7. SEM INVESTIGATIONS ON THE PARTIALLY DEGRADED POLLEN GRAINS OF FAMILY MALVACEAE

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Abstract

Pollen grains of *Malva sylvestris* and *Hibiscus syriacus* were partially degraded with 2-aminoethanol for durations of 30 minutes, 1, 5, 10 and 24 hours. Results of the light microscopic observations on these pollen grains have been published by KEDVES et al. (2003). The present study is based on scanning electron microscopic observations and provides new data on the morphological alterations and the superficial degradation of these pollen grains at fine structure level. The differences in degree of alterations on sculpture, ectexine, spines and thick foot layer with respect to resistance and susceptibility of the sporopollenin have also been discussed.

Based on these data, it is concluded that the pollen grains of *Malva sylvestris* are comparatively more sensitive than that of *Hibiscus syriacus*, as these show less response to 2-aminoethanol treatment.

Key words: Experimental Palynology, recent, Malvaceae, partial degradation, SEM.

Introduction

During earlier studies (KEDVES et al., 2003) the results on light microscopic observations of the partially degraded pollen grains of *Malva sylvestris* and *Althaea officinalis* were explained. Using the data of such studies, more detailed observations based on scanning electron microscopy of the superficial alterations caused by 2-aminoethanol for the periods of 30 minutes, 1, 5, 10 and 24 hours are illustrated here.

ROWLEY and PRIJANTO (1977) published TEM data on experimentally altered exine of Malvaceae pollen. DENIZOT (1978) studied effect of ethanolamine on the exine and sculpture under various durations on the pollen grains of *Malva sylvestris*. She observed a little alteration in the exinal sculpture of these pollen grains.

Morphological changes caused by 2-aminoethanol illustrated with SEM enable us to ascertain the differences in alterations and also the comparison of morphological features of both species. Comparison of non-altered, semi-altered and altered characters in these pollen ascertains degree of suceptibility of different ectexinal layers and differential behaviour of sporopollenin with 2-aminoethanol. These phenomena may vary with respect to the morphological structures as well as the pollen types.

Materials and Methods

The pollen material for these studies was collected in Szeged, September, 2002. The experiments with 2-aminoethanol were conducted in the Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged and SEM observations were made at Birbal Sahni Institute of Palaeobotany

Lucknow by using standard dehydration and preparation techniques. The present investigation is based on the earlier descriptions and methods used by KEDVES et al. (2003). The present study allows generation of more detailed data on changes in shape, size as well as the exinal features including spines and collumellae. In addition to these, the response on ectexinal characters, partial or severe alterations in pollen grains by 2-aminoethanol at 30°C for 30 minutes, 1, 5, 10 and 24 hours are described.

Results

Malva sylvestris LINN. (Plate 7.1., figs. 1-7, plate 7.2. and plate 7.3., figs. 1-6)

1. Fresh pollen grains (Plate 7.1., figs. 1,2). Amb circular, radially symmetrical, spheroidal. Size 80-95 µm. Exine ± smooth-echinate, spines 6-8 µm long, smooth, conical with pointed tips.

2. After 30 minutes of dissolution with 2-aminoethanol deformation in morphological features of the pollen grains are clearly distinct (Plate 7.1., figs. 3-5) and depressions at several places are common. The exoapertures (colpi) are clearly visible. In some pollen grains tectum gets completely dissolved and fibrillar structures attached with few spines at certain places forming net or exoskeleton are clearly visible (Plate 7.1., fig. 4). The exinal stratification showing distinct columellar elements with minor corrosion in outer spinal layer is noticed (Plate 7.1., fig. 5 and magnified photo, plate 7.2.). It is observed that the outer and inner part of the endoaperture is not identical. This kind of endopore was observed earlier by DENIZOT (1978) in *Malva sylvestris* LINN., CULHANE and BLACKMORE (1988), *Alcea rosea* LINN., *Althaea officinalis* LINN., LA SERNA RAMOS and DOMINGUEZ SANTANA (1991) and in two varieties of *Lavatera acerifolia*. In *Malva alcea* CULHANE and BLACKMORE (1988) observed that the outer and inner perforations of the foot layer are of equal diameter.

3. After one hour of treatment (Plate 7.1., figs. 6,7) more alterations were observed on the exinal surfaces with dissolutions and depressions in the tectum. The dissolution of tectum resulted in the uncovering of infratectal elements and free columellar ends at their upper surface are noticed. The endoaperture is unaltered and distinct between inter-columellar structures. The spines get detached at several places and infratectal layer also gets affected (Plate 7.1., fig. 7).

4. Five hours of treatment resulted in rupturing of pollen grains with detachment of maximum number of spines as well as tectal layer (Plate 7.3., figs. 1,2). Dissolution of tectum caused liberation of columellae at upper surface as observed in the previous experiment. Endoapertures clearly visible, partial degradation in infratectal layer also noticed.

5. The effect of 2-aminoethanol after 10 hours (Plate 7.3., figs. 3,4) resulted in deformation in exinal sculpture with dissolution of ectexinal surface to some extent, e.g. tectum is completely dissolved, upper part of columellae is also severely affected, maximum number of columellae are dissolved at their upper surfaces, thinning of spines and rupturing of the grain is usual.

6. Maximum degradation in morphology of the pollen grains is clearly visible after 24 hours of treatment with 2-aminoethanol (Plate 7.3., figs. 5,6). The infrasculptural elements are coalesced with each other due to their severe dissolution. The columellae and spines dissolved and appeared like gemmae or bulging structures or sometimes completely dissolved, coalesced and melted over the deformed columellar layer (Plate 7.3., fig. 5).

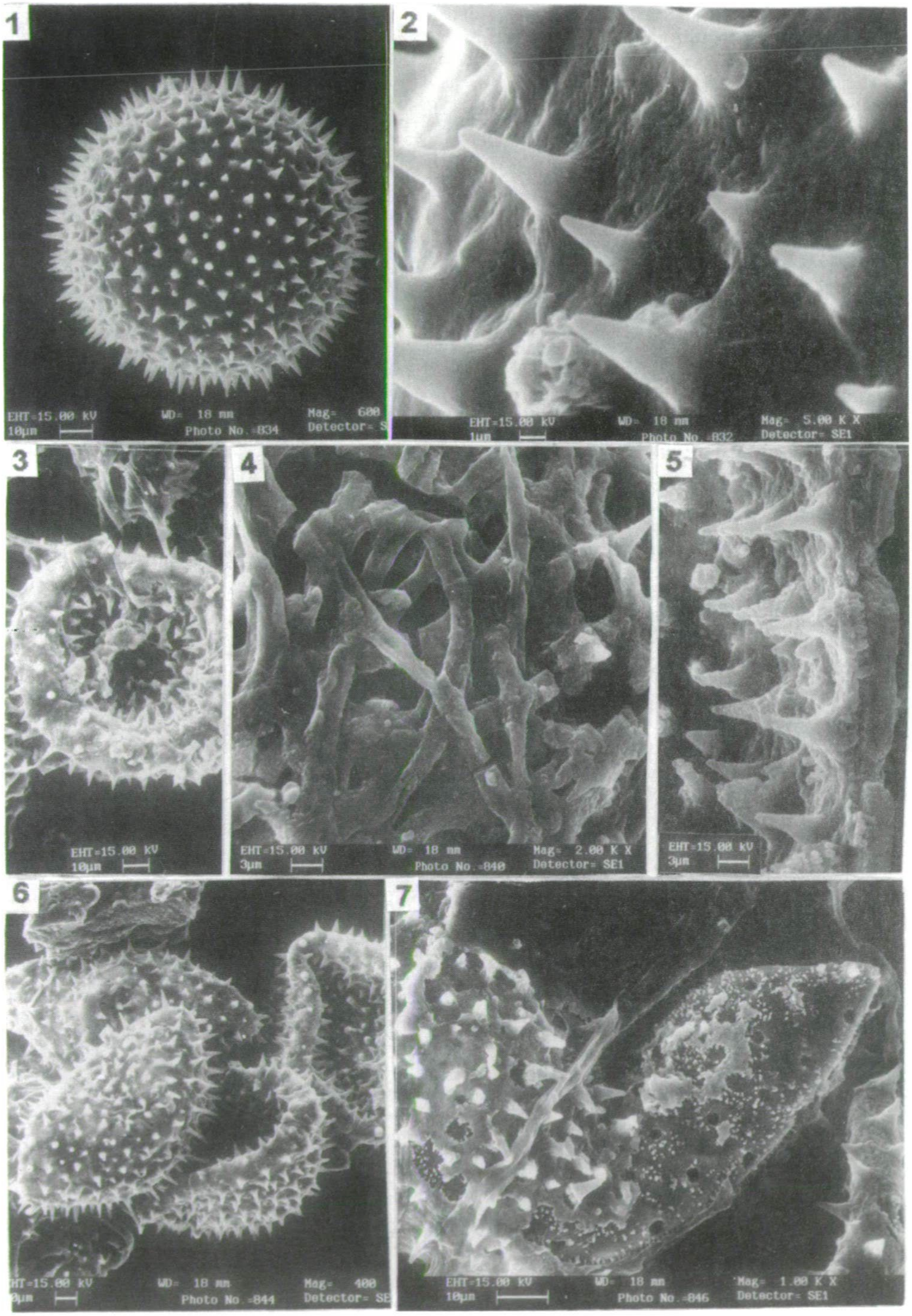
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Plate 7.1.

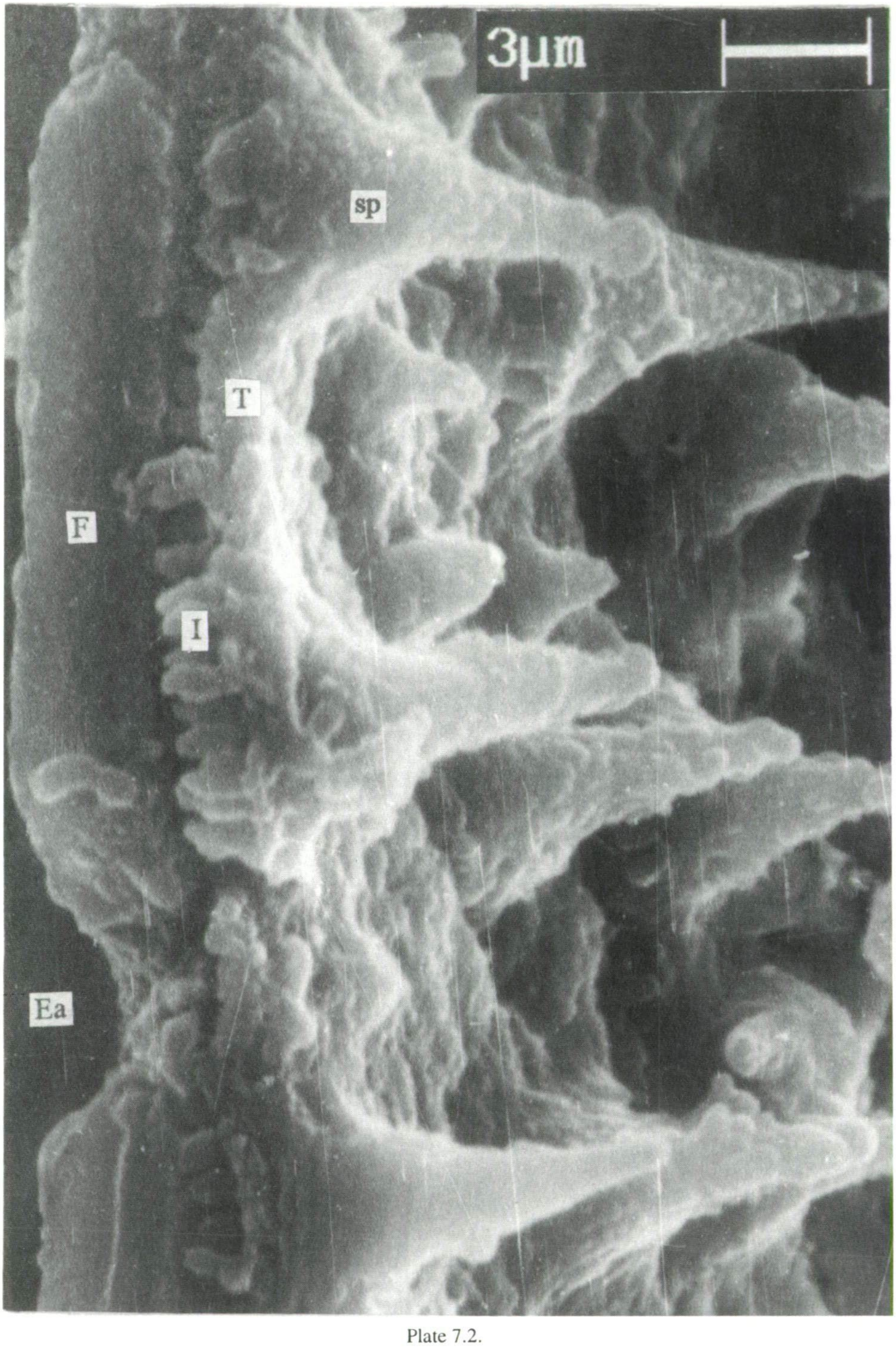
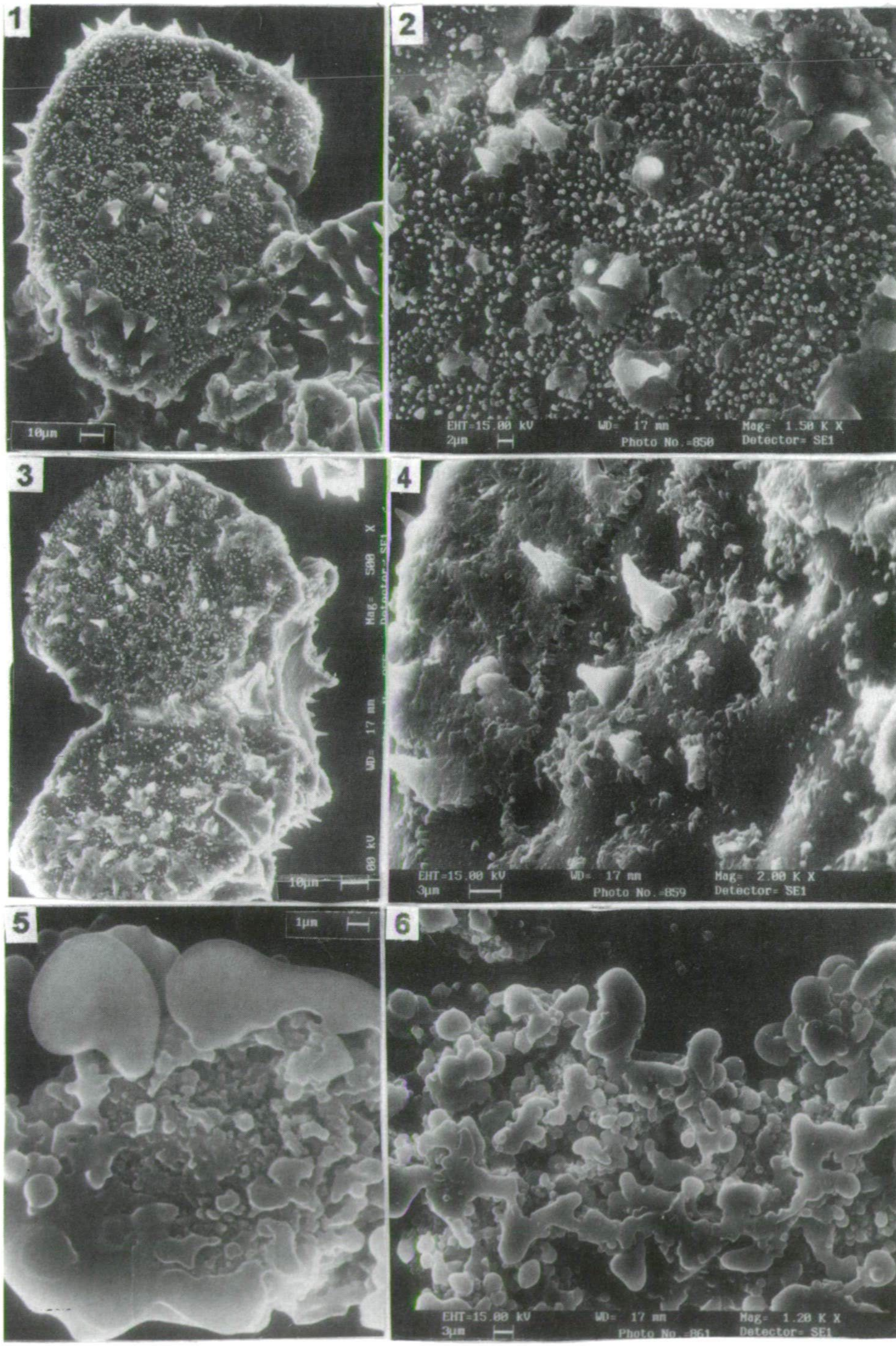


Plate 7.2.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Mag= 2.00 K X
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Photo No.=859
Detector= SE1
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EHT=15.00 kV
WD= 17 mm
Mag= 1.20 K X
3µm
Detector= SE1

Plate 7.3.

Hibiscus syriacus LINN. (Plate 7.4., figs. 1-6 and plate 7.5., figs. 1-6)

1. Fresh pollen grains: amb circular, radially symmetrical, spheroidal in shape. Size 80-110 µm. Spinose, spines 15-20 µm long, conical with blunt tips, polypontoporoidate. Exine granulate at interspinal and interapertural regions. Tectum perforate, mucilage around the apertures are visible (Plate 7.4., figs. 1,2).

2. After treatment with 2-aminoethanol for 30 minutes the deformation in the entire grain is noticed, depressions or shrinkage at several places are common. The apertures become distinct and mucilage disappeared (Plate 7.4., figs. 3,4).

3. The degradation after one hour caused further depressions and changes in the basic morphology of the pollen grains. Surface of tectum is same as in the previous one, but sometimes more bluntness in the tip of the spines is noticed (Plate 7.4., figs. 5,6).

4. Different kind of characteristic deformations are caused by five hours of treatment. Severe depressions at several places in pollen grains are common in highly shrunk grains. Spines are sometimes embedded or maybe detach from their base, the perforation of the tectum is very characteristic and exoapertures are also deformed or depressed (Plate 7.5., figs. 1,2).

5. After ten hours more deformations were observed as various secondary features, outer morphology appears like triradiate forms, some spines become sinuosus. The tectum shows differential depressions (Plate 7.5., figs. 3,4).

6. Treatment with 2-aminoethanol for 24 hours caused different kind of deformations in secondary features, such as depressions in tectal regions, detachment of spines and enlargement of sculptural structures at interspinal spaces and around the base of the spines (Plate 7.5., figs. 5,6).

Plate 7.1.

1-7. *Malva sylvestris* LINN.
1-2. Fresh pollen grains.
3-5. Pollen grains partially degraded with 2-aminoethanol for 30 minutes. 4. Please note the fibrillar network. 5. Change in ectexinal layers and thinning of spines.
6-7. Pollen grains partially degraded with 2-aminoethanol for 1 hour. 6. Shrinking of pollen grains. 7. Dissolution of tectum and detachment of spines.

Plate 7.2.

Malva sylvestris LINN. Highly magnified picture of Plate 7.1., fig. 5. sp = spine, T = tectum, I = infratectum, F = foot layer, En = endoaperture.

Plate 7.3.

1-6. *Malva sylvestris* LINN.
1-2. Pollen grains degraded with 2-aminoethanol for 5 hours. 1. Tectal layer completely dissolved. 2. Magnified view of fig. 1, showing free columellae and few spines.
3-4. Pollen grains degraded with 2-aminoethanol for 10 hours. 3. Ruptured pollen grains with free columellae. 4. Columellae dissolved to reduce their sizes and few thin spines attached to it.
5-6. Pollen grains degraded with 2-aminoethanol for 24 hours. 5. Severe alteration on columellae which are melted and fused with each other. 6. Coalescence of columellae, the effect of severe alteration by 2-aminoethanol.

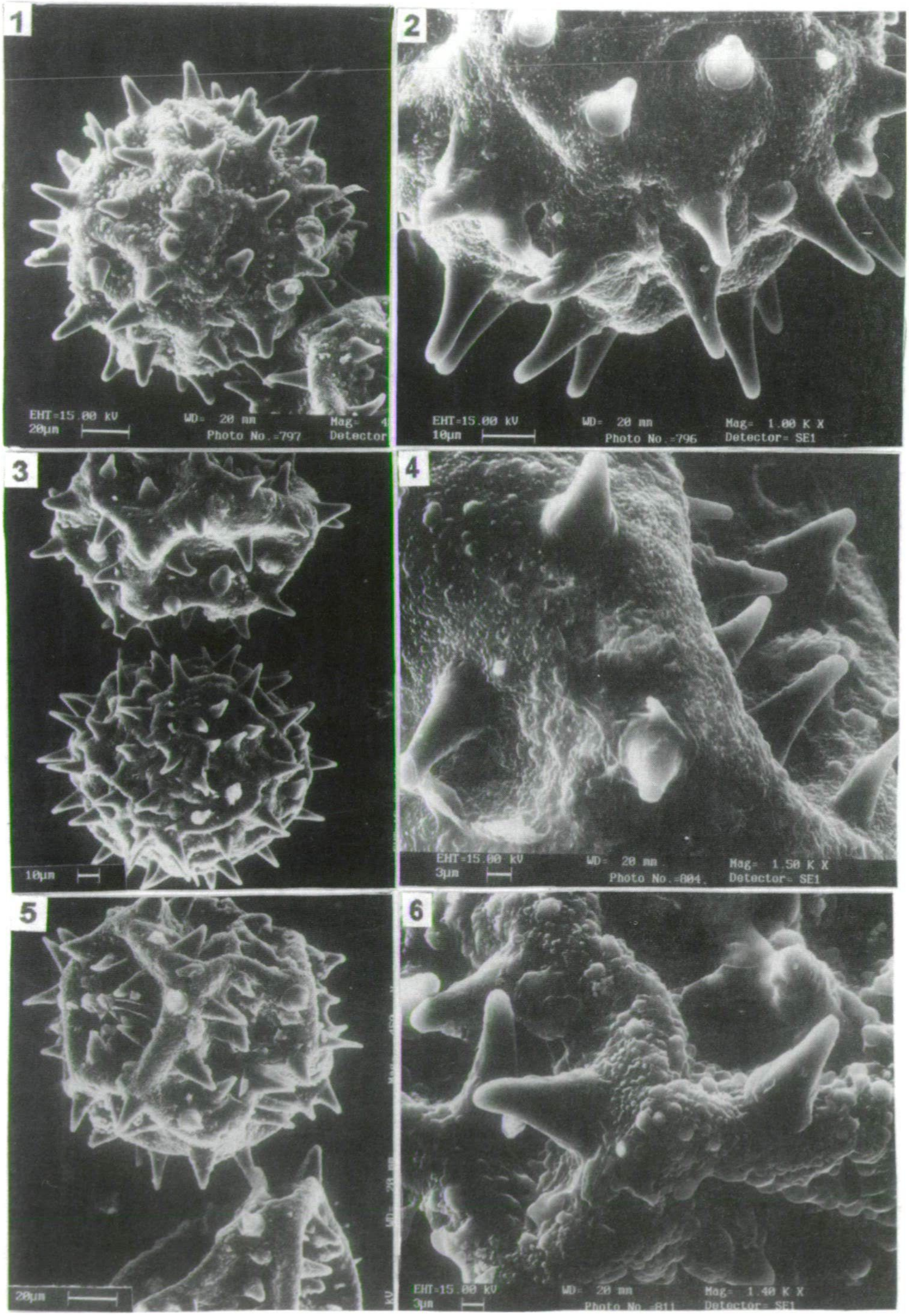
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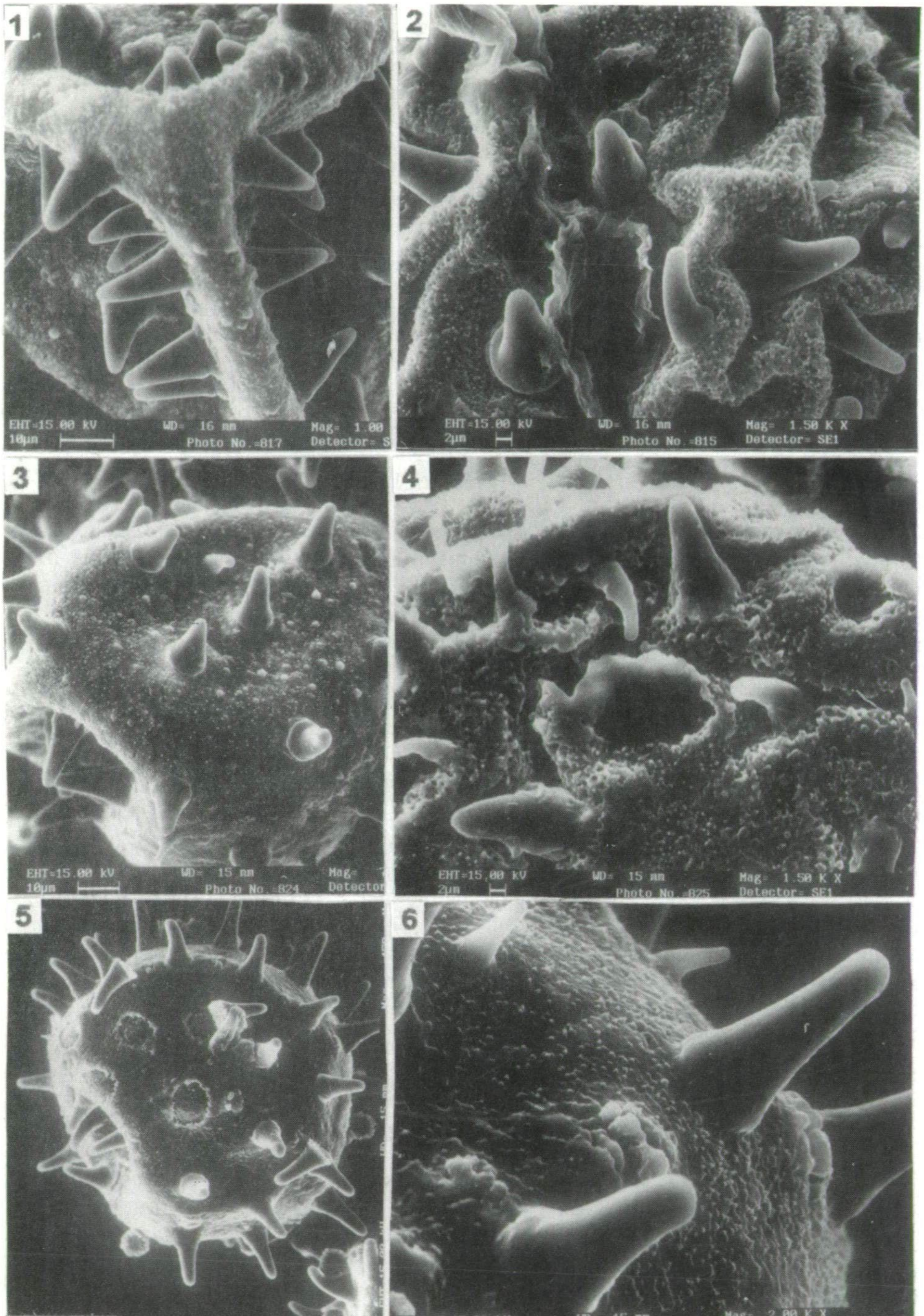
Plate 7.4.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Mag=
10µm
Photo No.=824
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4
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WD= 15 mm
Mag= 1.50 K X
2µm
Photo No.=825
Detector= SE1
5
10µm
6
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WD= 15 mm
Mag= 2.00 K X
3µm
Photo No.=828
Detector= SE1

Plate 7.5.

Plate 7.4.

1-6. *Hibiscus syriacus* LINN.
1-2. Fresh pollen grains
3-4. Pollen grains partially degraded with 2-aminoethanol for 30 minutes, a little depression is noticed.
5-6. Pollen grains partially degraded with 2-aminoethanol for 1 hour. Further depressions causing shrinking of the grains.

Plate 7.5.

1-6. *Hibiscus syriacus* LINN.
1-2. Pollen grains partially degraded with 2-aminoethanol for 5 hours. 1. Alteration caused to appear like triradiate forms. 2. Magnified view of fig.1, showing shrinking of tectum layer.
3-4. Pollen grains partially degraded with 2-aminoethanol for 10 hours, showing dissolution of mucilage, resulting in the appearance of aperture and partial detachment of some spines from their base.
5-6. Pollen grains partially degraded with 2-aminoethanol for 24 hours, showing not much alterations than the treatment for 10 hours. The granulate interspinal spaces and apertures are clearly visible.

Discussion and Conclusions

Treatment with 2-aminoethanol for various durations causes differential alterations and deformation of the morphological features in pollen grains of Malvaceae. Its effect under certain durations is very specific to different structures which are clearly visible in our experiments and results in pollen grains of *Hibiscus syriacus* and *Malva sylvestris.* These results provide a new concept to the differential behaviour of sporopollenin with 2-aminoethanol. These data enable us to understand that pollen grains of various species of a family constitute specific exinal structural patterns, which may or may not be altered after certain duration of treatment with 2-aminoethanol or other chemicals. In earlier stages of the treatment (after 30 minutes and 1 hour) pollen grains of *Hibiscus syriacus* show little alteration, whereas, at this stage almost maximum exinal characters are mostly deformed in *Malva sylvestris.* It shows that different taxa have a peculiar resistance system and secondary alterations are limited up to a certain level only. Likewise, some characters are severely affected or deformed in pollen grains of *Malva sylvestris* at the first stage of the treatment and at later stages (after 24 hours) almost all pollen characters are severly altered. But some pollen grains have resistant features which are not easily altered as observed in *Hibiscus syriacus.* The resistance in these pollen grains may be due to the presence of mucilage on the ectexine, which might serve as a protecting layer to the tectum and other superficial characters. In other words, the mucilaginous coating does not allow 2-aminoethanol to react with sporopollenin.

SOUTHWORTH (1974) and DENIZOT (1978) also observed differential solubility of pollen exine by various chemicals at certain durations, which modify their ultrastructures. KNOX and HESLOP-HARRISON (1969) opined that the sporopollenin of the pollen exhibits differential cytochemical localization of enzymes. Sometimes these enzymes are rapidly diffused by various chemicals. The present study (Plate 7.1., fig. 4) also supports the view of STANLEY and LINSKENS (1965) and KNOX and HESLOP-HARRISON (1969) regarding diffusion of pollen proteins by various chemicals. In our study the fibrillar structure is also seen on the pollen grains of *Malva sylvestris* after 30 minutes of treatment with 2-aminoethanol. The ectexinal characters of the pollens were rapidly degraded and a barren fibrillar skeleton network on the amb is clearly visible, representing a character of allergenic pollen (STANLEY and LINSKENS, 1965). This phenomenon is very important for understanding the differential behaviour of sporopollenin during preservation or fossilization of pollen grains under the influence of various ecological, chemical and edaphic factors.

Acknowledgements

The authors are grateful to the authorities of Hungarian Academy of Sciences, Budapest, Indian National Science Academy, Department of Science and Technology, New Delhi and Birbal Sahni Institute of Palaeobotany, Lucknow for their help which enabled us to carry out the present work under the scientist exchange programme between India and Hungary. This work was also supported by Grant OTKA T 31715.

References

CULHANE, K.J. and BLACKMORE, S. (1988): The Northwest European pollen flora, 41 Malvaceae - Rev. Palaeobot. Palynol. *57*, 45-74.

DENIZOT, J. (1978): Au sujet de la solubilité de l'exine dans l'éthanolamine. - Ann. des Mines de Belgique *2*, 23-31.

KEDVES, M., IMRE, ZS., FREY, K., BANGÓ, J., LUKÁCS, P., HAJNAL, E., GÉGÉNY, B., SZÉL, T., KRIZSÁN, T. and SCHULZ, G. (2003): Experimental investigations on the pollen grains of *Malva sylvestris* L. and *Hibiscus syriacus* L. I. - Plant Cell Biology and Development (Szeged) *15*, 59-68.

KNOX, R.B. and HESLOP-HARRISON, J. (1969): Cytochemical localization of enzymes in the wall of the pollen grains. Nature *223*, 92-94.

LA SERNA RAMOS, B.I. and DOMINGUEZ SANTANA, M.D. (1991): Contribution to the palynological study of the genus *Lavathera* L. (Malvaceae) in the Canary Islands. - In: Palaeoecology of Africa and the surrounding Islands. ed. K. HEINE *22*, 35-47.

ROWLEY, J.R. and PRIJANTO, B. (1977): Selective destruction of the exine of pollen grains. - Geophytology *7*, 1-23, 15 plates.

STANLEY, R.G. and LINSKENS, H.F. (1965): Pollen Biology Biochemistry Management. - Springer Verlag, Berlin, Heidelberg, New York.

SOUTHWORTH, D. (1974): Solubility of pollen exines. - Amer. J. Bot. *61*, 36-44.