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Behavioural studies on the pollen grains of *Pinus roxburghii* collected from Lucknow, India–A report

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ABSTRACT

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We have long recognized that natural climatic shifts influence the development of plants on earth. These slow temperature fluctuations have resulted in either the extinction or evolution of various species. A careful study of the comparative morphology of the living and fossil type suggests that the modern coniferous families originated independently from a group of extinct conifers called the transition conifers by Florin. The conifers reached their climax in the mid–Mesozoic times and have been on the decline ever since. Pinaceae is one such family whose pollen grains have been located as long back as in the Gondwana flora and have demonstrated morphological variations since then due to climatic changes.

Pinus is the most dominating genus of the family Pinaceae in gymnosperms. Pine grains are large due to their sacs or bladders, which make them one of the easiest pollen grains to identify. They are rich in non–enzymatic anti–oxidants, like provitamin A, B complex, C, D and E plus a host of minerals and amino acids. Apparently pine pollen is also a great defense against radioactive caesium that is appearing in dairy and other foods in the U.S.

The present study examines the changes occurring in one of the most sensitive phases of plant development in the living genus of *Pinus*, i.e. the behaviour of pollen, found to be dependent on number of genetic or environmental factors. The major environmental factors contributing to this abnormal behaviour include light intensity and quality, temperature and moisture and level of pollutants in atmosphere.

During present study, the *Pinus* cones were collected from five experimental areas of Lucknow that exhibited different level of deformities probably due to different levels of environmental stress or other genetic reasons. The clusters were unique in having deformed cones on the side facing the road while they were near normal on the side away from road indicating that the area around the roadside being more polluted and may be playing a detrimental role in cone morphology. Pollen of normal cones were bilateral, analept, usually bisaccate, spherical, slightly elliptical with verrucate corpus and sacci having pila, normal average size being 60μ–65μ including sacci and sacci attached almost full width of corpus, exine thick in proximal part of reticuloid corpus. While pollen grains of abnormal cones show abnormalities in their size, shape, size and number of wings, size and shape of corpus and development of pollen tube within microsporangium. The abnormalities were observed using scanning electron microscopic studies and its percentage was calculated using a light microscope.

Key–words—Deformities, elliptical, Gondwana, Mesozoic, pollutants, radioactive.

भारत में लखनऊ से संगृहीत *पाइनस रॉक्सबर्घीयाई* के पराग दानों पर व्यवहारपरक अध्ययन

पूजा दीक्षित, गौरी सक्सेना, दिनेश कुमार एवं लव सिंह

सारांश

हमने विस्तृत रूप में माना है कि पृथ्वी पर प्राकृतिक जलवायवी विस्थापन पौधों के विकास को प्रभावित करते हैं। ये मंद तापमान परिवर्तन विविध जाति के या तो विलोपन या विकास के परिगामी हैं। जीवित और जीवाश्म प्रकार की तुलनात्मक आकारिकी का ध्यानपूर्वक अध्ययन संकेत देता है कि फ्लोरिन द्‌वारा नाम दिए गए ये संक्रमणकालीन शंकुवृक्ष विलुप्त शंकुवृक्षों के समूह से स्वतंत्ररूप से आधुनिक शंकुधारी परिवारों से उद्‌गमित हुए। मध्य–मध्यजीवी कालों में शंकुवृक्ष अपने चरमबिंदु पर पहुंचे तथा उस समय से अवनति पर हैं। पिनेसी एक ऐसा परिवार है जिसके

परागकण गोंडवाना वनस्पतिजात की भांति से अवस्थित हैं तथा जलवायवी बदलावों की वजह से इन्होंने आकारिकीय उतार–चढ़ाव प्रदर्शित किए हैं।

अनावृतबीजियों में पिनेसी कुटुंब का वंश *पाइनस* सर्वाधिक प्रभुत्वी है। अपने कोष या थैलियों की वजह से चीड़ कण बड़े हैं, जो उन्हें पहचानने में सरलतम पराग कण विरचित करते हैं। वे खनिज एवं एमीनोएसिड के अतिथेय प्रोविटामिन ए, बी, सम्मिश्र, सी, डी एवं ई प्लस जैसे गैर–एन्ज़ाइमी प्रति–ऑक्सीकारकों में प्रचुर हैं। ऊपरी तौर से चीड़ पराग भी विकिरण सीजियम के विरूद्ध विशाल बचाव है जो यू.एस. में दुग्ध–उत्पाद एवं अन्य खाद्यों में प्रकट हो रही है।

आनुवंशिक या पर्यावरणीय गुणों की बड़ी संख्या में आश्रित पाए गए *पाइनस* के जीवित वंश अर्थात पराग के व्यवहार में पादप विकास की अति संवेदनशील प्रावस्थाओं में से एक में हो रहे बदलावों का मौजूदा अध्ययन जांच करता है। इस अपसामान्य व्यवहार में योगदान दे रहे विशाल पर्यावरणीय गुणों में प्रकाश तीव्रता व गुणवत्ता, तापमान व आर्द्रता तथा वातावरण में प्रदूषकों का स्तर समाहित है।

इस अध्ययन के दरम्यान, लखनऊ के पांच प्रायोगिक क्षेत्रों से *पाइनस* शंकुओं को संगृहीत किया गया जो कि संभवतः पर्यावरणीय दवाब या अन्य आनुवंशिक कारणों के विभिन्न स्तरों को विरूपताओं के विविध स्तरों से प्रदर्शित थी। मार्ग की तरफ वाले विरूपित शंकुओं में पुंज विशिष्ट थे जबकि मार्ग से अलग वे लगभग सामान्य थे इंगित कर रहे है कि मार्ग की तरफ वाला इलाका ज्यादा प्रदूषित था तथा शंकु आकारिकी में अहितकर भूमिका अदा कर रहा होगा। सामान्य शंकुओं के पराग द्विपार्शिवक, सजीव, सामान्यतया द्विसपुट, गोलाभकार, किणमय पिंड सहित थोड़े से दीर्घवृत्तीय, पाइला स्वत्व कोष, पिंड की लगभग पूरी चौड़ाई कोष व कोष संलग्न सहित 60 µm – 65 µm का सामान्य आकार होते हुए, जालिका पिंड के निकटस्थ भाग में बाह्य चोल चौड़े थे। जबकि अपसामान्य शंकुओं के पराग दाने, अपनी आमाप, आकृति, आमाप व पंखों की संख्या, पिंड की आमाप व आकृति तथा बीजाणुधानी के अंदर पराग ट्यूब के विकास में अपसामान्यताएं दर्शाते हैं। क्रमवीक्षण इलेक्ट्रान सूक्ष्मदर्शी अध्ययन प्रयुक्त करते हुए अपसामान्यताएं प्रेक्षित की गई थीं तथा लघु सूक्ष्मदर्शी प्रयुक्त करते हुए इसके प्रतिशत की गणना की गई।

सूचक शब्द—विरूपताएं, दीर्घवृत्तीय, गोंडवाना, मध्यजीवी, प्रदूषक, विकिरण।

INTRODUCTION

PINUS belongs to family Pinaceae of Coniferales, evolved in middle altitudes of the Northern Hemisphere in the middle Mesozoic. By the late Cretaceous pines had spread East and West throughout Laurasia, attaining high diversity in Eastern Asia, the Eastern United States and Western Europe, but having little representation at high northern latitudes. Changing climates in the early Tertiary established warm and humid tropical/subtropical conditions in a broad zone to 70º N throughout middle latitudes. Pines and their relatives disappeared from many middle–latitude areas during this time and were replaced by diverse angiosperm taxa of the boreotropical flora, which were adapted to the equable, tropical climate. The effect of this climate change and spread of boreotropical flora was to displace pines from their former habitats. A hypothesis is defended that pines shifted during the three warm periods of the Eocene, into three major refugial areas in the Northern Hemisphere: high latitudes, low latitudes, and upland regions of middle latitudes, especially in Western North America (Millar, 1993).

Pinaceae is one such family whose pollen grains have been located as long back as in the Gondwana flora and have demonstrated morphological variations since then due to

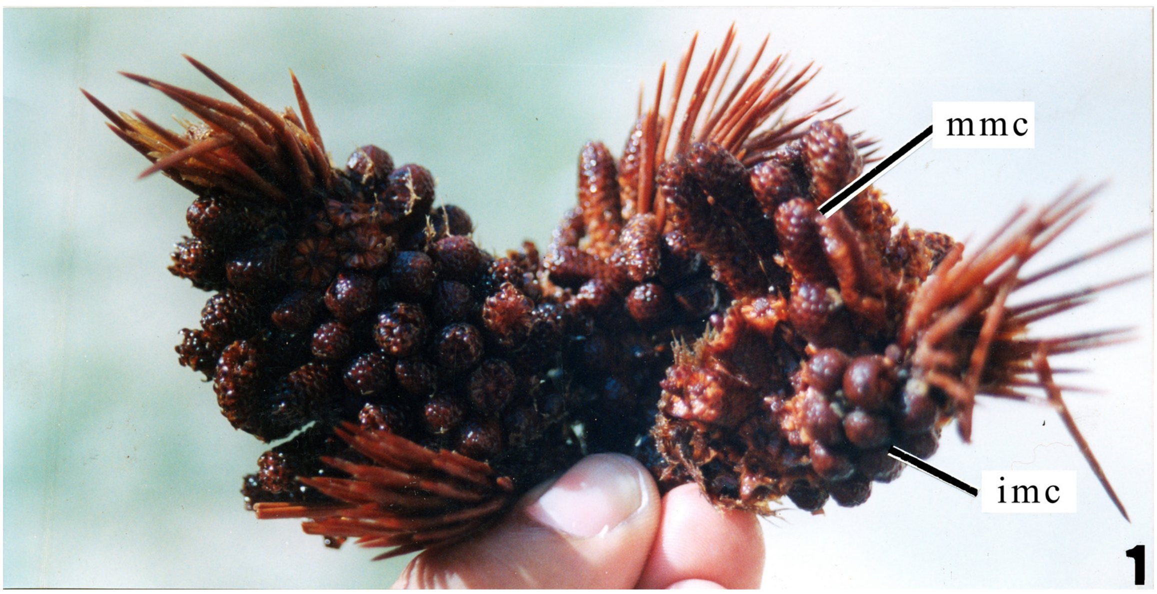

Fig. 1—Cluster of male cones of *Pinus roxburghii* showing both mature (m m c) and immature (i m c) cones.

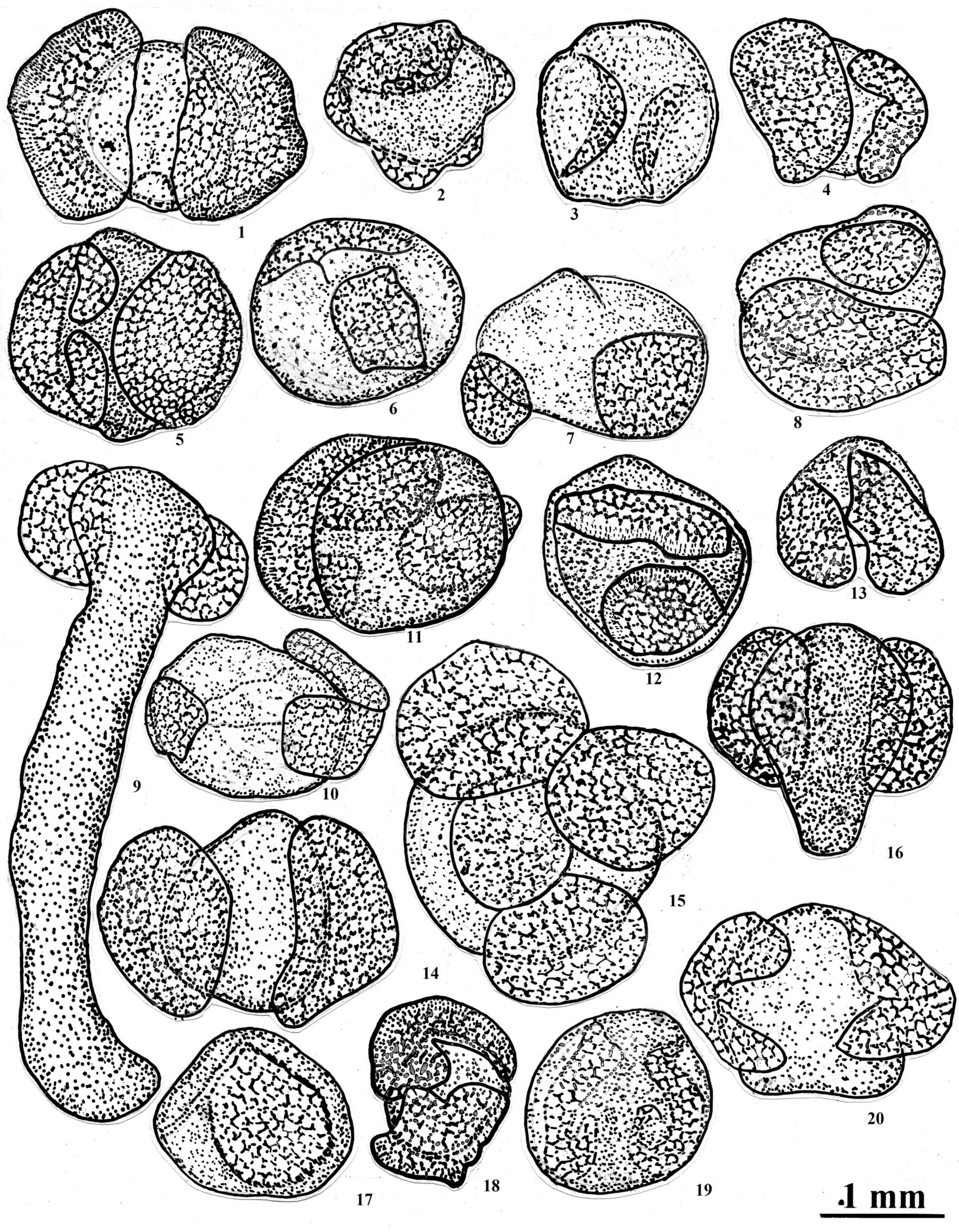

Fig. 2—Camera lucida drawing of pollen grains.

climatic changes. Pine grains are large due to their sacs or bladders, which make them one of the easiest pollen grains to identify. These sacs also allow them to be carried to great distances by the wind. The pollen is rich in non–enzymatic anti–oxidants, like pro–vitamin A, B complex, C, D and E plus a host of minerals and amino acids. Apparently, pine pollen is also a great defense against radioactive caesium that is appearing in dairy and other foods in the US (Sunny Soleil & Filed, 2012).

We have long recognized that natural climatic shifts influenced the development of plants on earth. These slow temperature fluctuations have resulted in either the extinction or evolution of various species. The present study however examines the changes occurring in one of the most sensitive phases of plant development that is pollen morphology. The major environmental factors contributing to this abnormal behaviour include light intensity and quality, temperature and moisture (Kristina *et al.*, 1998).

Among six native species, *Pinus roxburghii* Sargent, known as "Chir pine" growing as an ornamental tree in Lucknow, has been investigated for abnormalities with respect to number of wings, size and shape of corpus and size of pollen. It is a beautiful tree with horizontal branches in whorls giving the tree a pyramidal appearance. Tree is monoecious but the male and female cones are borne on separate branches. The male cones replace the dwarf shoot and occur in clusters. The number of male cones is approximately 140 in a cluster of *Pinus roxburghii*. Microsporophylls are spirally arranged on the central axis of each male cone and each microsporophyll bears two microsporangia on its abaxial surface. Large numbers of pollen grain are produced within microsporangium that show synchronous mode of development and liberate simultaneously.

The male cones were collected from trees in cultivated condition from different areas of Lucknow which exhibited unique type of abnormalities. The pollen grains of these cones show abnormalities in number of wings, size and shape of corpus, etc. The critical investigation on pollen morphology and their behaviour along with SEM details of *Pinus roxburghii* growing in cultivated condition are being reported for the first time in India.

MATERIAL AND METHOD

Survey, Collection and Preservation

Lucknow city in India lies on the geographical coordinates of 26°51'0" N, 80°55'0" E with geographical area 310 sq km and population 22.45 lakhs (approx.). During present investigation Trans–Gomti region of Lucknow has been divided in five areas according to pollution level for collection of pollen grains (area–1: Lucknow University, area–2: Nishatganj, area–3: Indira Nagar, area–4: Mahanagar, area–5: Gomti Nagar). Pollution level of area–2 to area–5 ranges from 158.9 to 195.6 $\mu gm/m^3$ while that of area–1 was negligible. Male cones were collected during the month of March/April. During these months temperature of Lucknow ranged between 30°C–35°C and relative humidity between 25%–30%. The freshly collected material was fixed in 1: 3 acetic acid and alcohol mixture for 2–4 hours then transferred in 70% alcohol.

Acetolysis

The pollen grains were obtained after dissecting the mature sporangia and subjected to acetolysis (Erdtman, 1943) for light microscopic studies and mounted in freshly prepared glycerine jelly. Microphotographs were taken using Nikon Camera (Fx35Dx) attached to the compound microscope (Nikon make Japan No. 013189).

Scanning Electron Microscopy

For SEM study the acetolysed samples of pollen grains were dehydrated through alcohol series and mounted on brass stubs. The stubs were coated with gold–palladium (Jones, 2012) and photographs were taken by scanning electron microscope (Philips 505, Holland) at Birbal Sahni Institute of Palaeobotany, Lucknow.

Calculation of Percentage of abnormality

Percentage of abnormality was calculated by the formula proposed by Waheed *et al.* (2012) i.e.

Percentage of abnormality=

$$\frac{\text{Number of abnormal pollen in an area}}{\text{Total number of pollen in that area}} \times 100$$

Observations

During present study it was observed that the cones collected from five experimental areas exhibited deformities due to environmental stress. Abnormalities even in the male cones were also noticed, the clusters were unique in having deformed cones on the side facing the road while they were near normal on the opposite side. The clusters of cones collected from area–1 only had normal cones while those collected from area–2 to area–5 had both normal and abnormal cones (Fig. 1).

Normal pollen grains were bilateral, analept, usually bisaccate, spherical, slightly elliptical, corpus verrucate (wart like processes) while sacci having pila (tube like growth), average size being 60–65 μ including sacci and sacci attached almost full width of corpus. Exine was thick in proximal part of corpus and corpus was reticuloid.

The pollen grains of area–1 showed normal characters while area–2 to area–5 showed different level of deformities (Figs 2, 3, 4, 5, 6, 7). The morphological characterization of pollen grains collected from different areas has been presented in Table–1.

The percentage of abnormality calculated under light microscope (10 x magnification) area wise was as follows–

Area 1: % of Abnormality= $\frac{0 \times 100}{12} = 0\%$

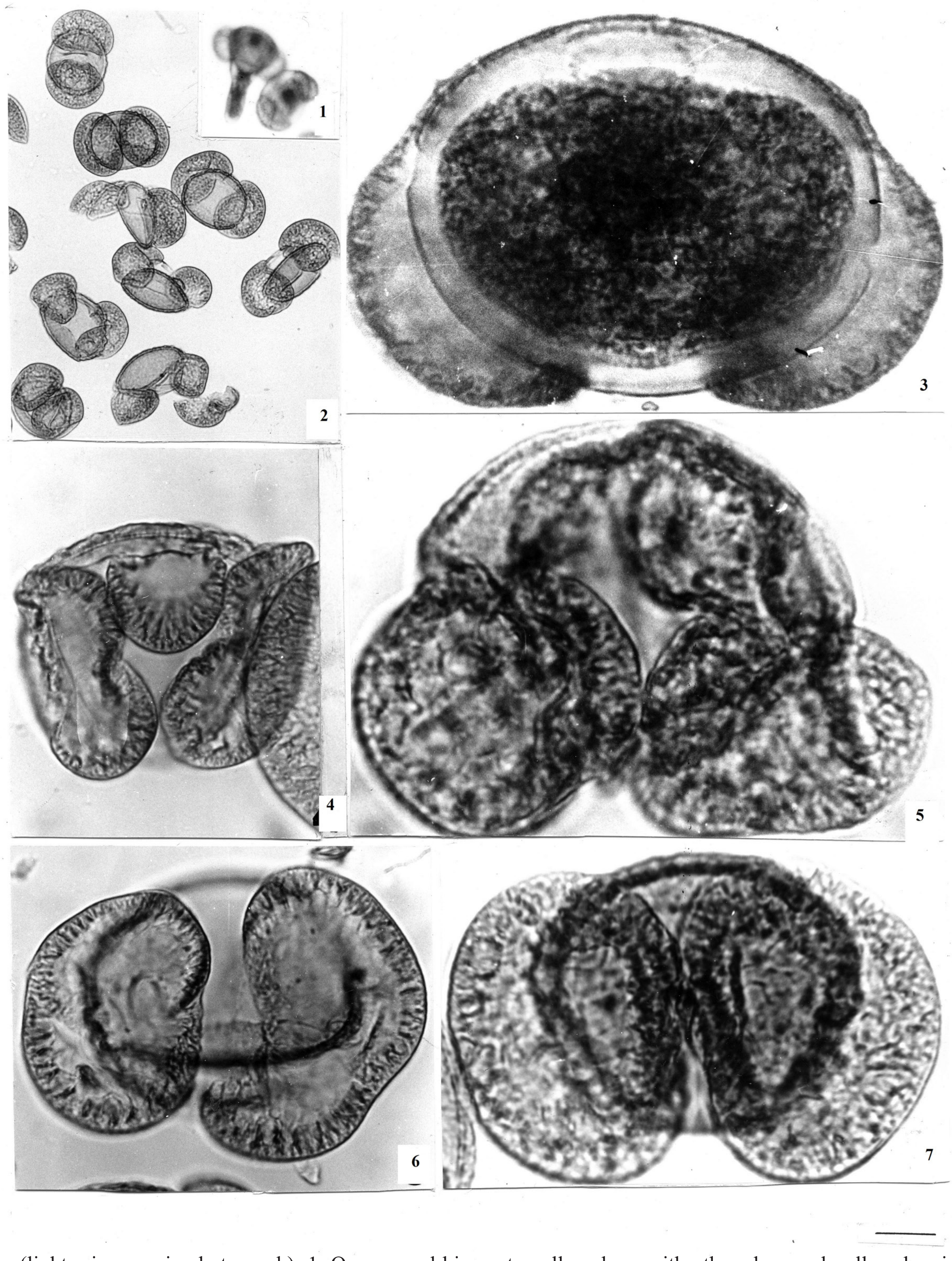

Fig. 3—(light microscopic photograph): 1. One normal bisaccate pollen along with other abnormal pollen showing pollen tube. x 700, 2. Normal pollen with two wings lying in various planes. x 700, 3. Abnormal pollen with corpus covering two lateral wings. x 1500, 4. abnormal pollen with three unequal wings two are in bifurcating condition. x 700, 5. Abnormal pollen showing two laterally attached normal wings and one reduced wing, corpus lying above the spherical wings. x 1500, 6. Bisaccate pollen with unequal wings. x 700, 7. Bisaccate pollen showing almost equal and elongated wings, corpus lying above the wings. x 1500. 1, 2: 0.1 mm ________ 3–7: 0.05 mm ____________

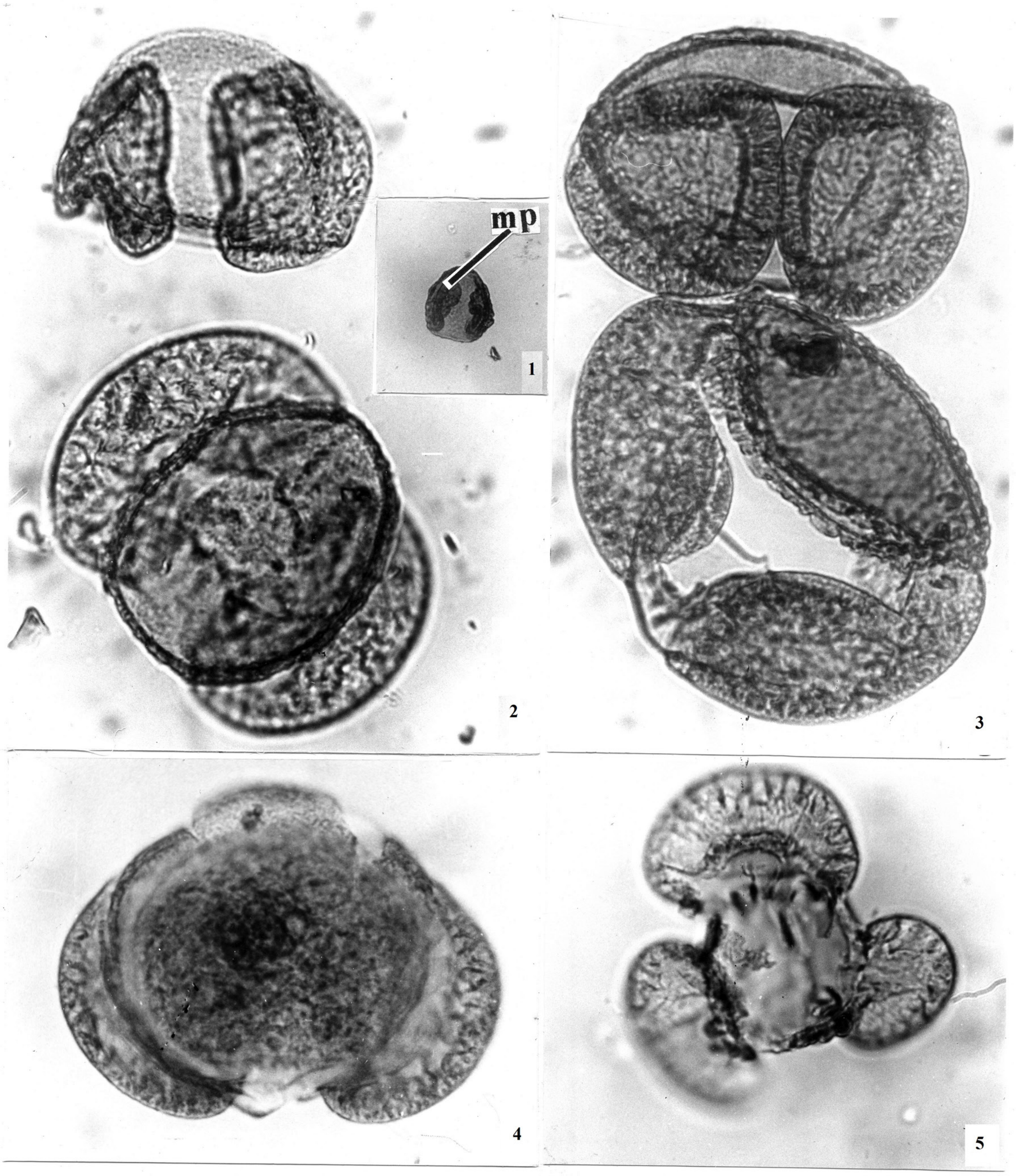

Fig. 4—(light microscopic photograph): 1. Abnormal bisaccate micropollen (mp) with two shrinked wings. x 700, 2. Two different size bisaccate pollen grains, one showing unsymmetrical wings lying below the corpus. x 700, 3. Two different sized bisaccate and trisaccate pollen grains. x 700, 4. Bisaccate pollen showing lateral wings, corpus lying above the wings. x 700, 5. Abnormal small pollen with three unequal wings. x 700. 1–5: .05 mm

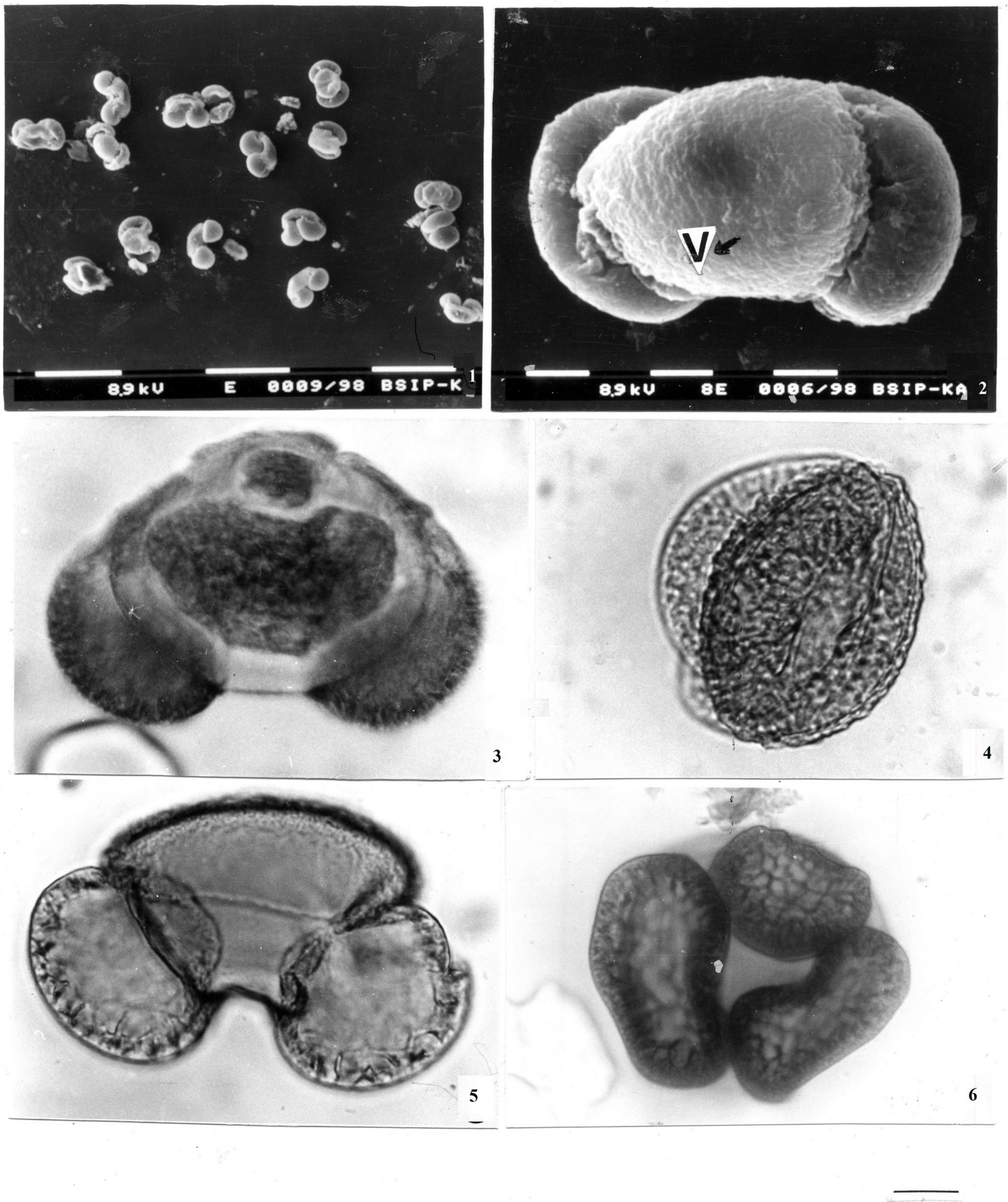

Fig. 5—1. Bisaccate pollen under SEM showing different size and shape in various planes, 2. Bisaccate pollen showing verrucate (v) texture of corpus. x 1350, 3. Bisaccate pollen showing prothallial cell. x 1400, 4. pollen with two overlapping wings. x 700, 5. Pollen showing two spherical wings, corpus lying above the wings. x 700, 6. Abnormal pollen with one small and two large wings. x 700. 3–6: .05 mm

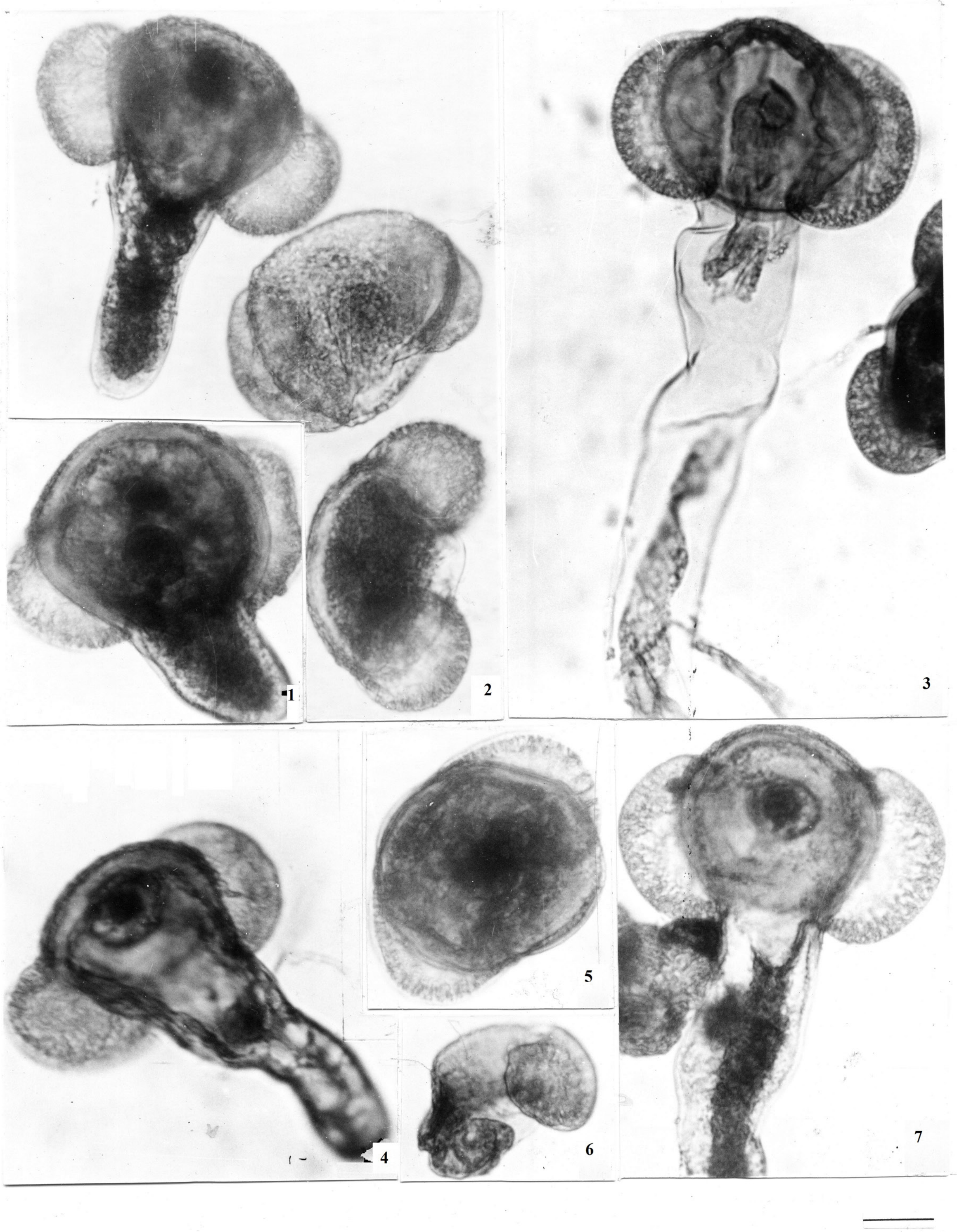

Fig. 6—(light microscopic photograph): 1. Abnormal bisaccate pollen showing pollen tube. x 700, 2. Three bisaccate pollen grains, one is normal other with small protuberance and third one showing pollen tube. x 700, 3. Abnormal bisaccate pollen showing long pollen tube. x 700, 4. Bisaccate pollen with pollen tube showing tube nucleus. x 700, 5. Abnormal pollen with small protuberance showing corpus lying above the lateral wings. x 700, 6. Very small bisaccate pollen having one reduced wing. x 700, 7. Bisaccate pollen with long tube. x 700. 1–7: 0.05 mm

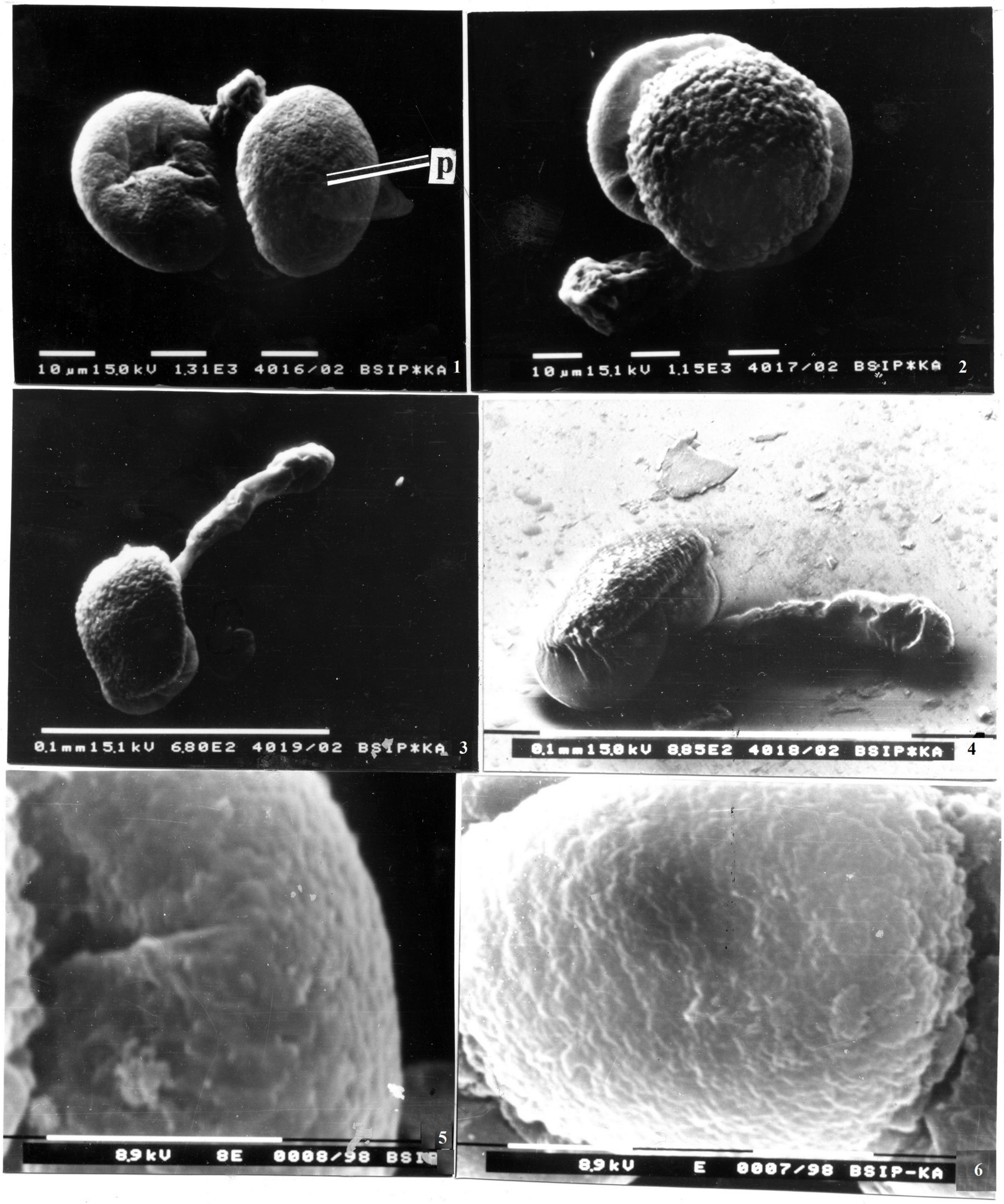

Fig. 7—(SEM photographs): 1. Pollen with two unequal wings having puncta (p) on surface and showing minute tube like growth, 2. Pollen with small pollen tube showing large verrucate (v) corpus covering two lateral wings, 3. Bisaccate pollen showing long tube 4. Bisaccate pollen showing origin of tube. 5. Wings of pollen with punctate (p) exine X 5200, 6. Corpus with verrucate (v) texture.

Table 1—Characterization of pollen of *Pinus roxburghii* collected from five different areas of Lucknow.

Characters	Area–1	Area–2	Area–3	Area–4	Area–5
Size of pollen	size ranging from 60 µ–65 µ including sacci	size ranging from 44 µ–88 µ including sacci, micropollen seen	size ranging from 65 µ–75µ including sacci	size ranging from 50 µ–75µ including sacci, micropollen seen	size ranging from 65 µ–75µ including sacci
Shape of pollen	spherical or slightly elliptical	spherical or slightly elliptical	spherical or slightly elliptical	spherical or slightly elliptical	spherical or slightly elliptical
Shape of corpus	typical biconvex with cappa and cappula	biconvex, oblong to triangular	Biconvex	oblong to triangular	typical biconvex
Wings or sacci	usually bisaccate, almost equal sized	bisaccate to tetrasaccate, difference in wing size	mostly bisaccate, some trisaccate, difference in wing size	bisaccate to trisaccate, difference in wing size	mostly bisaccate some trisaccate, difference in wing size
Aperture	aperture absent	aperture absent	aperture absent	aperture absent	aperture absent
Pollen wall	Exine thick in proximal part of corpus, sexine thicker than nexine, intine thin	exine thick in proximal part of corpus, sexine thicker than nexine, intine thin	exine thick in proximal part of corpus, sexine thicker than nexine, intine thin	exine thick in proximal part of corpus, sexine thicker than nexine, intine thin	exine thick in proximal part of corpus, sexine thicker than nexine, intine thin
Ornamentation	corpus finely granulate and sacci coarsely reticuloid	corpus finely granulate and sacci coarsely reticuloid	corpus finely granulate and sacci coarsely reticuloid	corpus finely granulate and sacci coarsely reticuloid	corpus finely granulate and sacci coarsely reticuloid
Symmetry	bilateral, analept	bilateral, analept	bilateral, analept	bilateral, analept	bilateral, analept
Germination	pollen tube absent	all the stages from initiation to fully developed pollen tube is formed within microsporangium	small protuberance of intine has been observed	tube like structure seen	small protuberance seen

Area 2: % of Abnormality= $\frac{5 \times 100}{11}$ = 45% (approx.)

Area 3: % of Abnormality= $\frac{2 \times 100}{13}$ = 15% (approx.)

Area 4: % of Abnormality= $\frac{4 \times 100}{15}$ = 27% (approx.)

Area 5: % of Abnormality= $\frac{2 \times 100}{16}$ = 12% (approx.)

DISCUSSION

The normal structure of pollen grains along with abnormalities in *Pinus roxburghii* growing in natural habitat has already been discussed earlier by workers like Puri (1945), Lakhanpal and Nair (1936), Erdtman (1957, 65, 69), Vishnu Mittre (1957), Srivastava (1960a, b) and Mehra (1966, 88) however their observation was restricted only to the occurrence of two or four winged pollen. During present study various other parameters like wings, size and shape of corpus and size of pollen have also been taken as criteria for calculating the percentage abnormality. Besides, the occurrence of *in situ* pollen tube which is an abnormal condition has also been reported for the first time.

During the present study, area–1 was nearly unpolluted and non-vehicular area of Lucknow University covered with green plants, because of which pollen of this area were normal however pollen of area–2 showed maximum (about 45%) abnormality probably due to pollution levels being highest owing to vehicular to and fro. The abnormality seems to be induced in the pollen grains that were mostly exposed to the road side. Considering it to be genetical phenomenon does not seem to be very viable because cones on the same plant exhibited different behaviour according to their position i.e those facing road exhibited abnormal characters while those away were near normal. Accordingly less abnormality (about 27%) had been observed in area–4 as compared to area–2 probably, because the pollution level was lower in area–4 as compared to area–2. Similarly area–5 and area–3 showed 12% and 15% abnormality respectively especially in pollen size, shape of corpus and number and size of wings.

Srivastava (1960b) reported 18% abnormality in *P. roxburghii* in relation to size, shape and number of wings in his study on fossil pollen of Shivalik wells. Chira (1967) found oversized pollen grains in *P. edulis*. She attributed the cause of abnormality in pollen to low temperature at the time of meiosis. Mehra (1988) reported abnormal pollen grains in the species of Indian pines, *Pinus griffithii*, *P. roxburghii* and *P. kesiya*. Tetrasaccate pollen grains were reported in *P. griffithii* while pollen grains with three or four wings in *P. roxburghii*. In some grains there was a single saccus or wing encircling the grain all over except at the distal end. In others, the saccus was poorly developed approaching nearly the nonsaccate condition. Bisaccate micropollens were also noticed in some cases. In *P. kesiya*, trisaccate pollen grains and pollen grains with poorly developed saccus on only one side were reported by him. The percentage of such abnormal grains was as low as 5.8% in *P. griffithii*, 7.51% in *P. roxburghii* and 3.26% in *P. kesiya*. In present study of *Pinus roxburghii* bisaccate to tetrasaccate pollen grains along with micropollen were observed and their percentage varied with the level of pollution.

Caiyun *et al.* (1989) examined the pollen morphology and ultra structure of pollen wall of *Pinus yunnanensis* Franch. by light microscope, scanning electron microscope and transmission electron microscope and found that the pollen grains were variable in shape and size. They also observed heteromorphic pollen grains without saccus, with 1–4 sacci and reported that the rate of the heteromorphic pollen grains was about 5%.

Wang *et al.* (2005) assessed the effects of brefeldin A (BFA) on pollen tube development in *Picea meyeri* using fluorescent marker FM4–64 as a membrane–inserted endocytic/recycling marker, together with ultra–structural studies and Fourier transform infrared analysis of cell walls. BFA inhibited pollen germination and pollen tube growth.

Hao *et al*. (2013) suggested that cellulose plays important role in pollen tube elongation in *Pinus bungeana* Zucc. using the specific inhibitor, 2, 6–dichlorobenzonitrile (DCB). They found that in the presence of DCB, the growth rate and morphology of pollen tubes distinctly changed and cellulose was involved in the establishment of growth direction of pollen tubes, and in the cell wall construction during pollen tube development despite its lower quantity.

CONCLUSION

In the present investigation percentage of such abnormalities in pollen grains along with pollen tube inside microsporangium was quite high. The possible cause might be due to environmental pollutants like suspended particulate matter, fine particles, sulphur dioxide, oxides of nitrogen, carbon and trace metals in air because of which there may be formation of some chemicals which induce growth or formation of some metabolites which provide the same environment as inside pollen chamber in the ovule; or some hormonal stimulation leading to formation of pollen tube; or fungal infection and production of fungal toxin induced tube growth. The occurrence of pollen tube before pollination as well as its development *in situ* was unique and interesting phenomena in *Pinus roxburghii* and had been observed for the first time using SEM. Such studies in the reproductive structures will open new avenues for further research in the developmental studies of pollen grains.

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