinting technology applicable to any organism without the need for prior sequence knowledge. It is a reproducible multilocus marker system with selective PCR amplification [40]. The main advantages of AFLPs are the high levels of polymorphism and high degrees of discriminative capacity for closely related accessions [41–45]. This technique was used successfully in *C. spinosa*, revealing genetic variations among 28 samples collected from six sites in the Aleppo and Lattakia provinces (Syria), and showed the presence of specific alleles for each province [46].

In Tunisia, *C. spinosa* is widely distributed. Although it has been extensively studied morphologically, *Capparis* taxonomy is also still highly controversial. Pottier-Alapetite [47] was the first to state four varieties (var. *canescens*, var. *rupestris*, var. *genuina* and, var. *aegyptiaca*) based on morphological traits. Later, Le Floc'h et al. [48], based on Inocencio et al.'s [8] and Yousfi's [49] phenotypic studies, sustained six *Capparis* species in Tunisia: *C. spinosa* subsp. *spinosa* var. *spinosa*, *C. sicula* subsp. *sicula*, *C. orientalis*, *C. ovata* subsp. *ovata*, *C. aegyptia*, and *C. zoharyi*, a new species mentioned for the first time. More recently, Fici's [10] works, based on morphological, ecological, biogeographical characteristics and on herbarium samples, suggested another classification and grouped intraspecific taxa of *C. spinosa* into two subspecies in Tunisia based on the presence of persistent thorns: *C. spinosa* subsp. *spinosa*, the thorny morphotype, with three varieties (var. *canescens*, var. *spinosa*, and var. *aegyptia*) and *C. spinosa* subsp. *rupestris*, the inerm morphotype, with two varieties (var. *ovata* and var. *rupestris*). According to Fici [10], *C. zoharyi* is not morphologically distinct from *C. aegyptia*. Until now genetic studies on Tunisian *Capparis* were rare and limited to one variety/species or one region, not helping to resolve this unclear classification. Khouildi et al. [50] was the first to study genetically *Capparis* species in Tunisia and showed a higher variability in Tunisian caper populations compared to Italian populations using RAPD analysis. In addition, Ghorbel et al. [51] subdivided 12 Tunisian *Capparis* populations into two genetic groups belonging to inerm and thorny morphotypes on the basis of RAPDs and isoenzymatic tools.

The objective of this study were:

- to molecularly characterize a representative panel of the morphologically identified *Capparis* accessions in Tunisia by AFLP analysis;
- to study the population structure, the phylogenetic relationship between accessions, and gene flow;
- to study the correlation of the genetic diversity with geographical and ecological suitability of *Capparis* in Tunisia.

2. Material and methods

2.1. Plant materials and DNA extraction

Two hundred and thirteen caper accessions, sampled from different climate zones, were selected for this study from the collection conserved at the Tunisian National Gene Bank (see details in supplementary material). Accessions belong to six distinct caper species (*C. sicula*, *C. spinosa*, *C. ovata*, *C. orientalis*, *C. zoharyi*, and *C. aegyptia*) and were chosen based on clear morphological characterizations [48]. Sampling site and GPS coordinates were recorded for each accession.

Total genomic DNA was extracted from frozen young leaves according to a modified CTAB procedure described by Saghai-Maroof et al. [52]. DNA concentration was estimated by spectrophotometer and by electrophoresis on [1% (w/v)] agarose gel [53].

2.2. AFLP genotyping

For each accession, 10 μL of genomic DNA (500 ng) was double digested with 5U of *Eco*RI restriction enzyme at 37 °C in a final volume of 20 μL for 4 h, then with 5U of *Mse*I restriction enzyme at 65 °C in a final volume of 40 μl for 4 hours. The resulting fragments were ligated to