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THE PALYNOMORPHOLOGICAL CHARACTERISTICS OF *PANCRATIUM MARITIMUM* L. (AMARYLLIDACEAE) IN ALBANIA

SUMMARY

This article provides data on the palynological study of *Pancratium maritimum* L. of the *Amaryllidaceae* family in Albania. *Pancratium* is a bulbous perennial herb that is very tolerant to dry climates. It grows mainly in the coastal sandy areas of our country. The material for the study was taken fresh from the sandy area of the beaches of Durres to Divjaka, Lushnje.

The processing of the material was done with the method of acetolysis and basic fuchsine. Fixation of pollen grains in microscopic preparations was done with the glycerin gelatin method.The microscopic study of the pollen grains as well as their photography was done with a microscope with X 1000 magnification.

The pollen grains of these plants varied from oblate ellipsoid to oblate spheroid, heteropolar, monocolpate. The exine appears thick and has two layers. The sculpture of exine is reticulate. The palynomonomological study of these representatives is given for the first time in the literature of palynology.

INTRODUCTION

The genus *Pancratium* L. is part of the *Amaryllidaceae* family. Referring to the resources of the fourth flora of Albania (Vangjeli *et al.*, 2000), the Amaryllidaceae family is represented by 5 genera, while the *Pancratium* genus is represented by the only maritimum species in Albania by *Pancratium maritimum* L. or sea lily.

Pancratium maritimum L. is an endangered species of the sandy coast of the Mediterranean Sea (Grassi, 2005). In addition to the Mediterranean, it is distributed from the Black Sea to a part of the Atlantic coast. This plant is receiving a lot of attention because of its value as a bioindicator, its chemical composition and its use as an ornamental plant (Paradiso *et al*., 2010; Giovino *et al*., 2015).

The Mediterranean basin can be considered as a natural laboratory with which to study the effects of ocean currents on the distribution of coastal plant species that propagate with hydrochory. *Pancratium maritimum* L. is a perennial geophyte, with a wide distribution along the Mediterranean coast (De Castro *et al*., 2020).

It is pollinated by autogamy, cross-pollination with insects, etc. In the Bolear Islands, the lizard *Podarcis lilfordi* also serves as a pollinating agent (Prez-Mellado *et al*., 2000).

During our observations, we noticed that the insect *Agrius convolvuli* L. (Lepidoptera) is one of the pollinating agents of this plant.

The study of the pollen grains of this plant is a continuation of the palynological studies of the main representatives of the Amaryllidaceae family in our country.

In order to accomplish the quantitative analysis of palynomorphological features, 31 pollen grains are taken in consideration. This study is focus on the type of pollen grains, shape, size, characteristics of furrow, sculpture of exine, etc. For the study of pollen grains is used light microscope Motic BA310. The measurements are performed with X 1000 power and the photos are taken with X1000 magnification.

MATERIAL AND METHODS

The material for the study was taken fresh from the sandy area of the beaches of Durres to Divjaka.To realize the study of morphological characteristics of pollen grains of plants taken in the study, we have used three analytical methods:

- Acetolysis of Erdtman method (Erdtman, 1960);
- Acetolysis of Avetisjan method (Avetisjan, 1950);
- Basic fuchsine of Smoljaninova and Gollubkova method (Smoljaninova and Gollubkova, 1953).

Two methods of acetolysis give very good results with respect to the elements sporoderme study, while basic fuchsine method gives very good results for the study of the shape, size and aperture, and in some cases even to the sculptural elements of exines.

Acetolysis Method of Erdtman (Erdtman, 1960)

During the implementation of this method, the flower or bud are put in alcohol 96 ° to separate the anthers from other parts of the flower. They can be shared within distilled water. Further, together with the anthers of pollen grains were dried on the thermostat, then moistened with a mixture of acetolysis which contains acetic anhydride with concentrated sulfuric acid and chemically clean with the ratio 9:1. Each

time it was prepared at the moment. Test tube in which the pollen grains were put and mixed with acetolysis were settled further in bain-marie, at a temperature of 70-80°C. The duration of the pollen grains in the bain-marie ranged in different types of plant. Afterwards, test tube was centrifuged, and pollen grains were washed several times with distillated water. They were observed under a microscope by adding drops of glycerin solution and water solution in ratio 1:1. When pollen grains are darkened during processing method, they are splitters into a tube material by adding another 1-2 points NaCl sodium chloride and sulfuric acid 1-2 drops of concentrated H_2SO_4 until it be clarified. Then it was realized the second washing with distillated water. By decantation and centrifugation, the material was made available to make preparation.

Simplified Acetolysis Method of Avetisjan (Avetisjan, 1950)

During the implementation of this method, we settled the anthers on the slide. We threw on them a few drops of 96% ethyl alcohol. We cleaned with blotting paper the fatty substances of pollen grains that were created due to the action with ethyl alcohol. New mixture of acetolysis was prepared every time. We threw them on the contents of the blade solution acetolysis 1-2 points and then warmed it in a thermostat or on the alcoholic lamp flames. We checked continuously the preparation on microscope during the usage of heat, so that the pollen grains are not darkened too much. Once it reached the desired color of the pollen grains, we realized the run off with 70% alcohol. Subsequently, the preparation was cleared of debris and was sealed with gelatin-glycerin glue prepared according to Kisser method (Avetisjan, 1950; Erdtman, 1960; Sladkov, 1967).

The colored method of basic fuchsine according to Smoljaninova and Gollubkova (Smoljaninova and Gollubkova, 1953)

During the implementation of this method the grains of pollen are placed under microscope. Later on we added a few drops of concentrated alcohol. If alcohol evaporate quickly, we added again any other drop. A little noticed that the fatty content of pollen grains scattered by alcohol toward the sides of the blade. This fatty substance was wiped with blotting paper. After the cleaning, we put on the it the basic coloring Fuchsine solution, which was prepared by 2 options, as follows:

1) Basic fuchsine alcohol, 75% of the phenol in this ratio 1: 700: 100

2) Basic fuchsine, 96% ethyl alcohol and xylol in ratio 1: 600: 800

Phenol and xylol substances were used to increase the transparency of coloring. Also, these substances were used and as antiseptics. After coloring, the materials were sticked with gelatin-glycerin, which was prepared according to the Kisser method.

Fixation finished preparations

To realize the fixing of preparations we used the adhesive method of preparation made of gelatin-glycerol (Kisser, 1937). To prepare the adhesive Kisser method, we used 50 g gelatin, 175 ml of distillated water, 150 g glycerin and 7 g phenol.

We warmed the distillated water in bain-marie at 50 °C temperature. Further on we mixed it with the gel and stired it a few times until the contents were dissolved completely. Later we added glycerin. We boiled the content until the liquid was thicken and became viscous. Before removing the mixture from bain-marie, we added in the mixture the solution of phenol and mixed them together. After the content was cooled, it formed a solid mass. The mixture was used in appropriate cases by heating it before hand in bain-marie. During the preparation of mixture, we could observe the formation of air bubbles. To eliminate them, the object where the preparation was put was warmed beforehand and then realized the final process of preparation. Prepared preparation after a few days was isolated with varnish or paraffin and after 2-3 days it became available for use and storage. With the above methods, 3-6 mixtures were prepared for each plant pollen grains.

RESULTS AND DISCUSSIONS

***Pancratium maritimum* L. -** Sea lily

Geophytes. Perennial herb. Bulbs of large size, tapering to a long neck. In the coastal desert, from Velipoja to Vlora. July - September. Chromozome 2n = 22 (Vangjeli *et al.*, 2000).

The pollination ecology of this plant is quite interesting. For this purpose, we made observations of *Pancratium maritimum* L. on August 13 and 14, 2021 (Fig. 1). On August 13 at 8:15 p.m., at the time when dusk had just begun, the plant emitted a very strong aroma that felt quite good in a distance about 2m away from it. The same phenomenon was repeated on August 14 at $20^{oo}13^{//}$.

In both cases, after a few minutes of releasing the aroma, two large insects came near this plant, which were equipped with a very long sucking proboscis (Abd El-Ghani *et al*., 2011; Giovino *et al*., 2015). These insects represent the pollinator *Agrius convolvuli* L. (Lepidoptera) (Fig. 2).

Fig. 1- *Pancratium maritimum* L. in different stages of development, the moments of cross pollination of *Pancratium maritimum* L. with *Agrius convolvuli* L. (Lepidoptera) on 13 and 14. 08. 2021 in Lalzi Bay, Durres.

Fig. 2- *Pancratium maritimum* L. and *Agrius convolvuli* L. *(Lepidoptera).*

Based on our observation, we judge that high air temperatures during the day as well as strong solar radiation prevent cross-pollination with insects. With the arrival of dusk, the solar radiation is gradually interrupted and the air temperatures drop. At this moment, *Pancratium maritimum* emits its strong aroma that serves as a signal to notify the insect *Agrius convolvuli* of its location. The arrival of the *Agrius* insect in the flowers of *Pancratium* achieves cross-pollination.

Pollen grains were monads as shown in the Figs. 3,4,5, in accordance with the data of the palynological literature (Sladkov, 1967; Erdtman, 1986; Faegri, 1989; Kapidani, 1996, Pupuleku, 2002; Kapidani, 2005; Dönmez *et al*., 2008; Golloshi *et al*., 2017; Halbritter *et al*., 2020).

The shape according to the contour of the pollen grain varies from elliptic to elongated elliptic. Pollen grains were monosulcate. The furrow is long, narrow, smooth and goes all the way to the pole. They form clear, uneven and slightly wavy edges. The edges of the furrows give the pollen grain its heteropolar shape.

Exine has two layers. Its layers are almost equal. The structure of exine is reticulate. The grid cells are mixed. There are large, closed cells with very angular, non-uniform contours that reach up to 5 μm. The large cells of the network alternate with small, closed, non-uniform cells, which range from 1.5 - 2 μm.

The network cells in the contact area between them are slightly higher than in other parts of the network. Exine's thickness is about 2 μm. The length of pollen grains varied from 65 to 75 (72) μm and their width varied from 35 to 45 (42) μm.

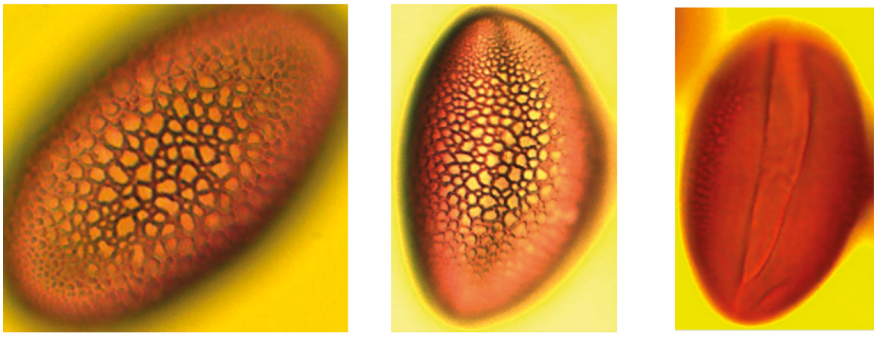

Fig. 3- Pollen of *Pancratium maritimum* L. X 1000 colored method of basic fuchsine.

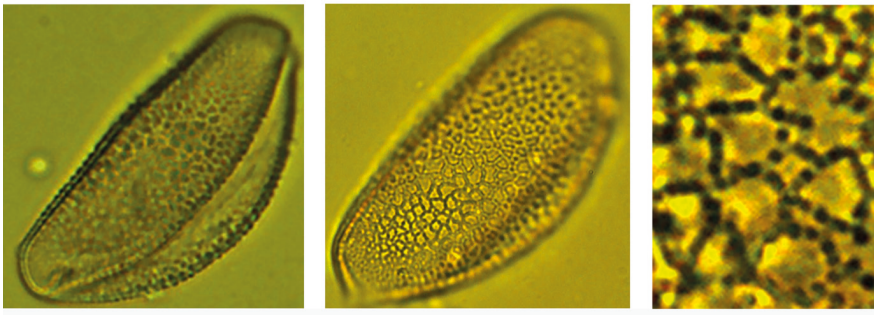

Fig. 4- Pollen *of Pancratium maritimum* L. X 1000 prepared by the acetolysis method.

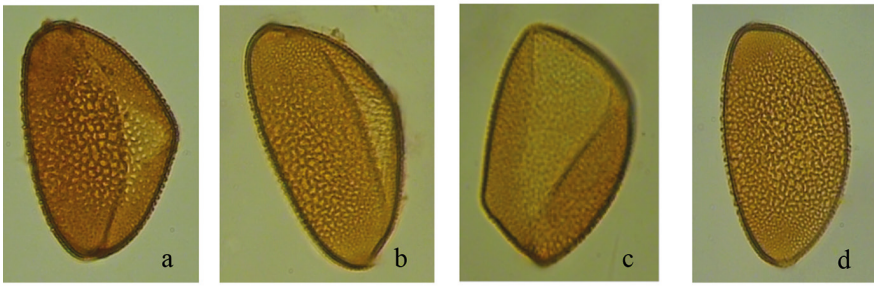

Fig. 5- Pollen grains of *Pancratium maritimum* **a, b, c**. Polar view; **d.** Equatorial view.

CONCLUSIONS

From the observation of pollination and the study of the pollen grains of *Pancratium maritimum* L. it results that:

1. *Pancratium maritimum* L. at dusk emits its strong aroma to attract the pollinating insect *Agrius convolvuli* L. (Lepidoptera);
2. From the analysis of the palynological features of *Pancratium maritimum*, it was found that the pollen grains varied in shape from elliptic to elongated elliptic;
3. The aperture was monosulcate, where the furrow was long, closed, smooth and went to the pole;
4. The exine is two-layered with reticulate sculptures, with closed cells but not uniform. The large reticle reached up to 5 μm while the small ones up to 2 μm;
5. Exine's thickness was about 2 μm, while the length of pollen grains varied from 65 to 75 (72) μm and their width varied from 35 to 45 (42) μm;
6. Data on pollination ecology and the study of *Pancreaticum* pollen grains are provided for the first time in the palynological literature of the country.

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