

## POLLEN MORPHOLOGY OF *Zygophyllum fabago* L. AND ITS RELATIONSHIP WITH POLLINATION

### Morphologie du pollen de *Zygophyllum fabago* L. et ses rapports avec la pollinisation de l'espèce.

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#### RESUME

Une étude relative à la morphologie pollinique de *Zygophyllum fabago* L. et sa relation avec la pollinisation de l'espèce a été réalisée.

Le grain de pollen est isopolaire, radialement symétrique et suboblate. Elliptique en vue équatoriale, il est trilobé en vue polaire. C'est un petit grain trizonocolporé; les colpes ayant des extrémités tranchantes qui limitent une petite zone polaire avec des marges et une membrane du colpe lisses. Les endopores sont allongés.

L'exine a un réticulum régulier et est lisse en surface. La marge aperturale a un tectum complet, parfois interrompu par une fine ponctuation. Dans le mésocolpium, l'exine est composée d'un tectum incomplet, régulièrement columellé et par une fine couche basale. L'endexine est peu épaisse, continue et homogène. L'ectexine est plus fine à la partie marginale à cause de columelles plus petites et la couche basale y est mince, fragmentée ou absente. Au centre de l'aperture, l'ectexine devient une fine membrane. Au niveau des colpes, l'endexine subit un élargissement progressif au centre de l'aperture; granulée près des endopores. Dans l'endopore, l'endexine est réduite à quelques granules ou absente. L'intine est fine tout autour du pollen. Le type de pollinisation de *Zygophyllum fabago* L. et son incidence sur des patients sujets aux allergies sont relatés.

#### ABSTRACT

A study about the morphology of *Zygophyllum fabago* L. pollen grains and their possible type of pollination has been carried out. The pollen grain is isopolar, radially symmetric and suboblate. Elliptic

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in equatorial view, trilobate in polar view. It is a small grain and 3-zonocolporate. The ectocolpi have sharp ends which limit a small polar area with margins and colpus membrane smooth. The endopore is lolongate. The exine has a regular reticulum and a psilate surface. The apertural margo has a complete tectum, sometimes interrupted by fine punctae. In the mesocolpium the ectexine is formed by an incomplete tectum, regular columellae and a thin foot-layer. The endexine is thin, continuous and homogeneous. The ectexine is thinner at the margin, because of shorter columellae and the foot-layer is thin, fragmented or absent. In the centre of the aperture, the ectexine becomes a thin aperural membrane. At the end of colpus, the endexine suffers a progressive enlargement towards the centre of the aperture. It is granulate and lax near the endopores with a few white lines. In the endopore, the endexine is reduced to some granules or absent. The intine is thin around all the pollen grains. The ambophilous character of pollination of *Z. fabago* is suggested, and its incidence in pollinosis patients related.

## INTRODUCTION

*Zygophyllum fabago* L. (Zygophyllaceae) can be found in Proximal Orient, in North Africa and in the arid zones of the Mediterranean region (FONT-QUER 1979, CRONQUIST 1981, VALDÉS *et al.* 1987b). It is a small wood bush with numerous branches measures 60-80 cm height. It is predominantly considered an insect pollinated plant. Nevertheless the pollen grains of *Z. fabago* are airborne in Cartagena (Spain) from April to July, and about 15% of patients who suffer from a clinic allergy were positive to *Z. fabago* in Cartagena (BELCHÍ *et al.* 1997, 1998). The pollen grains morphology of some genus of Zygophyllaceae has been described by BONNEFILLE & RIOLLET (1980), and VALDÉS *et al.* (1987 a). A study of morphology of *Z. fabago* pollen grains and their possible type of pollination has been carried out, in order to explain the presence of this pollen in the atmosphere.

## MATERIAL AND METHODS

Anthers were collected from wild plants of *Z. fabago* which were found in uncultivated soils from Cartagena and Murcia (Spain).

### *TEM-Transmission electron microscopy*

Mature anthers from *Z. fabago* were used. The samples were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer for 12 hours at room temperature. They were washed in 0.1 M cacodylate buffer and postfixed with 1% osmium tetroxide in 0.8% phosphate buffered  $K_3Fe(CN)_6$  for 7 hours at 4 °C (WEBER 1992). This process was followed by a dehydration in acetone and embedding in Spurr's resin. The osmium-thiocarbohydrazide-osmium (OTO) technique (SELIGMAN *et al.* 1966) was used to detect total lipids. Finally, the sections were stained with uranyl acetate for 15 min and lead citrate for 5 min and observed in a Philips EM301 and MT800.

### *SEM-Scanning electron microscopy*

Some pollen grains were acetolysed. Other pollen grains were fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer, at pH 7.4, for 24 hr at 4° C. After washing in cacodylate buffer, the samples were postfixed in 1% osmium tetroxide in the same buffer, at pH 7.4, for 1-2 hr at 4° C. Subsequently, the samples were dehydrated through an ethanol and amyl acetate series and critical point drying (CPD) was carried out with a Polaron E 3000. The pollen grains were sputtered with gold coat (Sputter Coater Polaron E 5000), and studied with a HITACHI S 2300 scanning electron microscope.

For the palynological terminology we refer to PUNT *et al.* (1994).

## RESULTS

The pollen grain of *Z. fabago* (Fig. 1, 2) is isopolar, radially symmetric and suboblate ( $P/E=0.80-0.87$ ). Elliptic in equatorial view, trilobate in polar view. It is a small grain ( $14-16 \times 16-20 \mu\text{m}$ ) and 3-zonocolporate. Ectocolpi with sharp ends limit a small polar area with the margins and colpus membrane smooth; the endopore is elongate. The exine is reticulate with lumina narrower than muri. The exine has a psilate surface, with regular reticulum. The apertural margo has a complete tectum, sometimes interrupted by fine punctae. In the mesocolpium the ectexine is formed by an incomplete tectum, regular columellae and an well defined foot-layer. The endexine is thin, continuous and homogeneous (Fig. 5, 7, 8). The ectexine is thinner at the margin, because of shorter columellae and the foot-layer is thin, fragmented or absent. In the centre of the aperture, the ectexine becomes a thin layer which coats the endexine (Fig. 9-11). The endexine that is located at the end of the colpus suffers a progressive enlargement towards the centre of the aperture (Fig. 9). It is granulate and lax near the endopores with a few white lines (Fig. 10). In the endopore, the endexine is reduced to some granules or absent (Fig. 12, 13). The intine is thin around all the pollen grains (Fig. 5), however, a slightly thick intine was observed under the aperture (Fig. 10, 12, 13).

In the dehiscent anther, pollen grains are in two morphological conditions: hydrated and dehydrated (Fig. 3, 4). Mostly of them are hydrated and are completely coated by pollenkitt (Fig. 3). The dehydrated pollen grains are less abundant; and, in this case, the pollenkitt coats partially the grains (Figs. 4). Abundant pollenkitt, which has low lipid content, is observed in the loculus of the anther (Fig. 6). Some droplets show however a higher lipidic density (Fig. 7).

## DISCUSSION

*Z. fabago* is a plant whose well developed flowers have nectar. These flowers are visited by insects (personal observations). The plant has nectaries which are very similar to that of the family's ones (VALDÉS *et al.* 1987 b). In the anthesis process, the stamens which are curved inside the corolla, projecting beyond the perianth, jut out notably. The anthers are born on long filaments outside the flowers and produce abundant pollen. Once the anthers are dehiscent, the petals and sepals fall down and the stamens straighten up. These stamens have a great quantity of

pollen grains; some of them are dry grains which may be easily dispersed by the wind (personal observations). This behaviour supports the presence of *Z. fabago* pollen grains in the atmosphere of Cartagena, where reaches a 6,06% (445 grains/m<sup>3</sup>) of total airborne during the pollination period, and its significative incidence in pollinosis patients (BELCHÍ *et al.* 1997, 1998)

The morphological characteristics of wind pollinated plants have been well-described (FAEGRI & VAN DER PIJL 1979). The flowers are small, and clustered in dense inflorescences on the outside of plants; the perianth is reduced to absent and when present it is often dully coloured. Nectaries are usually absent, but when they are present, they tend to be non-functional. The anthers are often borne on long filaments outside the flowers, produce abundant pollen and have explosive dehiscence mechanisms. In our opinion the flowers of *Z. fabago* have an intermediate situation, and a possible ambophilous pollination could be considered.

Pollen grains of most earthly species are released from anthers in a partially desiccated state (HESLOP-HARRISON 1979; DUMAS & GAUDE 1981; DUMAS *et al.* 1984). In wind-pollinated species, pollen grains may become further desiccated; and in extreme cases their water content may be as low as 3.2% of the fresh weight (LUZA & POLITO 1987). Desiccation is apparently a necessary step for pollen maturation (LIN & DICKINSON 1984) and maintenance of pollen viability (HOEKSTRA 1983). After contacting with a stigma, pollen grains experience several processes: rehydration, activation and finally germination (HESLOP-HARRISON 1987). The two grades of desiccation observed in the dehiscent anther of pollen grains of *Z. fabago*, would explain the presence of some of them in atmosphere (BELCHÍ *et al.* 1997).

Recent studies of animal pollinated plants have begun to elucidate the role of pollen surface sculpture in the control of pollenkitt distribution and thus pollen stickiness. The differences in pollenkitt and pollen surface structure have also been studied stabilishing a comparison between wind and animal pollinated taxa (HESSE 1979 a, b, c, 1980). These studies have shown that surface sculpture does interact with pollenkitt distribution to control pollen adhesion. HESSE (1978, 1980) pointed out that in most insect-pollinated species the pollenkitt is electron-dense and homogeneous, and forms a complete coating on the exine, rendering the pollen sticky. In anemophilous species, on the contrary, it is electron-transparent and not homogeneous; its quantity is also much smaller. The pollenkitt of *Z. fabago* has been located surrounding the pollen grains. In the hydrated pollen grains, the pollenkitt forms a thin layer over its surface, with some small droplets inside the cavities of reticulum. In dehydrated conditions the pollenkitt coating is not continuos and the pollen grains become less sticky. The pollenkitt has a medium lipid content and is heterogeneous. These features suggest that this pollen grain is mainly insect-pollinated, although this pollenkitt would not avoid wind dispersion in agree with LINDER (1998).

The exine of pollen transported by the wind is usually thinner than the exine of pollen transported by animals (WODEHOUSE 1935). An alternative that states that the exine of the pollen transported by the wind is not thinner than the exine of the pollen transported by animals if pollen of the same diameter is compared (BOLICK 1990). Foveolate or reticulate exines may be associated with beetle, fly or bee pollination (GRAYUM 1986). *Z. fabago* pollen grains are small, smooth and possess a thin and reticulate exine. Due to its features this pollen is easily transportated by the wind and by the animals. A secondary factor leading to a loss of ornamentation in anemophilous plants may be the energetic cost of sporopollenin. Sporopollenin, a lipid based substance (BROOKS & SHAW 1968), is energetically expensive if it is compared to other wall materials. Smooth pollen surfaces may be a cause of the selection process in order to achieve a metabolically "cheaper" pollen walls. *Z. fabago* has a thin exine but its sculpture is reticulated, probably due to the energy saving. This feature would justify its ambophilia.

## CONCLUSION

*Z. fabago* shows a set of features that may suggest a wind pollination, both on flowers and on pollen grains. That is to say, the anthers are born on long filaments outside the flowers and produce abundant pollen; the petals and sepals fall down and the stamens straighten up at the time of anther dehiscence. Moreover, the pollen grains are small and have a thin exine. Some of them are dry, and its pollenkit is heterogeneous and has a low lipid content.

On the other hand, the presence of nectaries, and large or complex flowers, visited by insects, and the reticulate exine and stickiness of some pollen grains, are interpreted as constraints, that would not be involved in wind pollination.

We suggest that the intermediate, ambophilous situation, would characterise the pollination of *Z. fabago*. In our opinion this pollen grain should be taken into account in aeropalynological studies in Proximal Orient, North Africa and in the arid zones of the Mediterranean region.

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Fig. 1-8. Mature pollen grains of *Zygophyllum fabago*. 1-4 SEM, 5-8 TEM. 1) Polar view of acetolyzed pollen grain. Bar: 5 mm. 2) Detail of the colporus. Bar: 5 mm. 3) Fully hydrated pollen grain (critical point drying). Bar: 5 mm. 4) Dehydrated pollen grains showing contracted aperture. Bar: 5 mm. 5) Section of entire pollen grain. (Stain UA/LC). Bar: 2 mm. 6) Abundant pollenkit (pk) in the loculus of the anther and pollenkit droplets in the cavities of the tectum (arrows). (Stain OTO). Bar: 2 mm. 7) Mesocolpium. Ectexine with tectum (t), columellae (c) and undifferentiated endexine and intine. The droplets of pollenkit (pk) are placed in the cavities of the tectum. (Stain OTO). Bar: 0,5 mm. 8) Mesocolpium. Ectexine with tectum (t), columellae (c), and foot-layer (f). Homogeneous and continuous endexine (en) and a thin intine (i) (Stain UA/LC). Bar: 0,5 mm



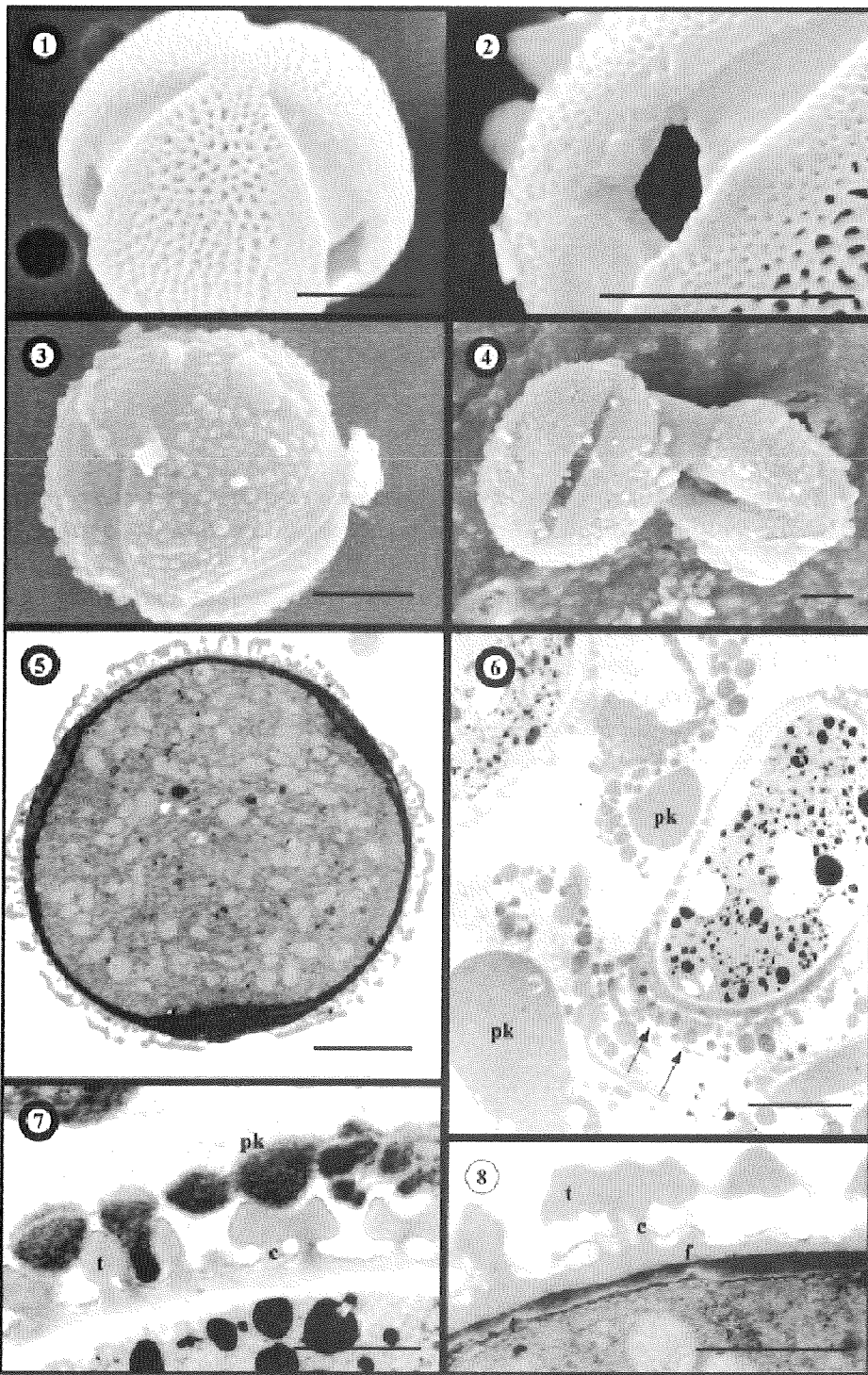


Fig. 9-13. TEM. 9) Apertural structure at end of colpus. Thin ectexine with tectum (t), short columellae (c) and thin or lacking foot-layer (arrows). Note a thin ectexinous apertural membrane (double arrows), a thick, spindle and homogeneous endexine (en) and thin intine (i). (Stain UA/LC). Bar: 0,5 mm. 10) Apertural structure near the endopore. Granulate and lax endexine (en) and clearly visible white lines (arrows). Tectum (t), columellae (c), foot-layer (f), apertural membrane (double arrows) and intine (i). (Stain UA/LC). Bar: 0,5. 11) Section across the end of colpus. Note the apertural membrane (arrow), tectum (t), endexine (en) and intine (i). (Stain UA/LC). Bar: 0,25. 12) Apertural structure around the endopore. Fragmented endexine (en) crossed by white lines (arrow) and intine (i). (Stain UA/LC). Bar: 0,25. 13) Apertural structure in the endopore. Only the intine (i) is present. (Stain UA/LC). Bar: 0,25.

UA/LC: uranyl acetate/ lead citrate. OTO: test for lipids.

