

PALYNOLOGICAL STUDY OF SALIX L. (SALICACEAE) IN IRAN

F. Babayi, M. Pakravan, A. A. Maassoumi & A. Tavasoli

Received 03.11.2011. Accepted for publication 21.02.2012.

Babayi F., Pakravan M., Maassoumi A. A. & Tavasoli A. 2012 06 30: Palynological study of *Salix* L. (*Salicaceae*) in Iran. -*Iran. J. Bot.* 18 (1): 118-126. Tehran.

Pollen grains in seven species of *Salix* are studied with light Microscope and Scanning Electron Microscope. The results of LM showed that pollen grains are tricolpate and the overall shape is spherical to subprolate. In polar view, most of pollen grains were 3-lobed with convex lobes. The size of pollen was very variable. The Exine was reticulate. The characters of muri such as shape and size of lumen were varied in different species. The lumen was isodiametric or heteromorphic and the patterns were orbicular, elliptic or polygonal with rounded angle. Based on former studies the pollen grains in this study were referable to the following types: *Salix alba* type 6, *S. issatissensis* type 1, *S. acmophylla*, *S. zygostemon* and *S. cinerea* type 2, *S. elbursensis* type 5, *S. excelsa* type 3 and 6. Our results showed that shape and size of pollen grains are similar but the exine sculpturing is different and can be used for distinguishing the species. In addition, the exine pattern in *S. zygostemon* which is a hybrid species showed that *S. cinerea* is the male parent of this hybrid and the female parent is *S. elbursensis*.

Fariba Babayi, Maneezheh Pakravan (correspondence <pakravan@alzahra.ac.ir>) & Akhtar Tavasoli, Biology department, Faculty of Science, Al-zahra University, Vanak, Tehran. -Ali Asghar Maassoumi, Research Institute of Forests & Rangelands, P. O. Box 13185-116, Tehran, Iran.

Key words. Plant systematics, *Salix*, palynology, Iran.

مطالعات گرده شناسی در برخی گونه های جنس *Salix* در ایران

فریبا بابایی، دانشجوی کارشناسی ارشد دانشگاه الزهراء (س).

منیژه پاکروان، استادیار دانشگاه الزهراء (س).

علی اصغر معصومی، استاد پژوهش موسسه تحقیقات جنگل ها و مراتع کشور.

اختر توسلی، استادیار دانشگاه الزهراء (س).

در این پژوهش دانه های گرده شش گونه از جنس *Salix* با استفاده از میکروسکوپ نوری و الکترونی اسکینینگ بررسی شدند. نتایج بررسی ها نشان داد که دانه های گرده سه شیاری بوده و شکل کلی آنها از کروی تا تقریباً مستطیلی و کشیده تغییر می کند. بیشتر دانه های گرده سه لوبه با لوب های محدب و اندازه دانه های گرده بسیار متنوع و با آگزین مشبک بود. ویژگی های موری مانند شکل و اندازه منفذ در گونه های مختلف متفاوت و منفذ دارای اقطار برابر یا نا برابر بود و الگوی آن از دایره ای، بیضوی تا چندضلعی دیده می شد. بر اساس مطالعات قبلی دانه های گرده در این مطالعه در تیپ های مختلف زیر قرار گرفتند: *Salix alba* در تیپ ۶، *S. issatissensis* در تیپ ۱، *S. zygostemon*، *S. cinerea*، *S. acmophylla* در تیپ ۲، *S. elbursensis* در تیپ ۵ و *S. excelsa* در تیپ ۳ و ۶. نتایج این پژوهش نشان داد که شکل و اندازه دانه گرده در گونه های مختلف مشابه بوده اما ساختار آگزین متفاوت است و می تواند در تشخیص گونه ها استفاده شود. همچنین با توجه به الگوی آگزین در گونه دورگه *S. zygostemon* می توان *S. cinerea* را والد نر و گونه *S. elbursensis* را والد ماده آن تشخیص داد.

Introduction

The species of the genus *Salix* are deciduous trees and shrubs with simple, stipulate leaves alternately arranged on woody stems. Based on several publications (Fang-Zhen 1987; Skvortsov 1969; Argus 1965; Ohashi, 2000) about 526 distinct species are recognized for the genus worldwide and 31 species and 6 hybrids for Iran (Maassoumi 2009). Recently, anatomical study have been done on *Salix* species in Iran (Khalili et al. 2010).

Description of pollen grains of the genus *Salix* has been given especially by Erdtman (1952), Straka (1952), Kuprianova (1965), Argus (1965), Kim (1984), Wang (1985), Kim, Koh & Zsuffa (1989) and Kim & Zsuffa (1989). Sohma (1993) examined the pollen grains of 72 taxa of the genus *Salix* and noted certain differences in the exine patterns and based on these differences described eight types. A summary of the pollen type characteristics are as follow:

Type 1 consists of grains with conspicuous keeled muri. Especially at the trifurcate points where the immediate neighbored meshes are jointed together, the muri are often acutely pointed. The side walls of the muri delimited the meshes may be more or less straight, curved, or sinuous. The lumina are more or less isodiametric and ellipsoidal to round polygonal in outline, and separated by relatively narrow muri.

Type 2 represents the muri which are wedge-shaped in section. The muri are variable in width considerably. The differences in the shape and the dimension of lumina are also considerable.

Type 3 is characterized by fairly fine reticulation consisting of elongate, curved lumina, and wedge-shaped and low relieved muri. The lumina with circular or triangular in outline are also observed together with them. The muri are as wide as the lumina.

Type 4 represents the grains with fairly wide, wedge-shaped muri, but low in relief. The width of muri is greater than that of lumina. The lumina are small circular, triangular, polygonal in outline and irregularly elongated.

Type 5 consists of grains with keeled muri which furnish a prominent sinuosity on the bases of their side walls. At the trifurcate points they are often pointed. The thickness is not constant and they take a zigzag course. The lumina are irregular in outline and size is much varied.

Type 6 made up of the grains with more or less coarse reticulation consisting of intricately winding muri which demarcate the margin of lumina. It gives an impression of that a few numbers of small lumina in size are located on the muri where they had come to broad. The cross section of muri may be keeled, wedge-shaped or almost semicircular.

Type 7 consists the grains characterized by curved muri with frequent gaps. In cross section they are varied from wedge-shape to keel with fairly high in relief. The lumina vary in size and shape on account of the irregularly curved muri with frequent gaps, and elongated lumina caused by an incomplete fusion of muri are sometimes present.

Type 8 is unique in having irregularly curved or hooked muri with gaps. The wide of muri is more or less the same as that of lumina which are also vary in size and shape. The muri are semicircular in section.

The purpose of this study is identifying of the pollen characters of some unstudied *Salix* species of Iran, and to clarify whether or not the pollen morphology can help to separation of each species.

Materials and Methods

Six species from two subgenera and four sections have been studied (table 1). *S. acmophylla* Boiss. (sect. *Humboldtianae* Pax), *S. alba* L., *S. issatissensis* Maassoumi, Moeeni & Rahiminejadi, *S. excelsa* S. G. Gmelin (all from Sect. *Salix*), belong to subgenus *Salix* and *S. elbursensis* Boiss. (Sect. *Helix* Dum.), and *S. cinerea* L., (Sect. *Cinerella* Duby in DC.) belong to subgenus *Vetrix* Dum., *S. zygostemon* is a hybrid taxon (*S. cinerea* × *S. elbursensis*).

The pollen grains were collected from plants cultivated in Alborz station of the Research Institute of Forests and Rangelands in Karaj. Five catkins and five flowers of each catkin were studied. For LM study pollen grains were preserved in farmer fixative for 24 hours and after that in 70% ethanol. Grains were observed by an Olympus BX51 light microscope with a video camera Olympus DP12 model.

For SEM study, samples were stored in glacial acetic acid and absolute ethanol solution. Pollen grains were mounted on standard aluminum stubs and sputter coated with gold and observed by means of SEM Philips XL30. This technique was used to study the exine and 3D shape of pollen grains. Terminology followed Punt et al. (2007) and Abdel Khalik et al. (2002).

Results and discussion

The pollen characters are given in Table 2 and Figs 1-6. All species had tricolpate grains. The shape of pollen grains was spheroid to subprolate in equatorial view. The smallest grain belongs to *S. zygostemon* with a polar axis length about 11.01 µm and largest one belongs to *S. cinerea* with a polar axis length about 22.16 µm. In polar view pollen grain shape was 3 lobed with rounded angles. The exine was reticulate and the lumen varied in shape and size. SEM study

Table 1. A list of *Salix* taxa investigated and their voucher specimens. Abbreviations: ALUH=Herbarium of Alzahra University.

| species | Section | Origin | Voucher specimens (ALUH) |
|-------------------------|----------------------|------------------------------------------|-------------------------------|
| <i>S. acmophylla</i> | <i>Humboldtianae</i> | Kurdestan | Babayi 5130 |
| <i>S. alba</i> | <i>Salix</i> | Markazi, Ardebil | Babayi 5131, 5132 |
| <i>S. cinerea</i> | <i>Cinerella</i> | Chaharmahal & Bakhtiari, Markazi | Babayi 8702, 8729 |
| <i>S. elbursensis</i> | <i>Helix</i> | Tehran | Babayi 5138, 5139, 5140 |
| <i>S. excelsa</i> | <i>Salix</i> | Tehran, Markazi, Chaharmahal & Bakhtiari | Babayi 5141, 5142, 5143, 5144 |
| <i>S. issatissensis</i> | <i>Salix</i> | Khorasan, Kerman | Babayi 5145, 5146, |
| <i>S. zygostemon</i> | | Chaharmahal & Bakhtiari, W. Azerbaijan | Babayi 5147, 5148 |

showed that muri was very variable. The differences were in height, wide, rounded or angled margin of muri. These characters were different between the species. Lumen had maximum size in the mesocolpia near the equatorial line and gradually or suddenly decreased in size toward the poles and colpi. The shapes of lumen were isodiametrical or heteromorphic and its patterns varied from spheroid to elliptic and polygonal with rounded angles or triangular (Figs. 1, 2, 3). The boarder of muri may be undulate or erect. The palynological study of the species studied showed that in equatorial view, the grains were much varied, being subprolate to spheroidal, but the outline was more common. In polar view, the outline was 3-lobed in all species. The lobes were convex. The size of pollen varied widely, so that the shape and size of pollen were not important for separating the species.

According to Sohma (1993) Sect. *Salix* is uniform in reticulation pattern with the type 6, but our results do not support it. Species showed heterogenous patterns, *S. alba* had type 6, *S. excelsa* type 3 and 6 and *S. issatissensis* type 1.

Our study showed that the exine characters were different among the species and can be used as a diagnostic character. Specially these characters can be used to separate *S. issatissensis* from *S. excelsa* (Fig. 4. e, f, g; Fig. 6. a, b, c, d). Skvortsov (1969) reduced *S. issatissensis* to a variety of *S. excelsa*, but Maassoumi (2009) and Abdollahzadeh (2008) recognized it as a distinct species. The SEM study showed different patterns in reticulation, in *S. excelsa* with type 6 and 3 and *S. issatissensis* with type 1.

Sohma (1993) noted that the hybrids as a rule exhibit exine reticulation pattern of the male parent. Our study showed that the exine pattern of *S. zygostemon* is type 2, *S. elbursensis* type 5 and *S. cinerea* type 2. Since *S. zygostemon* is the result of hybridization between *S. elbursensis* and *S. cinerea*,

therefore *S. cinerea* is the male parent of *S. zygostemon*. (Fig. 4 c, d, h).

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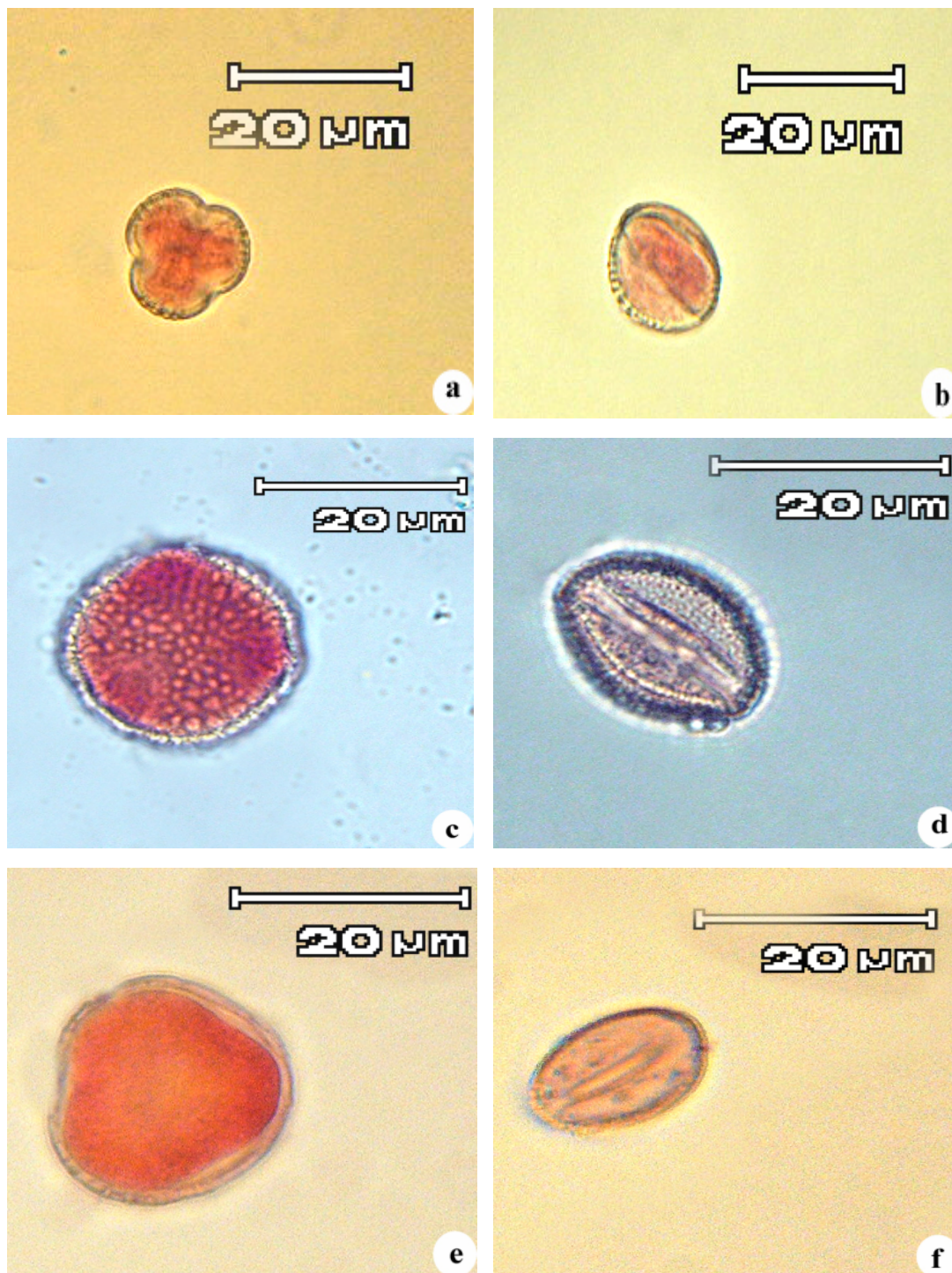


Fig. 1: LM of (a,b): *Salix acmophylla*, (c,d): *S. alba*, (e,f): *S. elbursensis*. (a,c,e) Polar view. (b,d,f) Equatorial view.

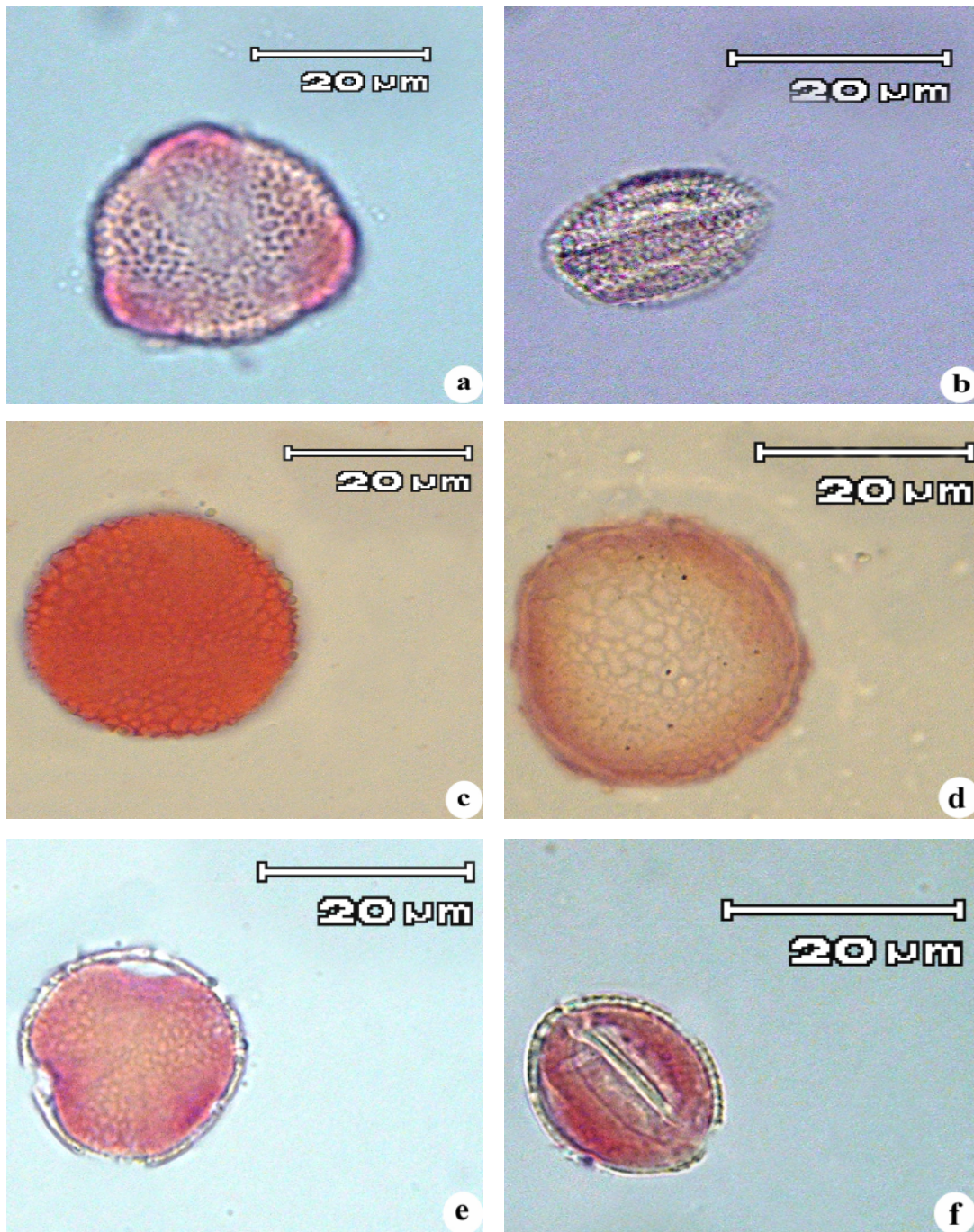


Fig. 2: LM of (a,b): *Salix excelsa*, (c,d): *S. issatissensis*, (e,f): *S. zygostemon*. (a,c,e) Polar view. (b,d,f) Equatorial view.

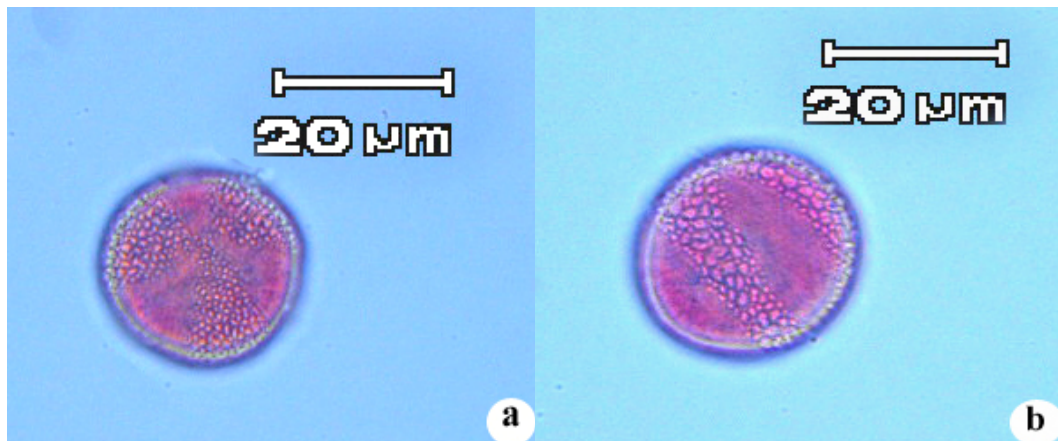


Fig. 3: LM of (a,b): *Salix cinerea*.

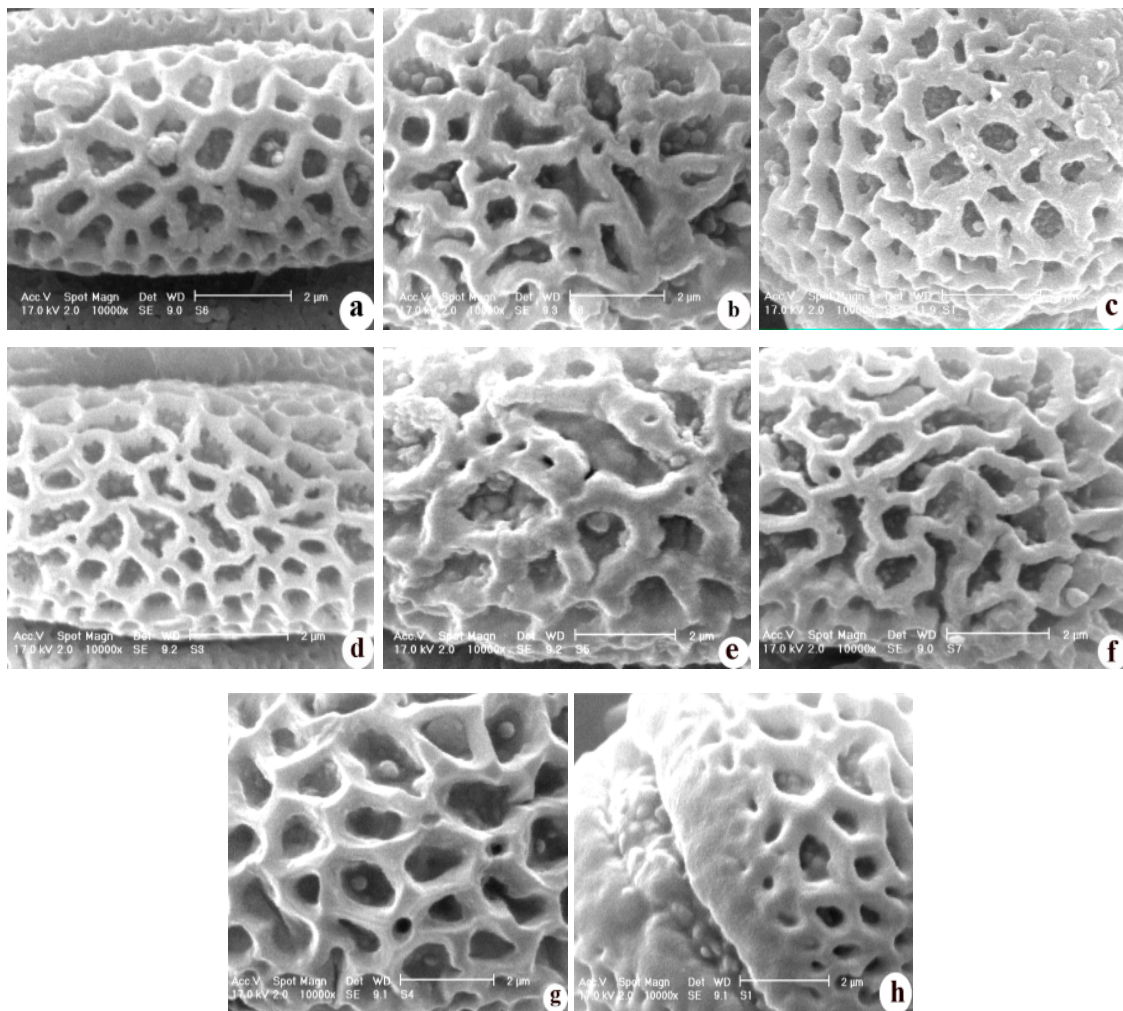


Fig. 4: The Reticulate pattern of (a): *Salix acmophylla* (type 2); (b): *S. alba* (type 6); (c): *S. cinerea* (type 2); (d): *S. elbursensis* (type 5); (e, f): *S. excelsa* (e) Type 6, (f) Type 3; (g): *S. issatissensis* (type 1); (h): *S. zygostemon* (type 2).

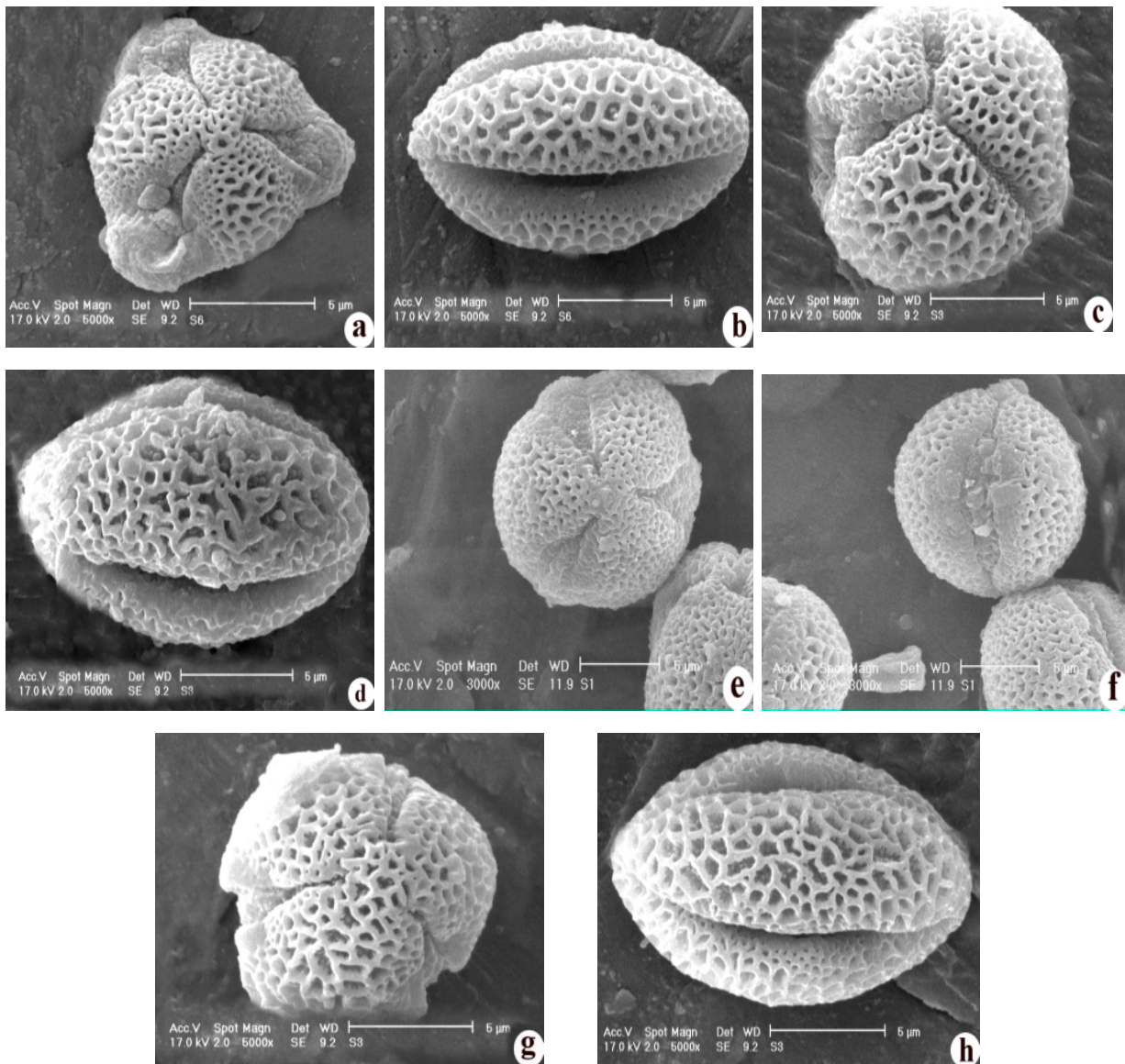


Fig. 5: SEM of (a, b): *Salix acmophylla*; (c, d): *S. alba*; (e, f): *S. cinerea*; (g, h): *S. elbursensis*. In each species the first image is Polar view and second image is Equatorial view.

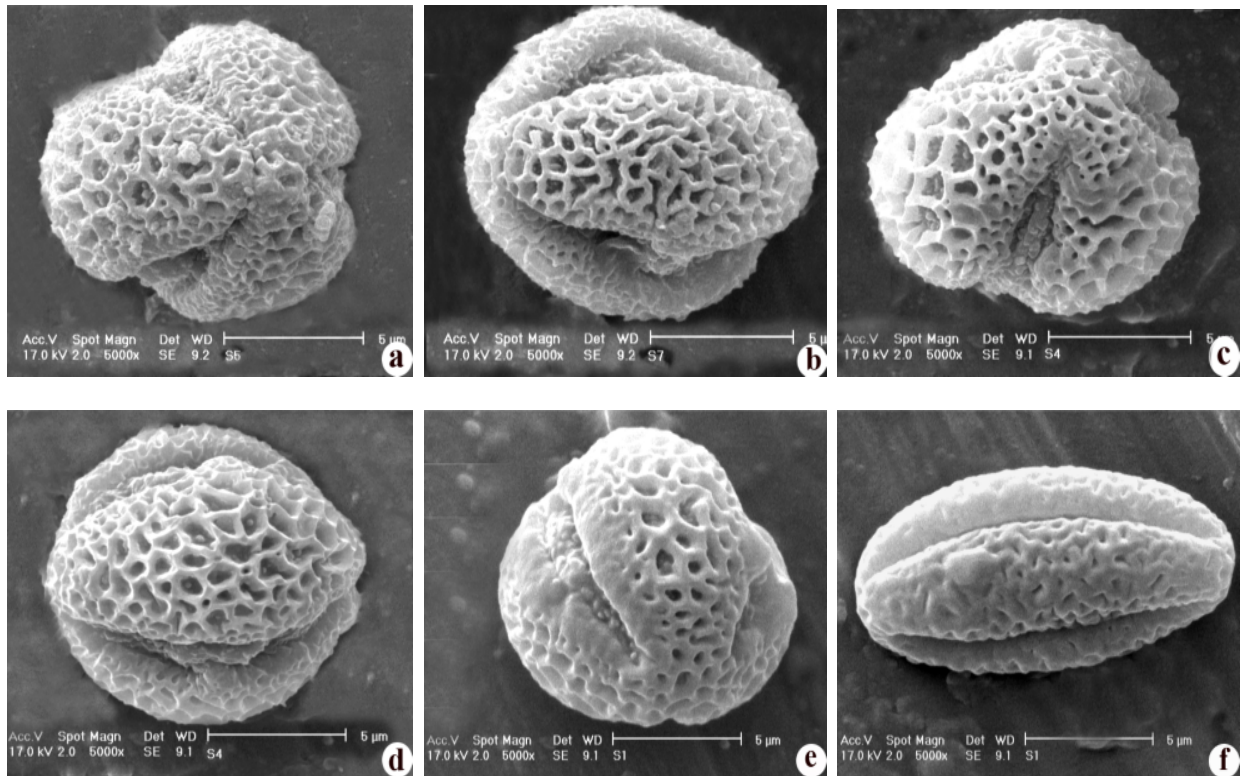


Fig. 6: SEM of (a, b): *Salix excelsa*; (c, d): *S. issatissensis*; (e, f): *S. zygostemon*. In each species the first image is Polar view and second image is Equatorial view.

Table 2. Pollen characters in six species of *Salix* L. with LM and SEM.

| species | Pollar axis length (μm) | | | Equatorial axis length (μm) | | | P/E | Total shape | Reticulate pattern |
|-------------------------|--------------------------------------|-------|-------|------------------------------------------|-------|-------|------|-------------|--------------------|
| | Max | Mean | Min | Max | Mean | Min | | | |
| <i>S. acmophylla</i> | 16.25 | 14.18 | 12.90 | 13.86 | 13.15 | 11.97 | 1.07 | Pr. Sph | Type 2 |
| <i>S. alba</i> | 18.41 | 15.08 | 12.87 | 15.77 | 14.11 | 13.08 | 1.01 | Pr. Sph | Type 6 |
| <i>S. cinerea</i> | 22.16 | 18.92 | 16.59 | 21.44 | 19.64 | 17.98 | 0.96 | Sph | Type 2 |
| <i>S. elbursensis</i> | 15.36 | 13.14 | 11.26 | 14.44 | 12.32 | 10.79 | 1.06 | Pr. Sph | Type 5 |
| <i>S. excelsa</i> | 20.12 | 16.43 | 11.47 | 20.30 | 15.72 | 12.07 | 1.04 | Pr. Sph | Type 3, 6 |
| <i>S. issatissensis</i> | 20.70 | 17.07 | 13.44 | 20.69 | 17.91 | 15.20 | 0.95 | Sph. | Type 1 |
| <i>S. zygostemon</i> | 16.14 | 13.55 | 11.01 | 17.57 | 12.73 | 9.21 | 1.06 | Pr. Sph | Type 2 |

Pr. Sph.=Prolate Spheroidal., Sph.= Spheroidal

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