# 9. LM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED POLLEN GRAINS OF TRITICUM AESTIVUM L.

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## Abstract

Pollen grains of *Triticum aestivum* L. were partially degraded with four series of experiments: 1. Degradation with 2-aminoethanol for 24, 48 and 72 hours. 2. After the treatment mentioned previously, $KMnO_4$ (1%) was added for 24 hours. 3. In this experiment merkaptoethanol was added to the residous after degradation with 2-aminoethanol. 4.Pollen grains partially dissolved with glycerine aq. dil. (50%). LM and TEM methods were used.

*Key words:* Experimental Palynology, *Triticum aestivum*, LM, TEM.

## Introduction

*Triticum* is an extremely important genus from several points of view. According to the "Index bibliographique" of THANIKAIMONI (1972, 1973) pollen grains of this genus were first investigated by MOHL (1835) and EDGEWORTH (1877). Important findings were published by ERDTMAN (1944, 1956), using the LM method. Phase contrast microscopy was applied by several researchers e.g. ERDTMAN and PRAGLOWSKI (1959), ERDTMAN, BERGLUND and PRAGLOWSKI (1961). FAEGRI and IVERSEN (1964) emphasized the importance of the phase contrast characteristics of the pollen grains in the differentiation of various cereals and wild grasses. SORSA (1968) used the interference contrast method for the pollen grains of *Triticum aestivum*. The organization and the polarity of the pollen mother cells were studied by DOVER (1972).

TEM studies. - The ultrastructural characteristic features of the pollen development of *Triticum aestivum* was investigated by SHIH-YI, MO-SHAN and LI-YUN (1977). The correlations between the development of the aperture and the interapertural part of the pollen grains were investigated by EL-GHAZALY and JENSEN (1985, 1986a,b). Comparison of exine development in normal and gametocide treated plants were investigated with the TEM method by EL-GHAZALY (1990). Cytological and genetical aspects in anther culture were investigated by SZAKÁCS (1992).

ANDERSEN and BERTELSEN (1972) used the scanning electron microscope for the pollen grains of cereals and other grasses. In this paper the importance of the identification of the fossil pollen of cereals in studies of postglacial vegetational history was emphasized. KÖHLER and LANGE (1979) published further data in this respect. The SEM method was used for selected Triticinae and intergeneric hybrids by RAJENDRA, TOMB,

MUJEEB and BATES (1978), and the usefulness of micromorphological pollen characteristics as genetic markers was established.

Taking into consideration the allergenic characters of the grass pollen grains, including the cereals (e.g.: RICHARD et al., 1986, DE LEONARDIS et al., 1986, NILSSON, PRAGLOWSKI and NILSSON, 1977, LEBBE et al., 1988, etc.), we also included the pollen grains of *Triticum aestivum* also within the experimental research program of our laboratory on allergenic pollen grains.

The aim of this paper is to establish the pollen morphological and ultrastructural alterations as a consequence of the experimental influences, in comparison to the previous results in this subject.

## Materials and Methods

The investigation material (*Triticum aestivum* L. cv. GK-Kalász) was collected by Dr. A. PALÁGYI and Mrs. B. VARGA on the 18th May 2001 Locality: Ságvári Experimental Research Station of the Cereal Research Institute, Szeged.

Fresh grains, (T-12-230) unstained (A) and stained with Methylviolet (B) were investigated. The experiments were as follows:

1. Treatment with 2-aminoethanol during 24, 48 and 72 hours, experiment numbers T-12-231, 232, 233.
2. Treatment with 2-aminoethanol as previously, but after this $KMnO_4$ (1%) was added for 24 hours.
3. Treatment with 2-aminoethanol as previously (1), after this 2 ml merkaptoethanol was added for the exinous remnants for 24 hours.
4. Partial dissolution with glycerine aq. dil. (50%) for 30 days. The pollen grains were mounted in glycerine-jelly, hydrated at 39.6%, and/or in Araldite. Embedding for TEM studies in Araldite after postfixation with $OsO_4$ aq. dil.(1%) The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome in the Cell Biological and Evolutionary Micropaleontological Laboratory. The pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences on a Tesla BS 540 instrument. All pictures are unretouched..

## Results

Percentages of the diameter of the pollen grains investigated.

| µm | 45.0 | 47.5 | 50.0 | 52.5 | 55.0 | 57.5 | 60.0 | 62.5 | 65.0 | 67.5 | 70.0 | 72.5 | 75.0 | 77.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T-12-230A | | | | | | 2.5 | 18.0 | 28.5 | 22.0 | 16.0 | 10.0 | 3.0 | | |
| T-12-230B | | | 12.0 | 27.5 | 25.5 | 20.0 | 12.5 | 2.5 | | | | | | |
| T-12-231A | | | 1.0 | 4.0 | 10.5 | 28.5 | 22.0 | 22.0 | 10.0 | 1.5 | | 0.5 | | |
| T-12-231B | | 0.5 | 3.0 | 8.5 | 13.0 | 31.0 | 24.5 | 16.5 | 3.0 | | | | | |
| T-12-231Ar | | | | | 2.5 | 12.0 | 22.0 | 33.5 | 21.5 | 6.5 | 2.0 | | | |
| T-12-232A | | | 1.0 | 1.5 | 7.0 | 13.0 | 17.0 | 32.5 | 15.0 | 11.5 | 1.5 | | | |
| T-12-232B | | | 0.5 | 2.0 | 6.5 | 15.0 | 26.0 | 25.0 | 12.5 | 9.0 | 3.5 | | | |
| T-12-232Ar | | | | | 3.0 | 6.5 | 18.0 | 24.0 | 23.0 | 15.5 | 6.5 | 3.5 | | |
| T-12-233A | | 1.0 | 3.0 | 3.5 | 7.5 | 21.0 | 24.5 | 26.5 | 10.0 | 2.5 | | 0.5 | | |
| T-12-233B | | 1.5 | 12.5 | 19.0 | 21.5 | 21.5 | 13.5 | 10.0 | 0.5 | | | | | |
| T-12-233Ar | | | | 2.5 | 10.5 | 19.5 | 21.5 | 27.0 | 12.5 | 6.5 | | | | |
| T-12-234A | | | 4.5 | 4.5 | 15.5 | 20.0 | 13.0 | 19.0 | 6.0 | 9.0 | 5.0 | 1.5 | 2.0 | |
| T-12-234Ar | | | | | | | 10,0 | 14.0 | 15.5 | 23.0 | 18.5 | 11.0 | 6.0 | 2.0 |
| T-12-235A | | 1.0 | 6.5 | 13.0 | 29.0 | 27.0 | 12.5 | 9.5 | 1.0 | 0.5 | | | | |
| T-12-235Ar | | | | | 4.0 | 13.5 | 21.5 | 19.0 | 21.0 | 14.0 | 5.0 | 1.5 | 0.5 | |
| T-12-236A | | 2.5 | 13.5 | 20.0 | 23.5 | 20.5 | 9.0 | 4.5 | 0.5 | 3.0 | 0.5 | 2.0 | 0.5 | |
| T-12-236Ar | | | | | | 6.0 | 25.0 | 25.0 | 20.0 | 10.0 | 11.5 | 2.5 | | |
| T-12-237A | | 1.5 | 3.5 | 7.0 | 21.5 | 23.0 | 23.0 | 15.0 | 5.0 | 0.5 | | | | |
| T-12-237B | | | | | 7.5 | 14.5 | 23.5 | 27.0 | 14.0 | 7.0 | 2.5 | 3.0 | 1.0 | |

| µm | 45.0 | 47.5 | 50.0 | 52.5 | 55.0 | 57.5 | 60.0 | 62.5 | 65.0 | 67.5 | 70.0 | 72.5 | 75.0 | 77.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T-12-237Ar | 0.5 | | 1.0 | 6.0 | 14.5 | 24.0 | 30.0 | 13.5 | 8.0 | 2.5 | | | | |
| T-12-238A | | 2.0 | 4.5 | 16.0 | 14.0 | 28.5 | 18.5 | 12.5 | 4.0 | | | | | |
| T-12-238B | | | | 2.5 | 15.5 | 26.5 | 28.0 | 25.5 | 2.0 | | | | | |
| T-12-238Ar | | | | | 12.0 | 12.5 | 23.5 | 27.0 | 15.5 | 9.5 | | | | |
| T-12-239A | | | 0.5 | 3.0 | 7.5 | 19.0 | 27.0 | 35.5 | 7.5 | | | | | |
| T-12-239B | | 0.5 | | 0.5 | 2.5 | 4.5 | 10.5 | 16.0 | 16.0 | 21.0 | 16.5 | 7.5 | 4.5 | |
| T-12-239Ar | | | | 0.5 | 3.0 | 9.5 | 19.0 | 32.0 | 20.5 | 12.5 | 3.0 | | | |
| T-12-240A | | | 5.0 | 11.5 | 19.5 | 25.0 | 24.0 | 14.5 | 0.5 | | | | | |
| T-12-240B | 0.5 | 0.5 | 2.0 | 11.0 | 26.0 | 29.5 | 23.0 | 7.0 | 0.5 | | | | | |
| T-12-240Ar | 1.5 | 6.0 | 20.0 | 33.5 | 22.0 | 12.0 | 3.0 | 1.5 | 0.5 | | | | | |

Table 9.1.

1. Fresh, untreated pollen grains (Plate 9.1., figs. 1-4, table 9.1)

Typically monoporate pollen grains. Diameter of the non-stained pollen grains from 57.5-72.5 µm, maximum (28.5%) at 62.5 µm. The stain changed the size of the pollen grains: diameter from 50,0 - 62.5 µm, maximum (27.5%) at 52.5 µm.

2. Partially degraded pollen grains with 2-aminoethanol (Plate 9.1., figs. 5-15, plate 9.2., figs. 1-6)

2.1. Experiment No.: T-12-231, length of time: 24 hours (Plate 9.1., figs. 5-10)

LM results (Plate 9.1., figs. 5-8, table 9.1.). The basic morphology of the pollen grains has not changed, only the protrusions are characteristic. The variation in the diameter of the non-stained and the stained pollen grains is nearly identical, but the embedding effect increased the size of the pollen grains.

TEM results.(Plate 9.1., figs. 9,10) The inter-apertural exine (Plate 9.1., fig. 9) is degraded. No orbiculi were observed on the surface of the tectum. Channels of the tectum are not so characteristic. Secondary thinning of the elements of the infratectal layer are perceptible. Well shown is the destruction of the outer part of the intine. In the apertural area (Plate 9.1., fig. 10), the elements of the annular system are more or less homogenized. Radially oriented light holes were present, which may indicated the presence of helical biopolymer systems.

2.2. Experiment No.: T-12-232, length of time 48 hours. (Plate 9.1., figs. 11-15, Plate 9.2., fig.1)

LM results (Plate 9.1., figs. 11-13, table 9.1.) No qualitative deformations were observed in the partially degraded pollen grains. In consequence of the stain, the diameter diminishes, but the size of the embedded pollen grains is nearly identical with the non-stained ones.

TEM results (Plate 9.1., figs. 14,15, plate 9.2., fig. 1) The degradation of the infratectal layer is characteristic in the inter-apertural ectexine (Plate 9.1., fig. 15). In the general survey picture (Plate 9.1, fig. 14) of the exine, remnants of the intine are illustrated. This layer was secondarily thickened and a degraded electron dense layer is located within the more or less homogeneous inner layer. The fine structure in the apertural area is more resistant than in the inter-apertural area (Plate 9.2., fig.1). The ectexinous elements of the operculi are well shown, as electron dense particles on a thin lamellar layer. The endannulus is homogeneous, the thickened ectintine is granular and a little less electron dense than the foot layer. Near the apertural area, the ectintine is well preserved and the inner border is wavy. The endintine is probably degraded. The ultrastructure of the protrusion is lamellar or irregular and, the elements are more or less oriented in the direction of the pore.

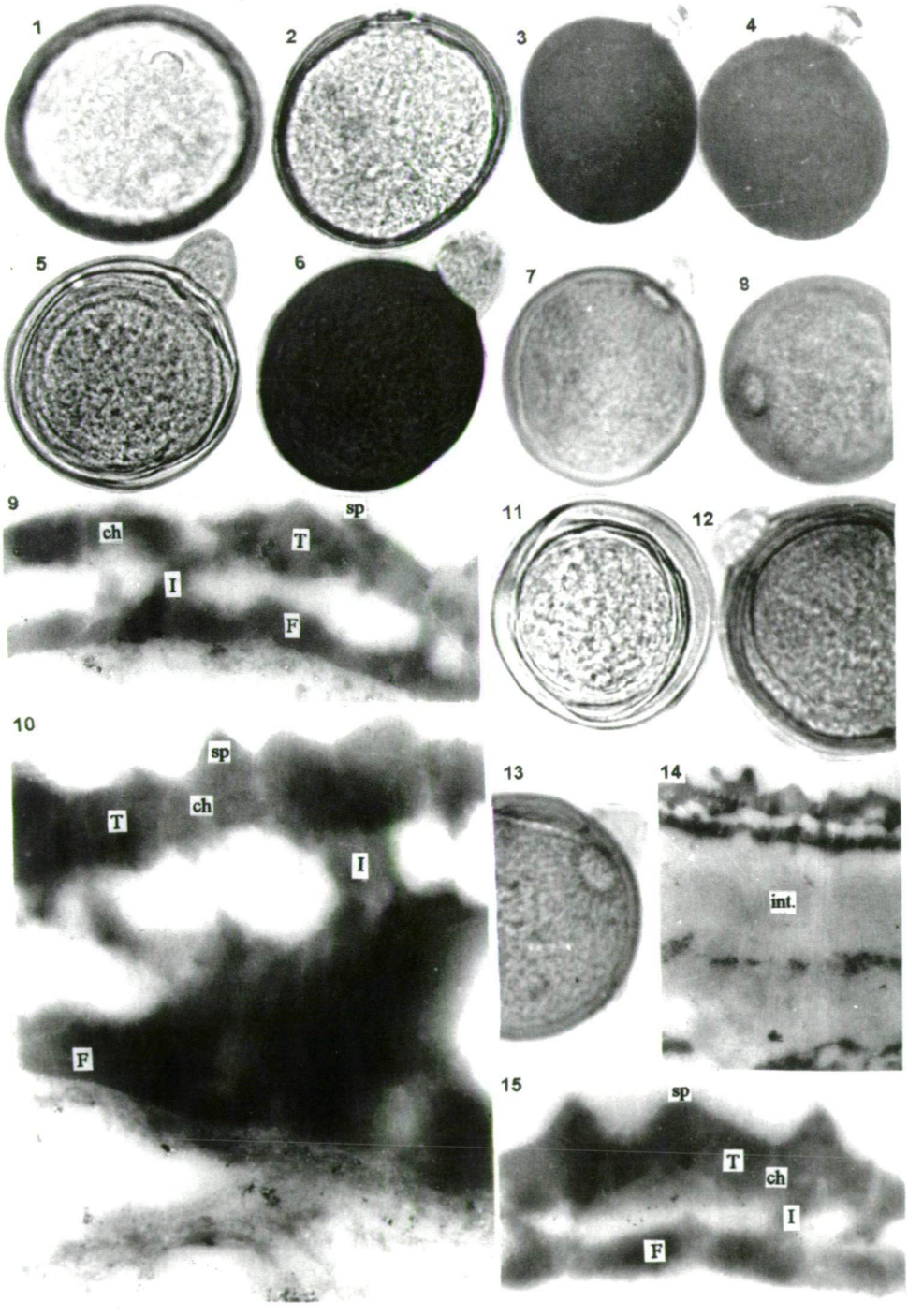


Plate 9.1.

Plate 9.1.

1-15. *Triticum aestivum* L.
1-4. LM pictures. Fresh pollen grains mounted in glycerine-jelly. 1,2. Unstained. 3,4. Stained pollen grains with Methylviolet., 660x.
5-10. Partially degraded pollen grains with 2-aminoethanol (24 hours)
5-8. LM pictures. 5. Unstained. 6. Stained pollen grains with Methylviolet. 7,8. Pollen grains after experimental processes, mounted in Araldite, 660x..
9,10. TEM pictures. 9. Detail from the inter-apertural ectexine. Negative No.: 9017, 33.035x. 10. Exine ultrastructure in the apertural area. Negative No.: 8017, 33.045x.
11-15. Partially degraded pollen grains with 2-aminoethanol (48 hours)
11-13. LM pictures. 11. Unstained. 12. Stained pollen grain with Methylviolet. 13. Pollen grain after experimental processes mounted in Araldite, 660x.
14,15. TEM pictures. 14. Detail from the inter-apertural exine. Negative No.: 9021, 9.910x. 15. Detal from the ectexine ultrastructure. Negative No.: 9023, 33.030x.
T = tectum, I = infratectum, F = foot layer, sp. = spinae, ch. = channel, int. = intine, pr. = protoplasm,

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2.3. Experiment No.: T-12-233, length of time: 72 hours (Plate 9.2., figs. 2-6)
LM results (Plate 9.2., figs. 2-4, table 9.1.) The deformations of the pollen grains started in this experiment. Characteristic wrinkled ectexine appeared in the stained pollen grains. The embedding effect swelled the intine and pro parte the protoplasm. The trend of the alterations of the diameter of the pollen grains is the same as previously.

TEM results (Plate 9.2., figs. 5,6) In the general survey picture of the inter-apertural area, the thickened intine is well illustrated (Plate 9.2., fig. 6). The electron dense ultrastructure of the ectintine is in all probability degraded. Within the more or less homogeneous intine, electron dense granular elements are perceptible, some of them are arranged in a layer. Remnants of the protoplasm are characteristic. In the apertural area (Plate 9.2., fig. 5) the elements of the protrusion are perceptible, but not in the previous preservations. The endannulus and the ectintine are degraded, granular remnants are perceptible. The protoplasmic lamellar elements are also degraded and some electron dense elements are present only.

3. Partial degradation with 2-aminoethanol and $KMnO_4$ aq. dil. (Plate 9.3., figs. 1-12)

3.1. Experiment No.: T-12-234, partial degradation with 2-aminoethanol, and $KMnO_4$ for 24 hours (Plate 9.3., figs. 1-4)

LM results (Plate 9.3., figs. 1,2, table 9.1.) Deformed and wrinkled ectexine was observed in the pollen grains mounted in glycerine-jelly. The "inner body" swelled during the embedding processes. Regarding the diameter of the pollen grains, secondarily larger forms were observed. The maximum is characteristically larger as previous.

TEM results (Plate 9.3., figs. 3,4) The ectexine, in particular the infratectal layer is degraded (Plate 9.3., fig. 4). The ornamental elements of the tectum are characteristic and of different size. In the general survey picture, the degradation of the infratectal layer is well illustrated (Plate 9.3., fig. 3). The remnants of the protoplasm are connected to the foot layer, the elements of the intine are not perceptible.

3.2. Experiment No.: T-12-235, partial degradation with 2-aminoethanol (48 hours) and with $KMnO_4$ (for 24 hours) (Plate 9.3., figs. 5-8)

LM results (Plate 9.3., figs. 5,6, table 9.1) The qualitative alterations are as previously. The trends of the quantitative data of the diameter of the pollen grains are identical with the previous experiment but the values are lesser.

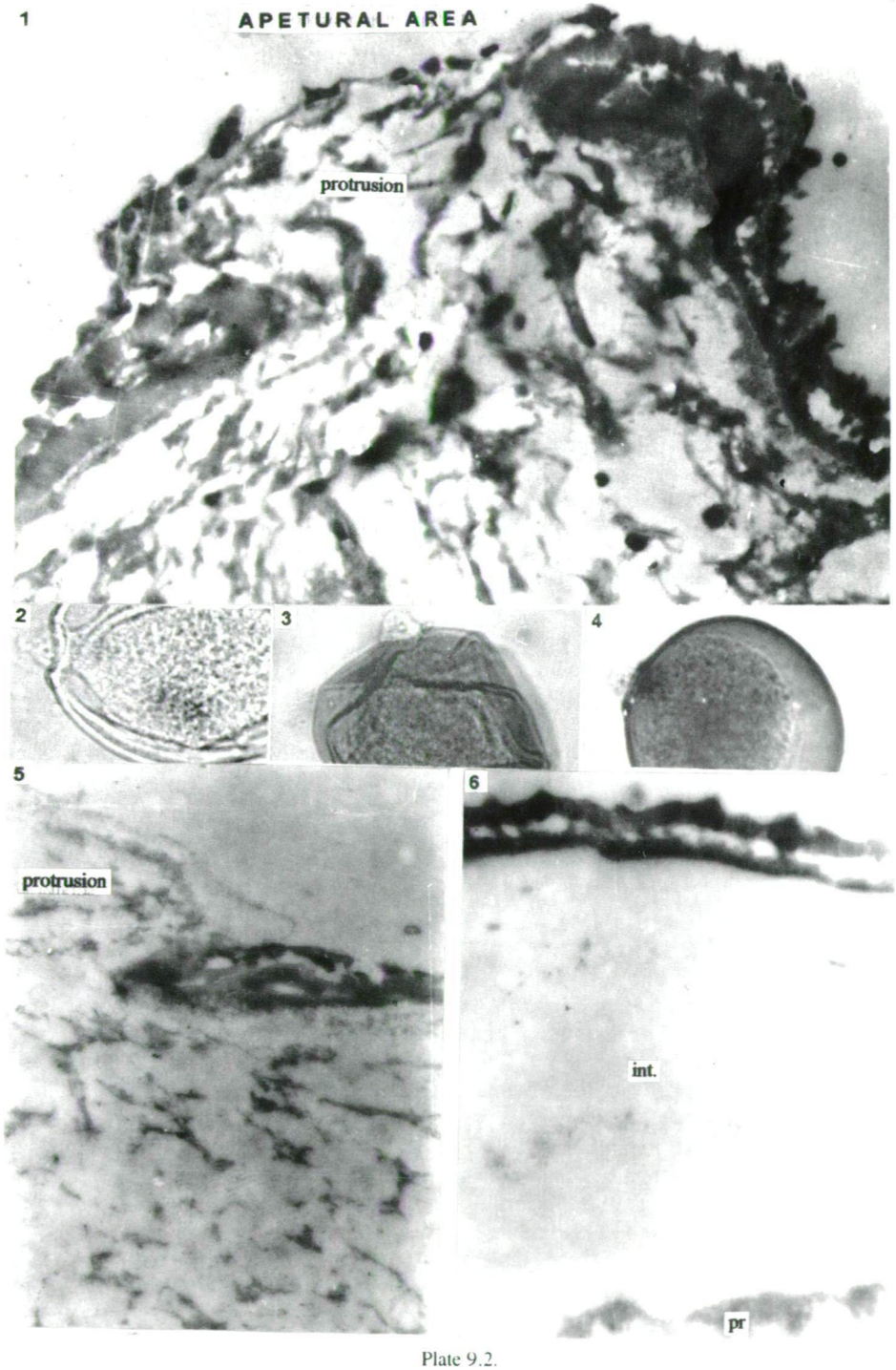


Plate 9.2.

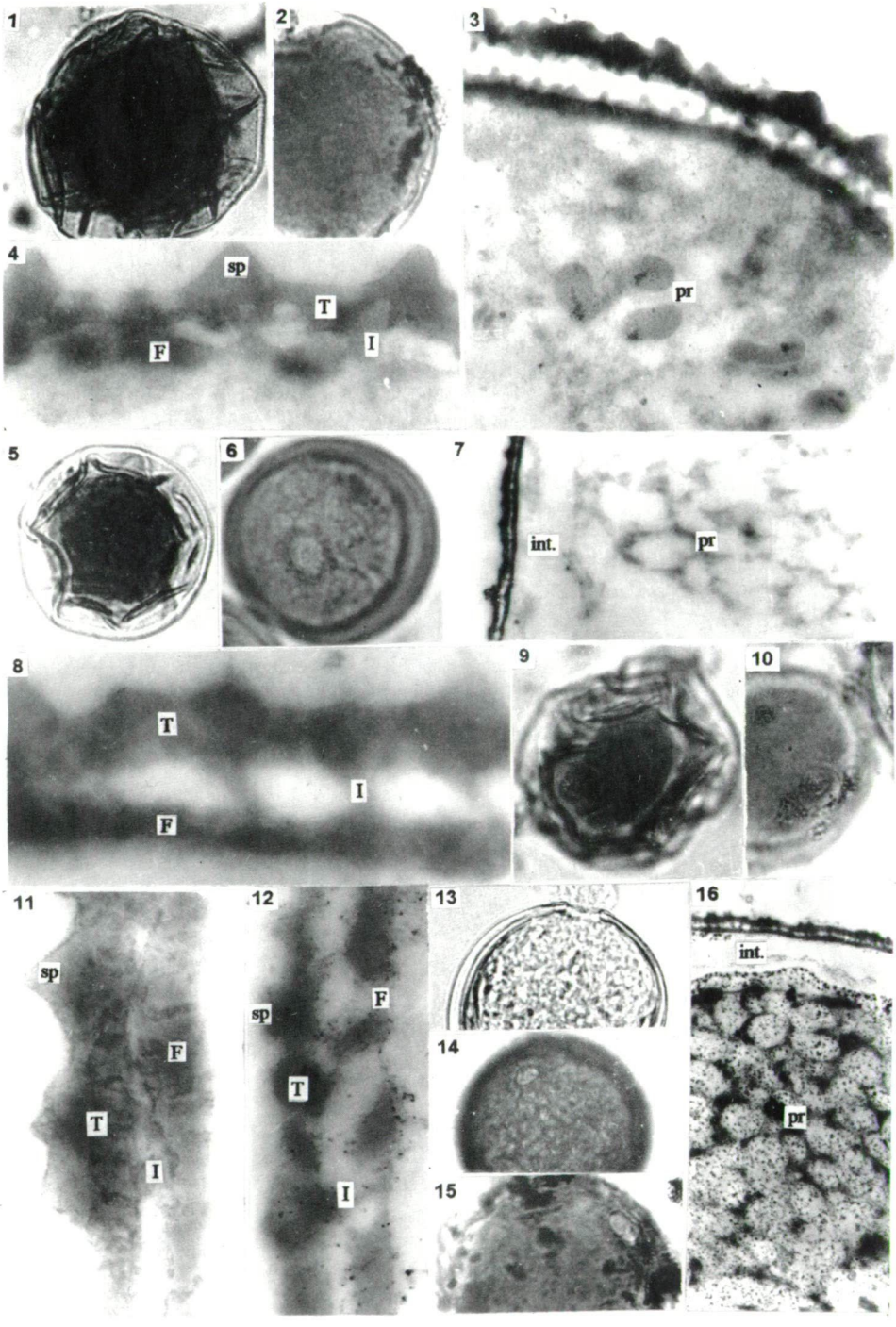
1
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Plate 9.3.

Plate 9.2.

1-6. *Triticum aestivum* L.
1. Partially degraded pollen grain with 2-aminoethanol (48 hours), ultrastructure of the apertural area. Negative No.: 9028, 9.910x.
2-6. Partially degraded pollen grains with 2-aminoethanol (72 hours)
2-4. LM pictures. 2. Unstained. 3. Stained pollen grain with Methylviolet. 4. Pollen grain after experiment processes mounted in Araldite, 660x.
5,6. TEM pictures. 5. Ultrastructure of the apertural area. Negative No.: 8997, 9.910x 6. Fine structure of the inter-apertural area. Negative No. 9000, 9.910x.

Plate 9.3.

1-16. *Triticum aestivum* L.
1-4. Partially degraded pollen grains with 2-aminoethanol (24 hours) and $KMnO_4$ (24 hours)
1,2. LM pictures. 1. Pollen grain mounted in glycerine-jelly after treatment. 2. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
3,4. TEM pictures. 3. Detail from the ultrastructure of the pollen grain. Negative No.:9008, 9.910x. 4. Detail from the partially degraded ectexine. Negative No.: 9007, 33.035x.
5-8. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with $KMnO_4$ (24 hours)
5,6. LM pictures. 5. Pollen grain mounted in glycerine-jelly after treatment. 6. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
7,8. TEM pictures. 7. General survey picture from the ultrastructure of the pollen grain. Negative No.: 9012, 3289x, 8. Ultrastructure of the partially degraded ectexine Negative No.: 9043, 33.035x.
9-12. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with $KMnO_4$ (24 hours)
9,10. LM pictures 9. Pollen grain after experiment mounted in glycerine-jelly. 10. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
11,12. TEM pictures. Detail from the partially degraded ectexine. 11. Negative No.: 9041, 33.035x, 12. Negative No.: 9044, 33.035x.
13-16. Partially degraded pollen grains with 2-aminoethanol (24 hours) and merkaptoethanol (24 hours)
13-15. LM pictures. 13. Unstained. 14. Stained pollen grain mounted in glycerine-jelly. 15. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
16. TEM picture. General survey picture from the inter-apertural part of the pollen grain. Negative No.: 9064, 3.289x.

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TEM data (Plate 9.3., figs. 7,8) In contrast to the previous experiment, remnants of the elements of the intine are relatively well shown. Within the light homogeneous part, a thin electron dense layer is perceptible, probably as a remnant of the ectintine (Plate 9.3., fig. 7). In the degraded protoplasm there are light ellipsoid holes. The ectexine is degraded, channels in the tectum are not perceptible. A trend of homogenization may be observed (Plate 9.3., fig. 8).

3.3. Experiment No.: T-12-236, partial degradation with 2-aminoethanol (72 hours) and with $KMnO_4$ for 24 hours (Plate 9.3, figs., 9-12)

LM results (Plate 9.3., figs. 9,10, table 9.1.) No important changes were observed in the qualitative characteristic features. The maximal value of the diameter of the pollen grains, mounted in glycerine-jelly, is a little smaller than in the previous experiment, but the diameter of the pollen grains mounted in Araldite, is essentially the same.

TEM results (Plate 9.3., figs. 11,12) The degradation of the ectexine is well illustrated it in both pictures. The degradation of the infratectal layer is advanced and sometimes has completely disappeared. Thinning or degradation of the foot layer is also perceptible in some parts of the ectexine (Plate 9.3., fig. 12). Ornamental elements of the tectum are characteristic. Channels were not observed during our investigations. The substance of the ectexine is partially degraded. Electron dense, globular large bio-

polymer units were observed. The arrangement of these units is irregular. It is worth mentioning that biopolymer structures, which are in general present around the channels, were not observed at the sites of the degraded channels.

4. Partial degradation with 2-aminoethanol and merkaptoethanol (Plate 9.3., figs. 13-16, plate 9.4., figs. 1-10)

4.1. Experiment No.: T-12-237, partial degradation with 2-aminoethanol (24 hours) and merkaptoethanol for 24 hours (Plate 9.3., figs. 12-16, plate, 9.4., fig. 1)

LM results (Plate 9.3., figs. 13-15, table, 9.1.) No important qualitative alterations were observed. The stained pollen grains are a little larger than the unstained and the pollen grains mounted in Araldite.

TEM results (Plate 9.3,. fig. 16, plate 9.4, fig. 1) The exine is relatively well preserved. Under the electron dense ectexine, a granular layer is embedded in the light homogeneous part of the intine. Below this layer a darker layer of the intine and another light part is seen. A characteristic layer, composed of electron dense globular units, is beneath this layer, which may be the inner part of the intine. The plasma membrane is electron dense and probably damaged. Organelles of the protoplasm are perceptible, there are vacuoles with tiny electron dense granules.

4.2. Experiment No.: T-12-238, partial degradation with 2-aminoethanol (48 hours) and with merkaptoethanol (24 hours) (Plate 9.4., figs. 2-6)

LM results (Plate 9.4., figs. 2-4, table, 9.1.) The qualitative and the quantitative alterations are essentialy indentical with the previous experiment.

TEM results (Plate 9.4., figs. 5,6) In the inter-apertural area (Plate 9.4., fig. 6) degradation of the ectexine was observed. Thinning of the elements of the infratectal layer and degradation of the superficial ornamental elements are illustrated in picture 6 of the Plate 9.4. Beneath the foot layer, the intine was also degraded. The ultrastructure of the apertural area is relatively well preserved (Plate 9.4., fig. 5). The ectexine seems to be in original preservation except for the inner layers beneath the foot layer. The more or less lamellar or filamentous elements of the protrusion are also perceptible.

4.3. Experiment No.: T-12-239, partial degradation with 2-aminoethanol (72 hours), and with merkaptoethanol for 24 hours (Plate 9.4., figs. 7-10)

LM results (Plate 9.4., figs. 7-9, table 9.1.) Qualitative characteristics are as previously. The maximum of the diameter of the unstained pollen grains is nearly the same in the pollen grains mounted in Araldite as in the previous experiment. Increasing consequences of the stain and embedding effect were observed.

TEM results (Plate 9.4., fig. 10) Degradation of the infratectal layer and the intine is well illustrated. The intine after this experiment is composed of two layers, an outer light and a darker, electron dense part. The plasma membrane is not perceptible. The degradation of the protoplasm is advanced. In the protoplasm there are holes, probably vacuoles.

5. Partial dissolution with glycerine (50%), (Plate 9.4., figs. 11-15)

LM pictures (Plate 9.4, figs., 11-13, table 9.1) Alterations in the quantitative characteristics were observed, in particular the pollen grains mounted in Araldite are smaller than the unstained and stained pollen grains.

TEM pictures (Plate 9.4., figs. 14,15) Degradation of the ectexine was observed (Plate 9.4., fig. 15). More or less radially oriented holes may be the remnants of the channels. Further light globular holes, of irregular arrangement are also perceptible. The infratectal and the foot layers are also damaged. Dark irregular elements are in the degraded intine (Plate 9.4., fig. 14).

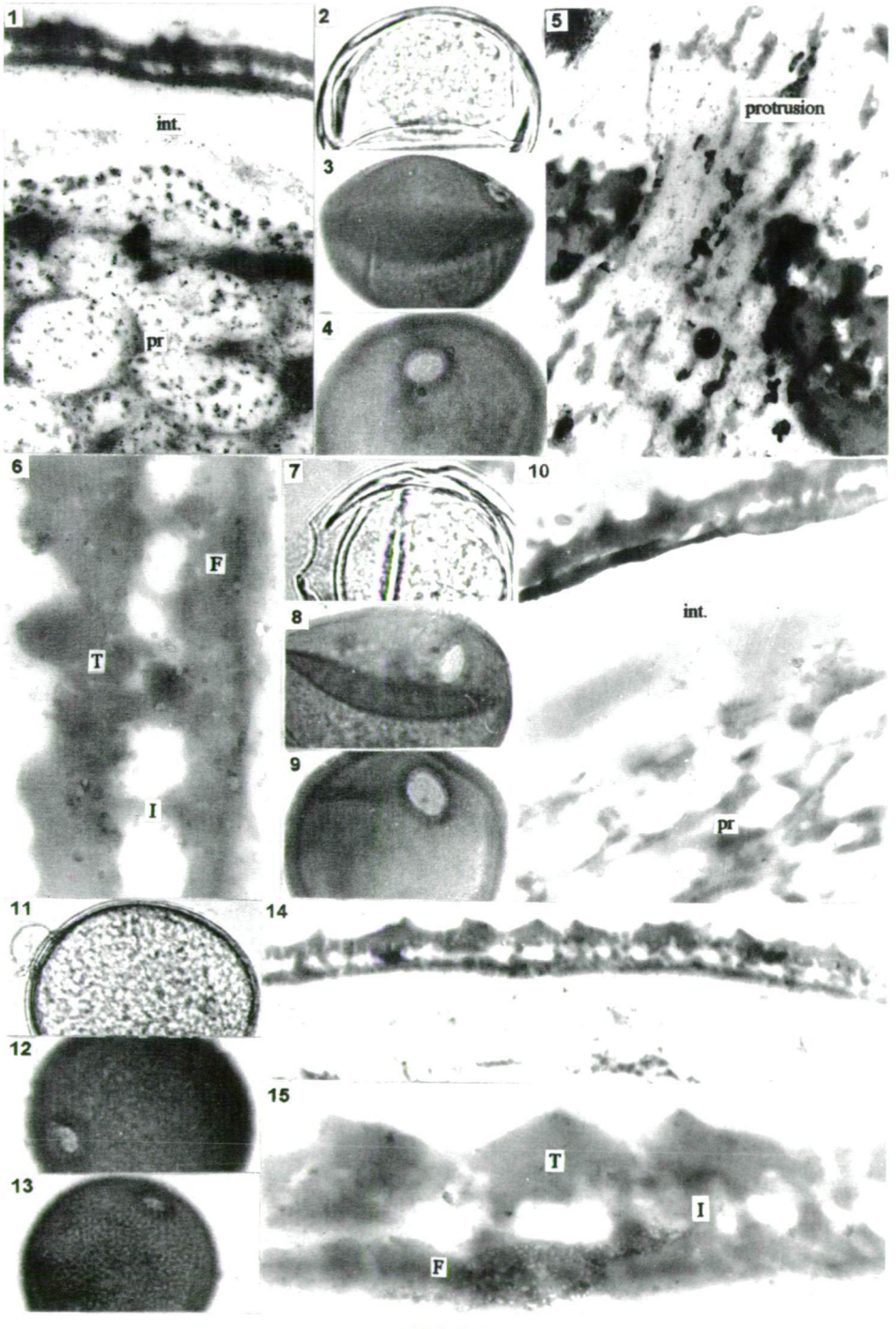


Plate 9.4.

Plate 9.4.

1-15. *Triticum aestivum* L.
1. Partially degraded pollen grain with 2-aminoethanol (24 hours) and merkaptoethanol (24 hours) TEM picture. Detail from the ultrastructure of the inter-apertural part of the pollen grain. Negative No.: 9035, 9.910x.
2-6. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with merkaptoethanol (24 hours)
2-4. LM pictures 2. Unstained. 3. Stained pollen grain with Methylviolet. 4. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
5,6. TEM pictures. 5. Ultrastructure of the apertural area with protrusion. Negative No.: 9049, 9.910x. 6. Detail from the partially degraded ectexine. Negative No.: 9051, 33.035x.
7-10. Partialy degraded pollen grains with 2-aminoethanol (72 hours) and merkaptoethanol (24 hours)
7-9. LM pictures. 7. Unstained. 8. Stained pollen grain with Methylviolet. 9. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
10. TEM picture. Detail of the ultrastructure of the pollen grain in the inter-apertural area. Negative No.: 9196, 9.910x.
11-15. Partially dissolved pollen grains with glycerine (50%) for 30 days
11-13. LM pictures. 11. Unstained. 12. Stained pollen grain with Methylviolet. 13. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
14,15. TEM pictures. 14. General survey picture from the interapertural part of the pollen grain. Negative No.: 9053, 9.910x. 15. Detail from the partially degraded ectexine. Negative No.: 9054, 33.035x.

## Discussion and Conclusions

Based on our new results we can point out the following:

1. The most important qualitative alterations were observed after the treatment with 2-aminoethanol and $KMnO_4$. The ectexine wrinkled. Orbiculi were not observed during our investigations.

2. As regards the diameter of the pollen grains, the stain altered in a remarkable measure the size of the fresh pollen grains. However in the partially degraded and dissolved pollen grains, a certain regularity was established in the alterations of the diameter of the pollen grains.

3. It is mentioning that the channels of the tectum are not evident after the experiment or have disappeared completely. The sporopollenin molecular system around the channels is less resistant. In contrast, to this, we observed helical biopolymer organization in channels of the tectum of partially degraded pollen grains of *Alnus glutinosa* (KEDVES, SZÉCSÉNYI and SASHALMI, 2002). The channels and the biopolymer organization around them may be important in the diffusion of the allergens from the pollen grains.

4. The sporopollenin of the infratectal layer and the inner lamellar structures in the apertural area are less resistant. In our first observations of fossil angiosperm pollen grains, we observed the degradation of the infratectal layer (KEDVES, STANLEY and ROJIK, 1974).

But our first opinion is that the ontogenetically first developed layer of the ectexine is less resistant was not always supported by our other experiments.

5. The cytoplasmic ultrastructural elements of the protrusions are more resistant than the other part of the protoplasm.

6. The dissolution with diluted glycerine did not reveal the organelles of the cytoplasm as well as we have previously established in the pollen grains of *Platanus hybrida* (KEDVES, PÁRDUTZ and TÓTH, 1999).

Finally, the pollen grains of *Dactylis glomerata* are also included in our research program, and the same experiments will be carried out during the next year. We hope that the comparative evaluation will be interesting and useful.

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## References

ANDERSEN, S.TH. and BERTELSEN, F. (1972): Scanning electron microscope studies of pollen of cereals and other grasses. - Grana *12*, 79-86.

DE LEONARDIS, W., LONGHITANO, N., MELI, R., PICCIONE, V., ZIZZA, A., CRIMI, N., PALERMO, F. and MISTRETTA, A. (1986): Flora of allergenic pollen grains in Italy. - 3rd Internat. Conf. on Aerobiology, Abstracts, 70.

DOVER, G.A. (1972): The organization and polarity of pollen mother cells of *Triticum aestivum*. - J. Cell Sci. *11*, 699-711.

EDGEWORTH, M. P. (1877): Pollen. – Hardwicke and Bogue, London.

EL-GHAZALY, G. (1990): A comparison of pollen wall development in normal and gametocide treated plants of *Triticum aestivum*. - Bull. Soc. bot. Fr., 137, Actual. bot. *2*, 65-70.

EL-GHAZALY, G. and JENSEN, W.A. (1985): Studies of the development of wheat *(Triticum aestivum)* pollen: III. Formation of microchannels in the exine. - Pollen et Spores *27*, 5-14.

EL-GHAZALY, G. and JENSEN, W.A. (1986a): Studies of the development of wheat *(Triticum aestivum)* pollen : I. Formation of the pollen wall and Ubisch bodies. - Grana *25*, 1-29.

EL-GHAZALY, G. and JENSEN, W. (1986b): Studies of the development of wheat *(Triticum aestivum)* pollen: formation of the pollen aperture. - Can. J. Bot. *64*, 3141-3154.

ERDTMAN, G. (1944): Sädesslagens pollenmorfologi. Pollen morphology of the cereals. With notes on the pollen morphology in Triticinae. - Svensk bot. Tidskrr. *38*,73-80.

ERDTMAN, G. (1956): Current trends in palynological research work. - Grana Palynologica *1*, 127-139.

ERDTMAN, G., BERGLUND B. and PRAGLOWSKI, J. (1961): An Introduction to a Scandinavian pollen flora. - Grana Palynologica 2, 3-92.

ERDTMAN, G. and PRAGLOWSKI, J. (1959): Six notes on pollen morphology and pollenmorphological techniques, - Bot. Notiser *112*, 175-184.

FAEGRI, K. and IVERSEN, J. (1964): Textbook of Pollen Analysis. 2nd edition. - Munksgaard, Copenhagen.

KEDVES, M., PÁRDUTZ, Á. and TÓTH, A. (1999): Ultrastructure of partially dissolved pollen grains of *Platanus hybrida* BROT. - Plant Cell Biology and Development (Szeged) *10*, 83-90.

KEDVES, M. STANLEY, E.A. and ROJIK, I. (1974): Observations nouvelles sur l'ectexine des pollens fossiles des Angiospermes de l'Eocène inférieur. - Pollen et Spores *16*, 425-437.

KEDVES, M., SZÉCSÉNYI, A. and SASHALMI, J. (2002): Helical biopolymer organization in channels of the tectum of partially degraded pollen grains of *Alnus glutinosa*. - Plant Cell Biology and Development (Szeged) *14*, 95-97.

KÖHLER, E. and LANGE, E. (1979): A contribution to distinguishing cereal from wild grass pollen grains by means by LM and SEM. - Grana *18*, 133-140.

LEBBE, J., VIGNES, R. et HIDEUX, M. (1988): Base de connaissances sur les pollens et spores atmosphériques de l'Europe du Nord et tempérée : identification et applications taxonomiques. - Ann. Sci. Nat. Bot., Paris 13 ser, 9, 107-123.

MOHL, H. (1835): Sur la structure des grains de pollen. – Ann. Sci. nat. ser. *2, 3*, 148-180, 220-226.

NILSSON, S., PRAGLOWSKI, J. and NILSSON, L. (1977): Atlas of Airborne Pollen Grains and Spores in Northern Europe. - Natur och Kultur, Stockholm.

RAJENDRA, B.R., TOMB, S.A., MUJEEB, K.A. and BATES, L.S. (1978): Pollen morphology of selected Triticeae and two intergeneric hybrids. - Pollen et Spores *20*, 145-156.

RICHARD, H., BIDAULT, M., KADMIRI, S., BOTELLA, N., SIMON-RIGAUD, M.L., CHAMBAUD, R. et ROUGEOT, J.C. (1986): Calendrier de la pluie pollinique à Besançon. - Publication du Lab. de Chrono-Ecologie, URA n° 35 du CNRS, Faculté des Sciences, Besançon, 1-32.

SHIH-YIH., MO-SHAN, W. and LI-YUN, H. (1977): Electron microscopic observations on the microsporogenesis in male sterile and its maintainer lines of wheat. - Sci. Sinica *20*, 625-638.

SORSA, P. (1968): Pollen morphology of four *Sedum telephium* L. subspecies studied by means of Nomarski's interference-contrast equipment. - Ann. Bot. Fenn. *5*, 98-101.

SZAKÁCS, É. (1992): Citológiai és genetikai vizsgálatok buza (*Triticum aestivum* L.) és kukorica (*Zea mays* L.) portokkulturákban. - Kand. Ért. Tézisei, MTA Mezőgazd, Kut. Int. Martonvásár, 1-10.

THANIKAIMONI, G. (1972): Index bibliographique sur la morphologie des pollens d'Angiospermes. - Trav. Sect. Sci. Tech, *12(1)*, 1-339.

THANIKAIMONI, G. (1973): Index bibliographique sur la morphologie des pollens d'Angiospermes. - Trav. Sect. Sci. Tech. *12(2)*, 1-164.