10. LM AND TEM INVESTIGATIONS ON EXPERIMENTALLY ALTERED POLLEN GRAINS OF PHOENIX DACTYLIFERA L.

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Abstract

Fresh and partially degraded pollen grains with 2-aminoethanol, $KMnO_4$, merkaptoethanol, and diluted glycerine (50%) were investigated with the LM and TEM method.The new results are the following: 1. The mounted pollen grains were observed in polar position and are opened. 2. Expansion and exudation of the intine and the protoplasm was not observed. 3. The partial degradation revealed the biopolymer system of the sporopollenin not only at the infratectal layer of the ectexine but in the outer and inner part of the foot layer. 4. Regular pentagon biopolymer units were observed. The diameter of these units are characteristically smaller than that of *Phoenix sylvestris* investigated previously. 5. The ultrastructure of the intine particularly in the apertural area is characteristic.

Key words: Experimental Palynology recent, *Phoenix dactylifera.*

Introduction

GRAHAM (1963, p. 36), in his paper concerning the function of pollen wrote the following, "The essential role of pollen grains in plant reproduction has been known for almost 5000 years. The ancient Assyrians were aware that trees of the date palm *(Phoenix dactylifera)* were of two kinds." Later he pointed out, that "Assyrian were not only the first known people to have practiced artificial pollination, but their writings reveal that they considered the pollen-producting plants male and the date-producting plants female." The first LM data of *Phoenix dactylifera* pollen grains were published by WODEHOUSE (1935). Several papers followed this pioneering work, e.g. ERDTMAN (1952), MAHABALÉ (1966), SOWUNMI (1968, 1972) and KEDVES (1980). Multidisciplinary studies were carried out on the palm pollen grains of *Phoenix dactylifera* by BOUGHEDIRI (1988, 1989, 1991, 1999), BOUGHEDIRI and BOUNAGA (1987), BOUGHEDIRI, CERCEAU-LARRIVAL and DORÉ (1995), BOUGHEDIRI et al. (1995), and MANAMANI, BOUGHEDIRI, DOGHMAN and BENOUARTH (2001). Concerning the chemistry of the pollen wall it is worth mentioning the finding published by SHAW and YEADON (1964), p. 247: "ZETZSCHE prepared other pollen membranes in a similar manner and from analytical results suggested that they could be represented by a general molecular formula which is varied from C90H13431 in *Secale cereale* pollen to C90H150O33 for *Phoenix dactylifera*".

During our experimental investigations on Indian palm pollen grains, in particular on the pollen grains of *Phoenix sylvestris* L., we observed a particular organization in the biopolymer structure of the ectexine (KEDVES, BORBOLA, TRIPATHI and MADHAV KUMAR 2000, KEDVES, HORVÁTH, TRIPATHI and MADHAV KUMAR 2001). Further LM data on some partially degraded palm pollen grains from India resulted in interesting alterations in the endexine and the protoplasm (KEDVES, PRISKIN, TRIPATHI and MADHAV KUMAR, 2002).

Taking into consideration the importance of *Phoenix dactylifera* L. and our previous experimental results on recent palm pollen grains, we started a research program on the pollen grains of this species. The first part of our results are presented in this contribution.

Materials and Methods

The pollen grains for our investigations were collected by Dr. Sekina AYYAD (Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt). LM and TEM methods were used. Fresh (T-12-98) and partially degraded pollen grains were investigated as follows:

1. Partial degradation with 2-aminoethanol for 24, 48 and 72 hours (Experiment numbers: T-12-99,100,101). 2. After the degradation with 2-aminoethanol, 10 ml 1% potassium permanganate were added for 24 hours (Experiment numbers: T-12-102, 103, 104). 3. 1 ml merkaptoethanol was added to the partially degraded pollen grains with 2-aminoethanol (Experiment numbers: T-12-105,106, 107). 4. Pollen grains were partially dissolved in glycerine (50%) for 30 days. For every experiment, 5 mg fresh pollen grains were used at temperature 30 °C. For LM studies pollen grains were mounted in glycerine-jelly hydrated at 39.6%, and/or mounted in Araldite after embedding. The ultrathin sections were made with glass knives in the Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, the pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences, Szeged. All pictures are unretouched.

Results

1. Fresh pollen grains

LM results (Plate 10.1., fig. 1) In polar position the pollen grains are generally opened, the thinning of the ectexine in the apertural area is well shown. The protoplasm is invaginated in the germinal region. TEM results (Plate 10.1., figs. 2-6) The complete general survey pictures (Plate 10.1., figs. 4, 6) illustrate well the submicroscopic characteristic features of the apertural area of the pollen grain. The colpus and the invaginated protoplasm are well shown. In picture 6 of Plate 10.1., the electron dense ectintine is separate from the foot layer. In highly magnified pictures (Plate 10.1., figs. 2,5) of the perforated tectum, the intine layers are illustrated.

2. Partial degradation with 2-aminoethanol

2.1. Partial degradation for 24 hours (T-12-99) LM results (Plate 10.2., fig. 1) Important alteration as a consequence of this experiment were not observed.TEM results (Plate 10.2., figs. 2-5) Characteristic electron dense granules (microbodies) are in the protoplasm (Plate 10.2., figs. 2,4). Alterations in the ultrastructure of the intine are not uniform. Light intine, sometimes with electron dense granular elements (Plate 10.2., figs. 2,5), or more or less radially oriented electron dense is probably ectintine remnant (Plate 10.2., fig. 4) in the apertural area (Plate 10.2., fig. 2) the electron dense part of the intine and the thin ectexine are well shown. The ectexine is seemingly damaged, the perforations of the tectum are much larger than in the fresh pollen grains (Plate 10.2., fig. 3).

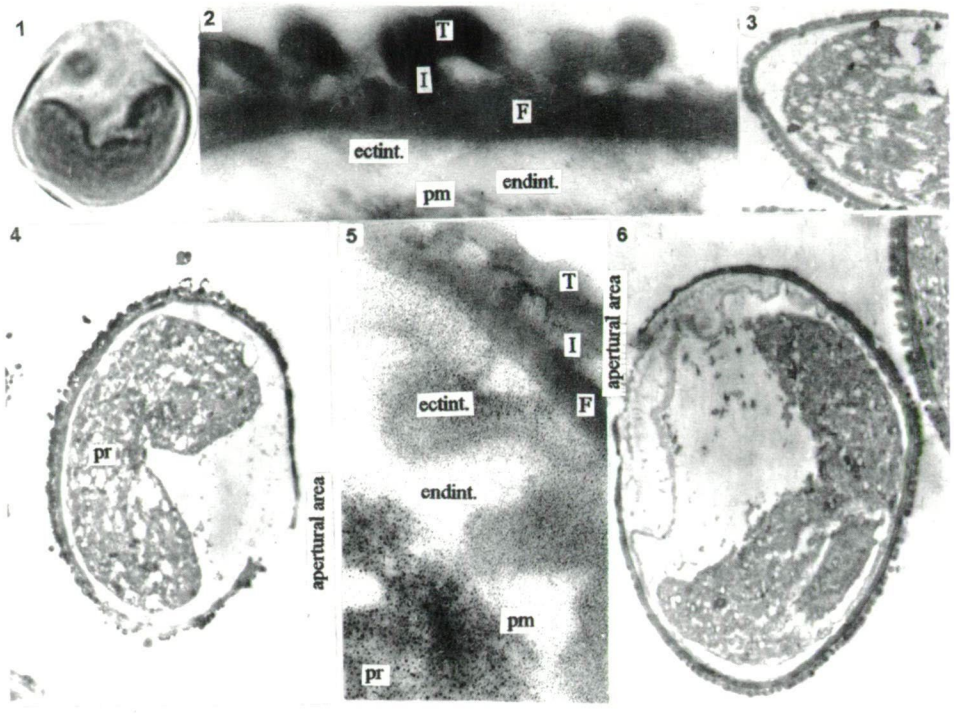
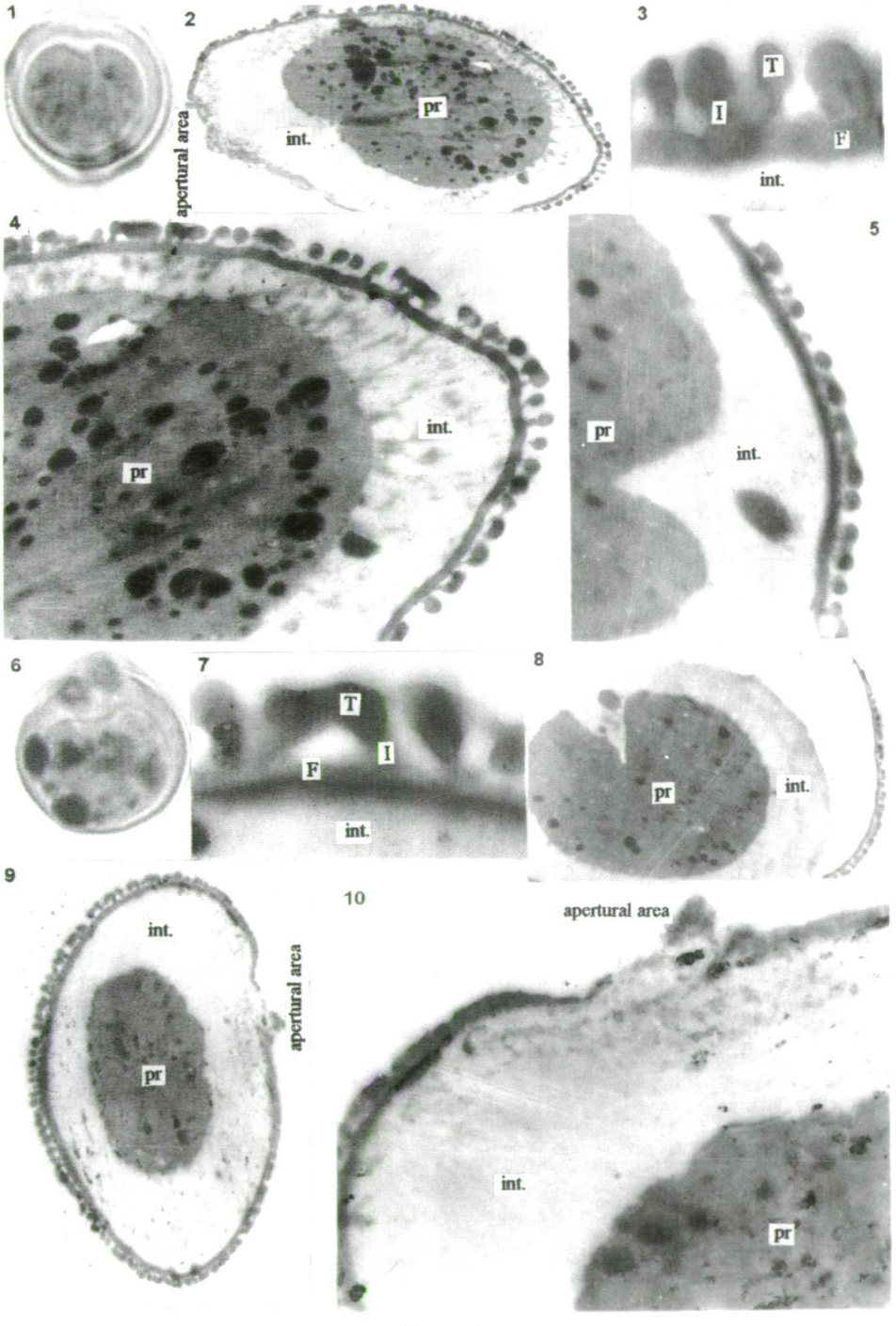


Plate 10.1

1-6. *Phoenix dactylifera* L., fresh pollen grains
1. LM picture, 1650x.
2-6. TEM pictures. 2. Detail from the inter-apertural exine. Negative No.: 8590, 33.035. 3, 4, 6. General survey pictures from the ultrastructure of the pollen grains. 3. Negative No.: 8587, 3.289x, 4. Negative No.: 8754, 3.890x, 6. Negative No.: 8763, 3.890x. 5. Detail from the fine structure of the exine. The ultrastructural characteristic features of the intine are well shown. Negative No.: 8499, 33.035x.
T = tectum, I = infratectum F = foot layer, ectint. = ectintine, endint. = endintine, pm = plasma membrane, pr = protoplasm, mb. = microbody.

Plate 10.2.

1-10. *Phoenix dactylifera* L.
1-5. Partially degraded pollen grains with 2-aminoethanol (24 hours)
1. LM picture. 1650x.
2-5. TEM pictures. 2. General survey picture of the pollen grain. Negative No.:8594, 3.289x, 3. Detail from the fine structure of the ectexine. Negative No.: 8507, 33.035x.. 4. Detail from the ultrastructure of the pollen grains. Negative No.: 8505, 9.910x. 5. Detail from the fine structure of the pollen grains. Negative No.: 8601, 9.910x.
6-10. Partially degraded pollen grains with 2-aminoethanol (48 hours)
6. LM picture 1650x.
7-10. TEM pictures. 7. Detail from the fine structure of the ectexine. Negative No.: 8516, 33.035x. 8,9. General survey pictures from the ultrastructure of the pollen grain. 8. Negative No.: 8602, 3.289x, 9. Negative No.: 8607, 3.289x. 10. Detail from the fine structure of the pollen grain in the apertural area. Negative No.: 8608, 9.910x.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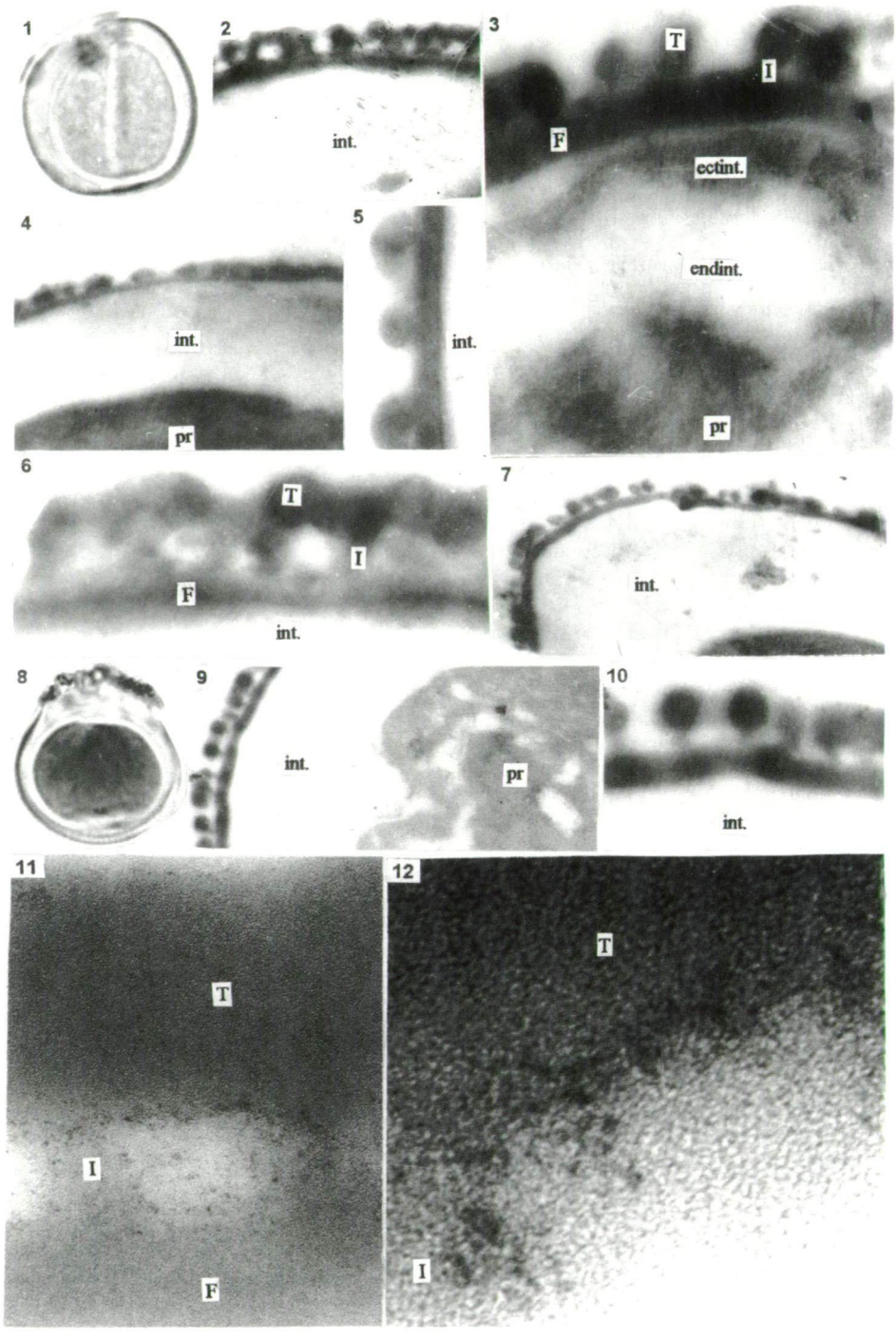
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Plate 10.3.

Plate 10.3.

1-12. *Phoenix dactylifera* L.
1-7. Partially degraded pollen grains with 2-aminoethanol (72 hours)
1. LM picture 1650x.
2-7. TEM pictures. 2-4. General survey picture from the exine ultrastructure of the pollen grain. 2. Negative No.: 8517, 9.910x. 3. Negative No.: 8523, 33.035x. 4. Negative No.: 8528, 9.910x. 5,6. Detail from the ultrastructure of the ectexine. 5. Negative No.: 8529, 33.035x. 6. Negative No.: 8531, 33.035x. 7. Detail from the fine structure of the pollen grain. Negative No.: 8515, 9.910x.
8-12. Partially degraded pollen grain with 2-aminoethanol (24 hours) and with $KMnO_4$ (24 hours)
8. LM picture 1650x.
9-12. TEM pictures. 9. Detail from the ultrastructure of the pollen grain. Negative No: 8578, 9.910x. 10. Ectexine ultrastructure. Negative No.: 8574, 33.035x. 11. Detail from the ectexine ultrastructure. The highly organized biopolymer units of the inner surface of the tectum and the infratectal layer are well shown. Negative No.: 10508, 66.714x. 12. Molecular system of the Inner surface of the tectum and the foot layer. Negative No.:10510, 820.000x.

2.2. Partial degradation for 48 hours (T-12-100). LM results (Plate 10.2., fig. 6) Characteristic electron dense granular elements are in the protoplasm, thinning of the ectexine is perceptible. TEM results (Plate 10.2., figs. 7-10) The ectexine degradation is well shown, the electron dense inner part of the foot layer is significant (Plate 10.2., fig. 7). Disintegration of the intine and the protoplasm are illustrated (Plate 10.2., figs. 8-10), but in the apertural area the outer part of the ultrastructural elements of the intine are resistant e.g.: Plate 10.2., fig. 10.

2.3. Partial degradation for 72 hours (T-12-101). LM results (Plate 10.3., fig. 1) The degradation of the pollen grains is perceptible. TEM results (Plate 10.3., figs. 2-7) The degradation of the ectexine is well shown, in particular in picture 6 of Plate 10.3. Different kinds of alterations in the intine ultrastructure were observed; 1. Electron dense characteristic ectintine and light endintine (Plate 10.3., fig. 3). 2. Not so well characteristic ectintine or more or less homogeneous intine (Plate 10.3., figs. 2,4,7). The plasma membrane seems to be damaged.

3. Partial degradation with 2-aminoethanol and $KMnO_4$

3.1. Partial degradation with 2-aminoethanol for 24 hours and with $KMnO_4$ for 24 hours (T-12-102). LM results (Plate 10.3., fig. 8) The wall of the apertural area and the inner body of the pollen grain is electron dense, the intine is light. TEM results (Plate 10.3., figs. 9-12) The intine is thickened and light, the electron dense elements are probably degraded (Plate 10.3., figs. 9,10). The pictures taken with a high resolution instrument illustrate well the molecular system of the ectexine at different organizational level (Plate 10.3., figs. 11,12). The globular biopolymer units at the infratectal layer and the inner surface of the tectum are well shown.

3.2. Partial degradation with 2-aminoethanol for 48 hours and with $KMnO_4$ for 24 hours (T-12-103). LM results (Plate 10.4., fig. 1) The wall in the apertural area and the inner body of the pollen grain is electron dense, similar to the previous experiment. The size is a little larger than previous. TEM results (Plate 10.4., figs. 2-6) The ultrastructure of the empty pollen grain was also observed (Plate 10.4., fig. 3). In the general survey picture, the light intine and the electron dense granules are well shown in the protoplasm (Plate 10.4., fig. 2). Pictures taken on a high resolution instrument (Plate 10.4., figs. 4-6) illustrate a peculiar process of partial degradation of the ectexine. The discovered biopolymer structures of the infratectal layer and the outer and inner surfaces of the foot layer

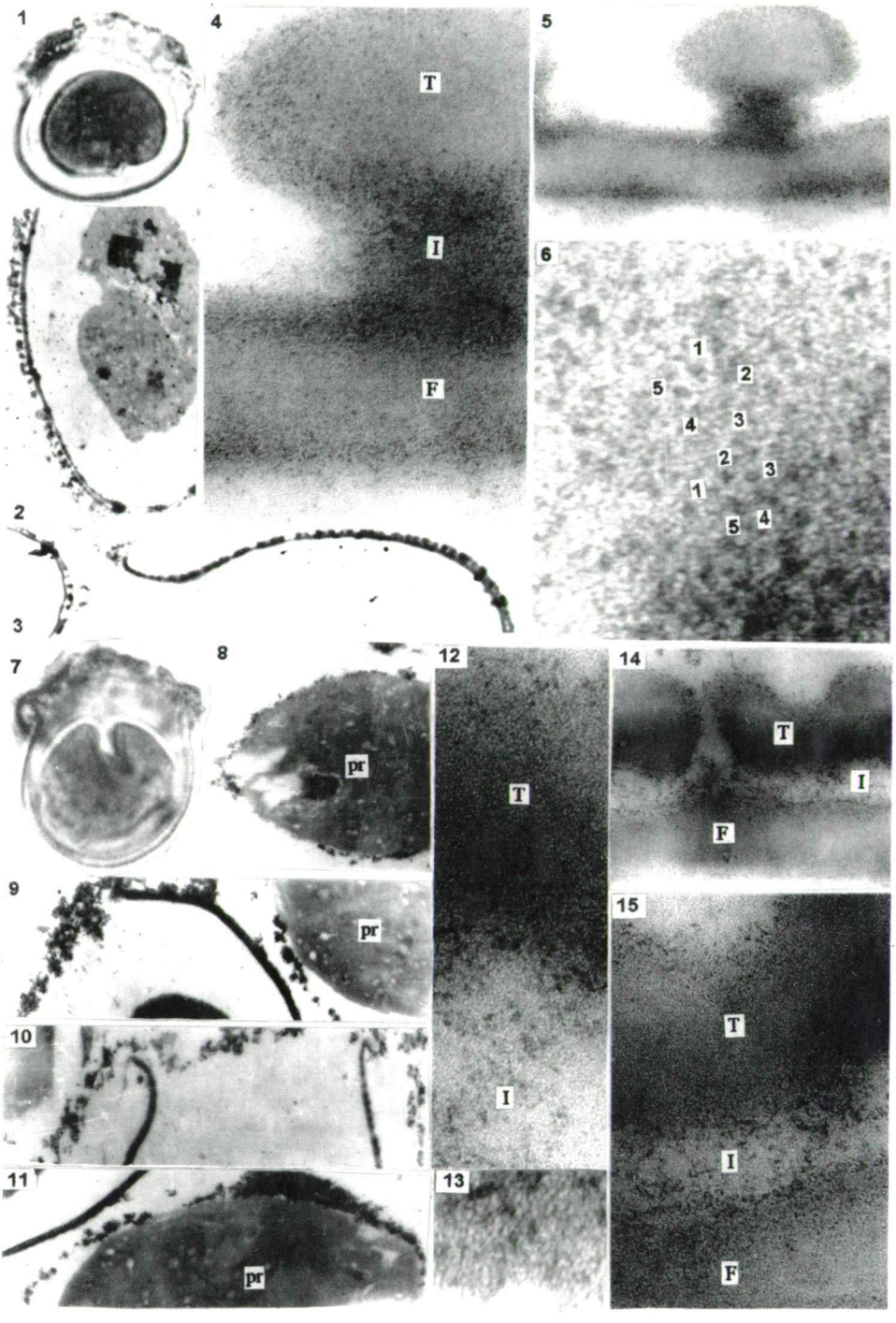

Plate 10.4.

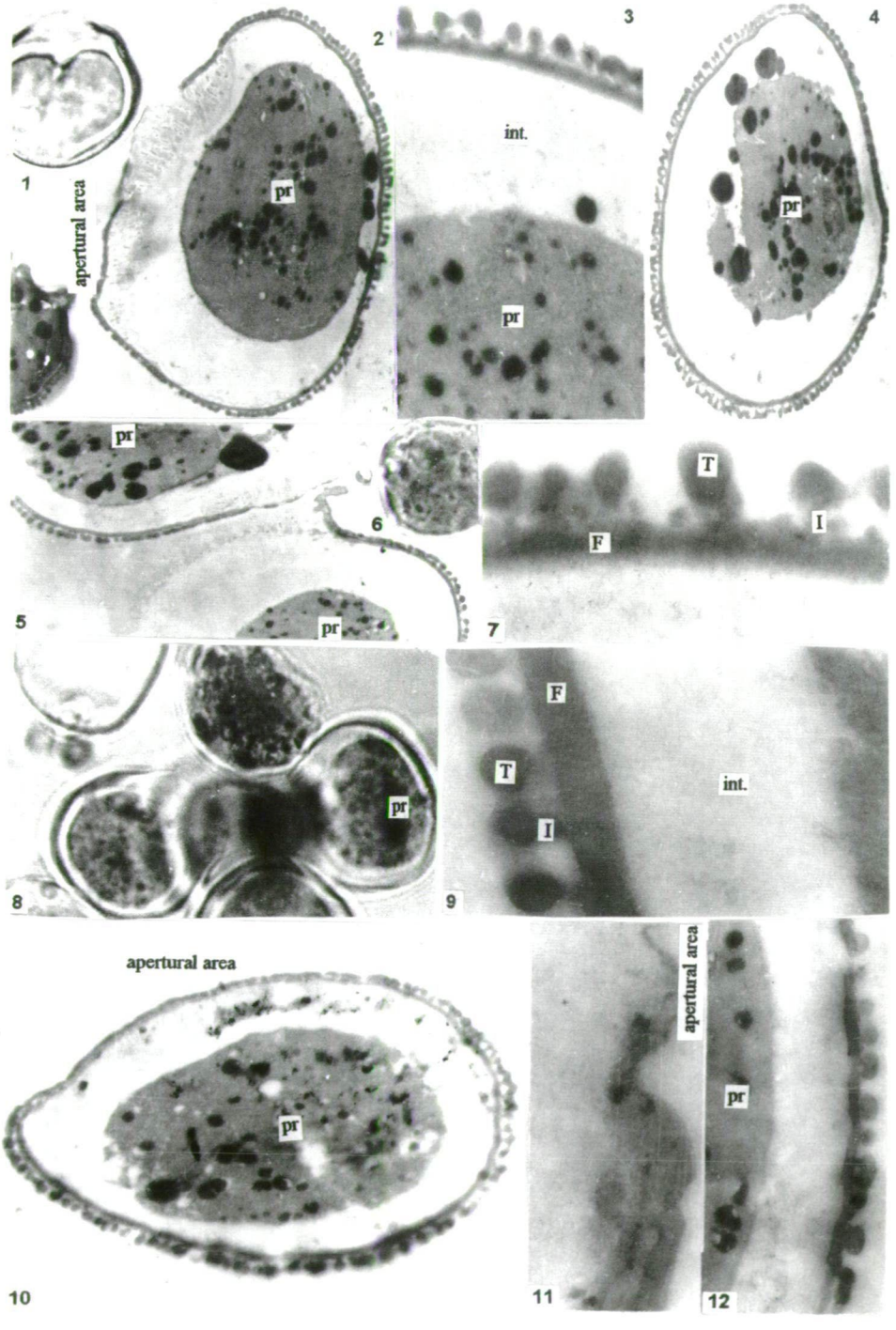
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Plate 10.5.

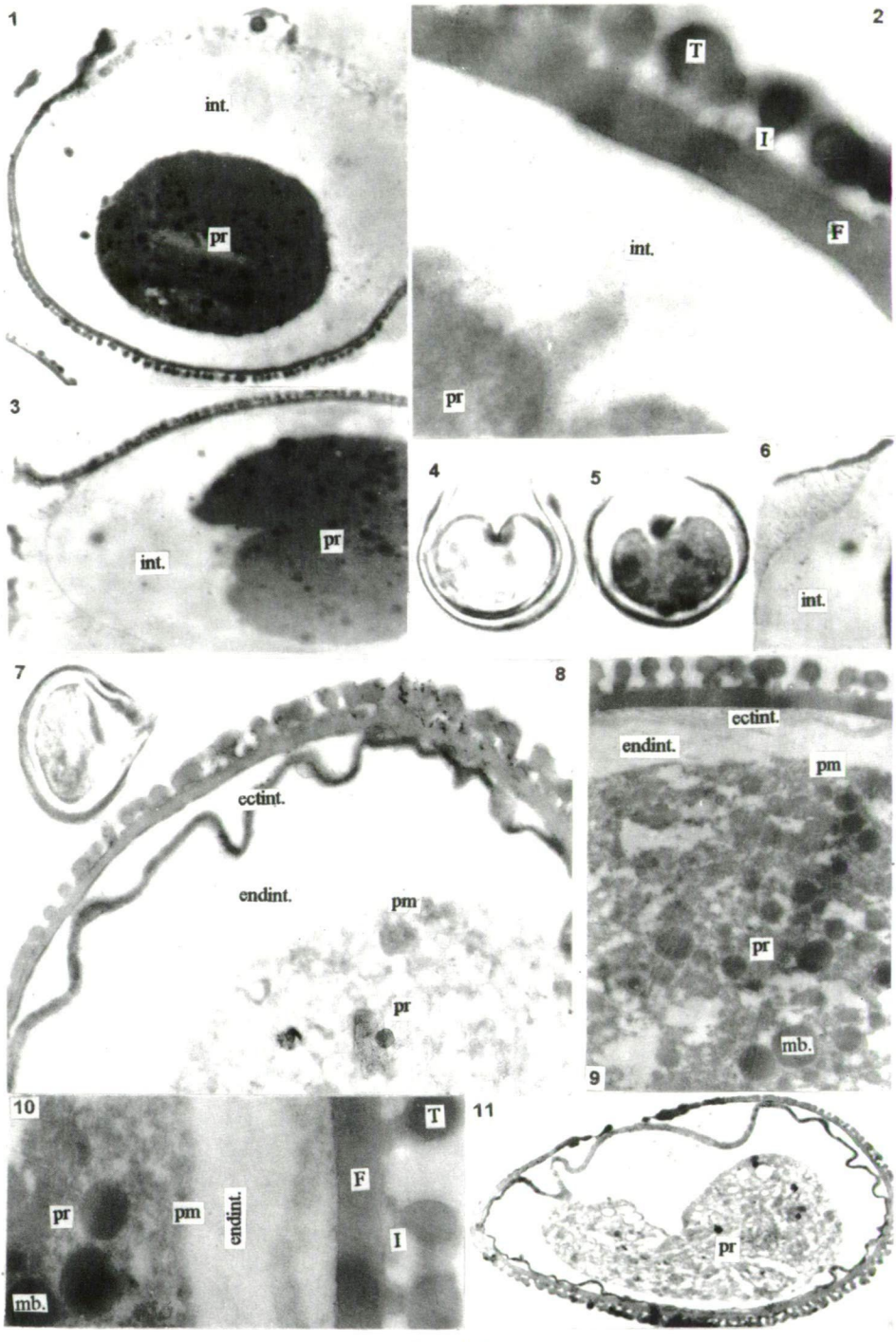
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Plate 10.6.

Plate 10.4.

1-10 *Phoenix dactylifera* L.
1-6. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with $KMnO_4$ (24 hours)
1. LM picture, 1650x.
2-6. TEM pictures. 2. General survey from tthe pollen grains with protoplasm. Negative No.: 8617, 3289x. 3. Detail from the empty pollen grain. Negative No.: 8617, 3289x. 4,5. Detail from the partially degraded ectexine. 4. Negative No.: 10528, 123.000x. 5. Negative No.: 10507, 66.714x. 6. Biopolymer system of the infratectal layer. Negative No.: 10529, 820.000x.
7-15. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with $KMnO_4$ (24 hours)
7. LM picture, 1650x.
8-15. TEM pictures. 8. Detail from the protoplasm of the pollen grain without exine. Negative No.: 8585, 3.289x. 9-11. Detail from the general survey picture of the partially degraded pollen grains. 9. Negative No.: 8581, 3.289x. 10. Negative No. 8583, 3.289x. 11. Negative No.: 8582, 3.289x. 12-15. Detail from the partially degraded ectexine. 12,13. Negative No.:10514, 820.000x. 14. Negative No.: 10513, 33.035x. 15. Negative No.: 10512, 123.100x.

Plate 10.5.

1-12. *Phoenix dactylifera* L.
1-7. Partially degraded pollen grains with 2-aminoethanol (24 hours) and with merkaptoethanol (24 hours)
1,6. LM picture, 1650x.
2-7. TEM pictures. 2,4,5. General survey picture from the partially degraded pollen grain. 2. Negative No.: 8559, 3.289x, 4. Negative No.: 8557, 3.289x. 3. Detail from the ultrastructure of the pollen grain. Negative No.: 8556, 9.910x. 5. Negative No.: 8555, 3.289x. 7. Negaive No.: 8558, 33.035x.
8-12. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with merkaptoethanol (24 hours)
8. LM picture. 1650x.
9-12. TEM pictures. 9-12. Detail from the ultrastructure of the partially degraded pollen grain. 9. Negative No.: 8566, 33.035x, 11. 8569, 33.035x, 12. 8622, 9.910x. 10 General survey picture from the ultrastructure of the pollen grain. Negative No.: 8620, 4780x.

Plate 10.6.

1-11. *Phoenix dactylifera* L.
1-6. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with merkaptoethanol (24 hours)
1-3, 6. TEM pictures. 1,3. General survey pictures from the ultrastructure of the pollen grain. 1. Negative No.: 8568, 3.289x, 3. Negative No.: 8569, 3.289x. 2. Detail from the ultrastructure of the inter-apertural exine. Negative No.: 8629, 33.035x. 6. Detail from the ultrastructure in the apertural area. Negative No.: 8570, 3.289x.
4,5. LM pictures, 1650x.
7-11. Partially dissolved pollen grains with glycerine (50%) for 30 days
7. LM picture. 1650x.
8-11. TEM pictures. 8-10. Details from the ultrastructure of the partially dissolved pollen grain. 8. Negative No.: 8945, 9.910x. 9. Negative No.: 8648, 9.910x. 10. Negative No.: 8649, 33.035x. 11. General survey picture from the ultrastructure of the pollen grain. Negative No.: 8744, 3.289x.

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are well shown. Globular biopolymer units, arranged in regular pentagons, were also observed (Plate 10.4., fig. 6). These pentagons are relatively small, 11-13 Å in diameter.

3.3. Partial degradation with 2-aminoethanol for 72 hours and with $KMnO_4$ for 24 hours (T-12-104). LM results are similar to the previous one (Plate 10.4., fig. 7). TEM results (Plate 10.4., figs. 8-15) The general survey pictures (Plate 10.4., figs. 8-11) illustrate the strong degradation. Pollen grain without ectexine (Plate 10.4., fig. 8), empty pollen grain with degraded ectexine (Plate 10.4., fig. 10) and pollen grains with degraded ectexine (Plate 10.4., figs. 9,11) were observed. In highly magnified pictures

(Plate 10.4., figs. 12-15), the advanced degradation of the tectum is well shown by the globular biopolymer structures.

4. Partial degradation with 2-aminoethanol and with merkaptoethanol

4.1. Partial degradation with 2-aminoethanol for 24 hours and with merkaptoethanol for 24 hours (T-12-105). LM results (Plate 10.5., fig. 1,6) In contrast to the previous series of experiments the inner body is light. In the apertural area, the outer part of the intine is more electron dense than the inner one. TEM results (Plate 10.5., figs. 2-7) In the general survey pictures (Plate 10.5., figs. 2-5) the following may be pointed out: 1. There are dark globular units (microbodies) in the protoplasm and sometimes in the inner part of the intine (Plate 10.5., figs. 2-4). 2. The electron dense part of the outer part of the intine is also well shown in these pictures (Plate 10.5., fig. 2). Degradation of the ectexine was also observed (Plate 10.5., fig. 7).

4.2. Partial degradation with 2-aminoethanol for 48 hours and with merkaptoethanol for 24 hours. (T-12-106). LM results (Plate 10.5., fig. 8): The dark granules in the protoplasm are well shown in this picture. TEM results (Plate 10.5., figs. 9-12). These results are essentially identical with the previous experiments.

4.3. Partial degradation with 2-aminoethanol for 72 hours and with merkaptoethanol for 24 hours (T-12-107). LM results (Plate 10.6., figs. 4,5): The dark granular units in the protoplasm are well shown after staining (Plate 10.6., fig. 5). TEM results (Plate 10.6., figs. 1-3, 6) The outer part of the intine in the apertural area is less electron dense (Plate 10.6., fig. 1). In several places, there is an outer thin layer ot the intine in the apertural region (Plate 10.6., fig. 6). The microbodies are present in the intine and in the protoplasm.

5. Partial dissolution with diluted glycerine (50%) for 30 days (T-12-108)

LM results (Plate 10.6., fig. 7) The pollen grains after this experiment were not completely opened as with the previous ones. TEM results (Plate 10.6., figs. 8-11) The general survey pictures illustrate interesting alterations in the electron density and the swelling of the ectintine (Plate 10.6, figs. 8,11). The protoplasm, including the plasma membrane, is well preserved (Plate 10.6., figs. 9,10). Plasma membrane, microbodies and sometimes mitochondria were observed.

Discussion and Conclusions

1. Concerning the LM data, we can point out that, the peculiar swelling and exudation of the protoplasm and intine, which was observed previously (KEDVES, PRISKIN, TRIPATHI and MADHAV KUMAR, 2002) on Indian palm pollen grains, was not observed in the present investigations.

2. The discovery of the biopolymer structure of the ectexine is different from the pollen grains of *Phoenix sylvestris*, but the biopolymer system of both *Phoenix* species investigated up till now, is particular. The regular pentagons in *Phoenix sylvestris* are unusually large, those of *Phoenix dactylifera* are small.

3. After dissolution with diluted glycerine, the protoplasmic organelles are relatively well preserved, similar to our first observations on the pollen grains of *Platanus hybrida* BROT. (KEDVES, PÁRDUTZ and TÓTH, 1999).

Finally, further experimental investigations will be carried out, we hope that the partial degradation using the C60 fullerene/benzol solution will bring interesting new data concerning biopolymer organization of the ectexine.

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