Industrial Crops and Products 60 (2014) 75–78

Contents lists available at ScienceDirect

Industrial Crops and Products

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

# *Pancratium maritimum* L. in Tunisia: Genetic and chemical studies among the threatened populations

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ARTICLE INFO

*Article history:*
Received 16 April 2014
Received in revised form 1 June 2014
Accepted 5 June 2014
Available online 26 June 2014

*Keywords:*
Essential oil
Genetic
Island
*Pancratium maritimum*
Structure

ABSTRACT

Genetic and volatile variations in 13 populations of *Pancratium maritimum* growing wild in mainland and island habitats were assessed using seven isozymes and 18 volatile compounds. Allozymes were revealed by 13% gel electrophoresis. Volatiles were analyzed by GC and GC–MS.

Genetice and volatile data were not correlated. The volatile variation may result either from the genetic variability or from the influence of micro-ecological factors contributing to the selection of particular compounds dictated by the isolation.

The continuous eradication of *P. maritimum* populations reduced their size and contributed to enhancing their differentiation level unless *in situ* and *ex situ* conservation measures are adopted very fast.



## 1. Introduction

The genus *Pancratium* (Amaryllidaceae) comprises about20 species, extending from the Canary Islands through the Mediterranean region to tropical Asia, and southwards through West Africa to Namibia (Walters et al., 1986; De Castro et al., 2012). *Pancratium maritimum* L. is distributed in the Mediterranean, the Atlantic, the Black and Caspian coasts (Dothan, 1986). The habitat of *P. maritimum* in the immediate vicinity of the sea is characterized by direct exposure to sea breezes and to continuous sprays by high air humidity and by strong radiation (Eisikowitch and Galil, 1971). The species is severely threatened in its original range, the sandy coasts of the Mediterranean Sea by over collection, urbanization and tourism development. The current status of *P. maritimum* in Lebanon is vulnerable. In Italy, France, Spain and Crete, populations of this species have significantly decreased in number and size and the species are considered endangered (Zahreddine et al., 2004).

*P. maritimum* L. is a well-studied species. Genetic diversity and population structure were studied by Grassi et al. (2004) and Sanaa and Ben Fadhel (2010). Pollination spectrum was studied by Medrano et al. (1999). The seeds and pollen morphology and dispersion were reported by Keren and Evenari (1974) and Perez-Mellado et al. (2000). Alkaloids, essential oils and polymers were investigated by Berkov et al. (2004) and Sanaa et al. (2012, 2013).

In Tunisia, *P. maritimum* populations are at present endangered and represented by scattered individuals as a result of coastal habitat destruction, especially those on prime tourist areas such as sandy beaches, and overharvesting for its significant ornamental interest (Sanaa and Ben Fadhel, 2010).

The adult plant is 30–60 cm high on sand surface. In summer, it produces 3–14 white scented flowers which are grouped in inflorescences. The stem forms big bulbs that sink down to 140 cm in the sand. The seeds are produced from late September to early December. They are black and extremely light (50 mg each). The seeds are carried away by the wind or floating in the sea, which transport them to other distant beaches.

The present study is a comparative analysis of *P. maritimum* population structure by volatiles and genetic markers. We address the following questions: (i) Do isozymic and chemical markers provide similar conclusions about population structure? (ii) Is there a relationship between chemical and isozymic data?

## 2. Materials and methods

### 2.1. Surveyed populations and sampling

We assessed 5 island and 8 mainland populations of *P. maritimum* located in different geographical regions belonging to the

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http://dx.doi.org/10.1016/j.indcrop.2014.06.006
0926-6690/

**Table 1**
Location of *Pancratium maritimum* populations analyzed.

| Populations | Population code | Geographic region | Latitude (N) | Longitude (E) | Rainfall (mm/year) | Habitat and degradation level[a] |
|---|---|---|---|---|---|---|
| *Mainland* | | | | | | |
| Cap Serrat | $M_1$ | North/Coral Coast | 37°12′48.09″ | 9°14′29.57″ | 1030 | +++ |
| Bizerte | $M_2$ | North/Gulf of Tunis | 37°18′37.68″ | 9°51′24.24″ | 635 | ++ |
| Oued Laabid | $M_3$ | Cap Bon/Gulf of Tunis | 36°52′18.64″ | 10°36′36.36″ | 600 | ++ |
| Dar Allouch | $M_4$ | Cap Bon/Gulf of Hammamet | 36°59′15.67″ | 11° 4′49.36″ | 450 | + |
| Monastir | $M_5$ | Sahel/Gulf of Hammamet | 35°43′0.33″ | 10°49′6.47″ | 300 | ++ |
| Mahdia | $M_6$ | Sahel/Gulf of Hammamet | 35°30′48.29″ | 11° 3′0.91″ | 320 | + |
| Chaffar | $M_7$ | South/Gulf of Gabes | 34°31′30.57″ | 10°33′39.19″ | 200,175 | ++ |
| Zarzis | $M_8$ | South/Gulf of Gabes | 33°35′27.02″ | 11°5′0.28″ | 175 | ++ |
| *Islands* | | | | | | |
| Galite | $I_1$ | North/Coral Coast | 37°31′4.67″ | 8°55′28.12″ | 525 | +++ |
| Zembra | $I_2$ | Cap Bon/Gulf of Tunis | 37°7′5.19″ | 10°48′31.41″ | 575 | +++ |
| Kuriat | $I_3$ | Sahel/Gulf of Hammamet | 35°46′6.35″ | 11°0′42.53″ | 370 | +++ |
| Karkennah | $I_4$ | South/Gulf of Gabes | 34°42′3.77″ | 11°8′18.48″ | 250 | + |
| Djerba | $I_5$ | South/Gulf of Gabes | 33°46′21.33″ | 11°2′14.76″ | 225 | + |

[a] +++: population highly distributed; ++: site partially lost to development; +: degraded site.

Coral Coast, Gulf of Tunis, Gulf of Hammamet and Gulf of Gabes. The habitat of *P. maritimum* in the close proximity of the sea is marked by direct exposure to breezes and salt water droplets carried by the wind, by strong radiation and by high air humidity (Table 1). Although the underdeveloped area of Cap Serrat ($M_1$) and the uninhabited islands populations of ($I_1$, $I_2$ and $I_3$; Galite, Zembra and Kuriat, respectively) are highly distributed, the inhabited Karkennah island and the well-known touristic island of Djerba ($I_4$ and $I_5$, respectively) populations added to many mainland ones are characterized by their small size and growing in destroyed habitats and replaced with beach resorts and large commercial and industrial ports. Because of the vegetative propagation by bulbs of the species, flowering samples were collected at a distance exceeding 20 m from each other to avoid collecting multiple plants from the same parent. For each population, allozyme analysis was performed on 20 individuals; volatiles were assessed on 10 plants taken at random from those used for the isozyme study. The limited number of samples analyzed was due to the small size of the existing populations. Bulbs from the analyzed populations were also deposited in our laboratory at the National Institute of Applied Sciences and Technology of Tunis.

### 2.2. Electrophoresis and revealed isozymes

Using horizontal starch gel electrophoresis (13%), we analyzed the variation of seven isozymes: isocitrate dehydrogenase (Icd, E.C.1.1.1.42), 6-phosphogluconate dehydrogenase (6-Pgd, E.C.1.1.1.44), phosphoglucomutase (Pgm, E.C.2.7.5.1), Malate dehydrogenase (MDH, E.C.1.1.1.37), phosphoglucoisomerase (Pgi, E.C.5.3.1.9), glutamate oxaloacetate transaminase (Got, E.C.2.6.1.1) and esterase (EST, E.C.3.1.1). Enzyme extraction, staining procedures and zymogram genetic interpretation were carried out according to the methods of Sanaa and Ben Fadhel (2010).

### 2.3. Flower volatiles analysis

Samples were finely ground in liquid $N_2$ and then macerated in 150 ml of hexane for 12 h. We have avoided steam distillation in order to obtain a maximum of constituents to estimate the variation among populations using minor and major compounds. After maceration, extracts were transferred to test tubes and centrifuged at 1300 rpm for 10 min. The supernatant was dried by evaporation using a rotary evaporator (50 °C) and then diluted in 2 ml of hexane before GC and GC/MS analysis. Measurements were repeated twice for each sample. GC Analysis was accomplished with a gas chromatograph AGILENT 6980 series II system. GC/MS Analyses were performed on a Hewlett-Packard 5972 mass spectrometer connected to a Hewlett-Packard 5890 series II gas chromatograph. For each plant, volatiles were identified by comparing their retention times with those of some authentic standards injected under the same chromatographic conditions and by comparison of their retention indices (RI), relative to alkanes, and their mass spectra with the HP Chemstation database HP NBS 75 K.L. Library. The quantification of the compounds was accomplished from their GC peak areas without correction factors.

### 2.4. Data analysis

A principal component analysis (PCA) was used to assess population structure based on the combined data of volatile percentages and allelic frequencies. Using the program TFPGA 1.3 (Miller, 1997), a Mantel's test (at $P < 0.05$ and after 1000 permutations) was performed to assess the correlation between *Euclidean* and genetic distances matrices calculated on volatile and allozyme data, respectively.

## 3. Results and discussion

### 3.1. Genetic and essential oil analysis

The species exhibited relatively high levels of genetic diversity ($A_p = 1.36$, $P = 37.17\%$, and $He = 0.100$) were comparable or slightly less than those reported by Hamrick and Godt for animal-pollinated-outcrossing seed plants ($A_p = 1.54$, $P = 35.90\%$ and $He = 0.124$). The low genetic divergence among populations and their high structuring indicate their recent isolation as a result of coastal habitat destruction by anthropic pressures. Moreover, essential oil composition varied highly among populations. The major components at the species level were *n*-heptacosane (12.07%), hexadecanoic acid (11.91%), benzyl benzoate (8.17%), *n*-octacosane (8.13%), and *n*-hexacosane (7.28%). They are largely used in cosmetics, in pharmaceutical and in food industries (Hay and Waterman, 1993).

### 3.2. Combined analysis

The first three axes of the principal component analysis (PCA), based on the averages of the constituents of the essential oil and allelic frequencies matrices, represent 57.37% of the total variation (Table 2). The plot obtained according to axes 1 and 2 (41.93% of the inertia) revealed three population aggregates (Fig. 1). The first group is formed by island population $I_1$. The