ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Pollen Morphology of Pepper Cultivars and their wild allies from Southern Western Ghats, Kerala, India

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Manuscript Info

Manuscript History:

Received: 10 January 2015
Final Accepted: 25 February 2015
Published Online: March 2015

Key words:

Piper, Pepper cultivars, Pollen Morphology, Wild allies of Piper

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Abstract

The pollen morphology of nine species and six cultivars of *Piper nigrum* var. *nigrum* were elucidated with scanning electron microscopy (SEM), supplemented by light microscopy (LM) and micrometry. The pollen grains of all the taxa are monosulcate, with considerable variations in exine ornamentation. Two distinct lines of exine ornamentation were observed in both wild allies and the cultivars. All the cultivars of *Piper nigrum* L. var. *nigrum* studied were tuberculate or granulate with very little variations, while the different wild species studied either possessed granulate or tuberculate pollen grains.

INTRODUCTION

Piperales includes the family Piperaceae which contains around 4300 species, and is considered to be morphologically the most diverse group in Magnolids (Isnard et al., 2012). Genus *Piper* coming shows pantropical distribution and is one of the richest genera of flowering plants. *Piper* includes more than two thousand species, along with the most valuable and economically important spice crop, Black pepper (*Piper nigrum* L. var. *nigrum*), and known as 'the King of Spices' which is widely consumed throughout the world (Kubitzki et al., 1993) The species of this genus are shrubs, herbs or climbers (Sasidharan 2010; Gamble and Fischer 1915-1936; Hooker 1886). In South East Asia, believed to be the center of origin of *Piper nigrum* and *Piper betel*, the number of species amounts to around 300 (Jaramillo and Manos 2001). This economically and ecologically important genus possesses high species diversity which is unique to traditional Magnoliidae and may be the best example of diversification in basal angiosperms (Sanderson and Donoghue 1994).

Kerala, the southernmost State of India occupies an extensive share of the Western Ghats which is a rich repository of wild relatives of tropical spices, especially of the genus *Piper*, which occurs among the moist deciduous semi-evergreen and evergreen forests (Ravindran 2000; Sasidharan 2010). Kerala contributes about 97% of the India's pepper production. Sixteen species of the genus are found distributed in various ecosystems of the State, of which eight species are endemic to the Western Ghats (Parthasarathy et al., 2006).

In spite of the great importance in diet, medicine and excessive commercial importance, palynological research on spices including *Piper* is meager. However, studies on intraspecific variability in *Piper nigrum* (Chandy et al., 1984; Kanakaswamy et al., 1985; Mathew and Mathew 2001), size and pollen types in *Piper* (Erdtmann 1952; Walker and Doyle 1975), cytological studies on *Piper* in the Western Ghats (Mathew, 1958) and the biosystematics and morphometric studies of *Piper* in the Western Ghats (Ravindran et al., 1996, 1997) are recorded in the literature. Even though palynological studies on Piperaceae has been carried out by different authors (Tseng-Cheung Huang 1966; Lei and Xing 1998; Ligong and Hanxing 1998; Suwanphakdee et al., 2008), a detailed

descriptive and comprehensive palynological study of the various *Piper* species occurring in the Western Ghats with the help of SEM has been seldom attempted except for the work of Mathew and Mathew (2001).

Worldwide demands on *Piper* necessitate to work on conservation of germplasm and their further genetic improvement for which pollen grains are an inevitable resource. Therefore, proper characterization of different promising *Piper* species and evaluation of genetic variations are essential. In such a backdrop, the present work focuses on the pollen morphological characterization of nine wild species of the genus *Piper* along with six cultivars of *Piper nigrum* L.

2. Materials and methods

The polliniferous materials of nine species of *Piper* and six cultivars of *Piper nigrum* var. *nigrum* were procured from the Conservatory of Kerala Agricultural University's Pepper Research Station at Panniyur, Kannur District and from identified locations of southern Western Ghats. The taxa selected for the study were *Piper argyrophyllum, P. barberi, P. chaba, P. colubrinum, P. galeatum, P. hymenophyllum, P. mullesua, P. nigrum,* and *P. trioicum*. The freshly collected materials were fixed and preserved in 70% Ethanol followed by processing for microscopic preparation as per the standard method of acetolysis (Erdtman 1952; Nair 1960). The herbarium of the materials were prepared and deposited at ERRC, Thiruvananthapuram. In addition to this, unacetolysed preparations contained in 70% alcohol and stained by were made use of in comparing the pollen features with acetolysed grains. Micro-morphological data was generated by both light microscopic (Olympus CX27i), scanning electron microscopic (JSM, JOEL SEM) and micrometric means. In deducting the size of the pollen grains, 20 grains taken at random were considered. The measurements were taken at the magnification of 1000 X. The technical description of the pollen grains was carried out in accordance with the terminology used by Punt et al.,(2007).

3. Results

The comparative analysis revealed that the pollen grains of *Piper* were monads, heteropolar, spheroidal and monosulcate. The shape of pollen varied from spheroidal to sub-spheroidal with a size range from P (polar diameter) = 11-16 µm to E1 (equatorial diameter) = 10 - 14µm and E2 (second equatorial diameter) = 8-12 µm. There was an increase in the equatorial diameter in acetolysed grains (*Ac*) as compared to unacetolysed grains (*UAc*). The characterization of pollen grains of different cultivars of *Piper nigrum* var. *nigrum* and the species are summarized below.

3.1. Pollen morphological characterization of different species of the genus *Piper*

3.1.1. *Piper argyrophyllum* Miq. (Figure 1: 1A, 1B)

Distribution: Sri Lanka and Western Ghats, India, in evergreen and semi-evergreen forests.

Grains monosulcate. Exine ornamentation was faintly micro granulate and fossulate. Exine thickness 1µm. Size of pollen: *Acetolysed* (*Ac*): P: 10-11 µm; E1: 13-14 µm; E2: 11 µm. *Unacetolysed* (*UAc*): P: 9-10 µm; E1: 12 µm; E2: 9 µm. Expansion due to acetolysis 1µm.

3.1.2. *Piper barberi* Gamble (Figure 1: 2B, 2B)

Distribution: Endemic to southern Western Ghats, India, in evergreen forests;
Status: Critically endangered.

Grains monosulcate. Exine ornamentation granulate with faintly micro granulate condition. Randomly micro-verrucate projections were seen. Exine thickness 1 µm. Size of pollen: *Ac*: P: 12-13 µm; E1: 14-15 µm; E2: 12 µm. *UAc*: P: 10-11 µm; E1: 13 µm; E2: 10 µm. Expansion due to acetolysis was 1µm.

3.1.3. *Piper chaba* Hunter (Figure 1: 3A, 3B)

Distribution: Indo-Malaysia; cultivated in various parts of India

Grains monosulcate. Exine ornamentation tuberculate; surface spotted with dense granulate, sparsely micro echinate projections. Exine thickness 2 µm. Size of pollen: *Ac*: P: 11-12 µm, E1:10 µm, E2: 9 µm. *UAc*: P: 10 µm, E1: 9 µm, E2: 8 µm. Expansion due to acetolysis was 1µm.

3.1.4. *Piper colubrinum* Link. (Figure 1: 4A, 4B)

Distribution: Exotic, Native to South America introduced elsewhere; often cultivated in Kerala.

Note: The species is distantly related to Black pepper (*P. nigrum*), and forms the only species reported to be totally immune to *Phytophthora* causing Foot rot disease. Grafting of Black pepper on *P. colubrinum* rootstock is an important aspect of the disease management

Grains monosulcate. Exine ornamentation granulate. Surface was spotted with dense granules all over. Exine thickness 2µm. Size of pollen: *Ac*: P: 10-11 µm, E1: 12 µm, E2: 10 µm. *UAc*: P: 9-10 µm, E1:10 µm, E2: 9 µm. Expansion due to acetolysis was 2 µm.

3.1.5. *Piper galeatum* (Miq.) DC. (Figure 2: 1A, 1B)

Distribution: Endemic to South Western Ghats, India, in evergreen and semi-evergreen forests

Grains monosulcate. Exine ornamentation tuberculate ridged. Ridges sinuate. Exine thickness: 1 µm. Size of pollen: *Ac*: P: 9-10 µm; E1: 11-12 µm; E2: 11 µm. *UAc*: P: 9 µm; E1: 10 µm; E2: 9 µm. Expansion due to acetolysis was 1µm.

3.1.6. *Piper hymenophyllum* Miq. (Figure 2: 2A, 2B)

Distribution: Endemic to Peninsular India, in evergreen and semi-evergreen forests.

Grains monosulcate. Exine pattern tuberculate ridged, ridges being tuberculate. Exine thickness: 2 µm. Plano-convex grains with ectexine thicker than endexine. Size of pollen grain *Ac*: P: 12-13 µm, E1:11 µm, E2: 10 µm. *UAc*: P 11-12 µm, E1: 11µm, E2: 10 µm. Expansion due to acetolysis was 1µm.

3.1.7. *Piper mullesua* Buch.-Ham ex D. Don. (Figure 2: 3A, 3B)

Distribution: Peninsular and Northeast India, in evergreen and shola forests.

Grains monosulcate. Exine ornamentation tuberculate ridged with segmented ridges. Exine thickness 2 µm. Size of pollen: ***Ac***: P: 11-12 µm, E1: 11 µm, E2: 10 µm. *UAc*: P: 10 µm, E1: 9 µm, E2: 8 µm. Expansion due to acetolysis was 1 µm (E1).

3.1.8. *Piper nigrum* L. var. *nigrum* Hook (wild) **(Figure 2: 4B, 4B)**

Distribution: Peninsular India and Sri Lanka, in evergreen and semi-evergreen forests; cultivated throughout Kerala

Grains monosulcate. Exine ornamentation tuberculate, segments hemispherical. Dense microverrucae present. Exine thickness: 1.5 µm. Size of pollen: *Ac*: P: 11-12 µm, E1:11 µm, E2:10 µm. *UAc*: P: 11 µm, E1: 10 µm, E2:10 µm. Expansion due to acetolysis was 1 µm.

3.1.9. *Piper trioicum* Roxb.(Figure 3: 1A, 1B)

Distribution: Western Ghats, India, in evergreen forests

Grains monosulcate. Exine ornamentation tuberculate striate, ridges being segmented. Spots representing low spinules present at random. Exine thickness: 1 µm. Size of pollen: *Ac*: P: 11-12 µm; E1: 10 µm; E2: 8 µm. *UAc*: P: 9 µm; E1: 8 µm; E2: 8µm. Expansion due to acetolysis was 2 µm.

3.2. Pollen morphological characterization of different cultivars of *Piper nigrum* L.

3.2.1. *Piper nigrum* L. cv. Panniyur 1 (Figure 3: 2A, 2B)

Grains monosulcate. Exine ornamentation verrucate-spinulate with prominent inconspicuous to conspicuous hemispherical hold, and very small column. Exine thickness: 1 µm. Size of pollen: *Ac*: P: 10-11 µm; E1: 10 µm; E2: 8 µm. *UAc*: P: 9-10 µm; E1: 8 µm; E2: 6 µm. Expansion due to acetolysis is 2 µm.

3.2.2. *Piper nigrum* L. cv. Panniyur 2 (Figure 3: 3A, 3B)

Grains monosulcate. Exine ornamentation tuberculate with sinuate ridges which are cut into differently sized segments. Size of pollen: *Ac*: P: 10 - 11 µm; E1: 12 µm; E2: 10 µm. *UAc*: P: 9 µm; E1:11 µm; E2: 10 µm. Expansion due to acetolysis was 1 µm.

3.2.3. *Piper nigrum* L. cv. Panniyur 3 (Figure 3: 4A, 4B)

Grains monosulcate. Exine ornamentation tuberculate. The tubercles are fused to form plate like structures. Exine thickness 1 µm. Size of pollen: *Ac*: P: 10 - 11 µm; E1:10 - 12 µm; E2: 10 µm. *UAc*: P: 9 µm; E1: 11 µm; E2: 10 µm. Expansion due to acetolysis was 1 µm.

3.2.4. *Piper nigrum* L.cv. Panniyur 4 (Figure 4: 1A, 1B)

Grains monosulcate. Exine ornamentation pilate to sympilate. Spinulate projections spread all over the grain. Exine thickness measured 1 µm. Size of grain *Ac*: P: 10 - 11 µm; E1: 10 µm; E2: 9 µm; *UAc*: 9-10 µm; E1: 9 µm; E2: 9 µm. Expansion due to acetolysis was 1µm.

3.2.5. *Piper nigrum* L.cv. Panniyur 6 (Figure 4: 2A, 2B)

Grains monosulcate. Exine ornamentation tuberculate - verrucate. Microspinulate projections spread all over the grain. Exine thickness 1 µm. Size of pollen: *Ac*: P: 12 - 14µm; E1: 12 - 13 µm; E2: 10 -11µm. *UAc*: 10-11 µm; E1: 11-12 µm; E2: 10 µm. Expansion due to acetolysis was 1µm.

3.2.6. *Piper nigrum* L.cv. Panniyur 7 (Figure 4: 3A, 3B)

Grains monosulcate. Exine ornamentation tuberculate with sparsely distributed inconspicuous spinules. Exine thickness 1µm. Size of pollen: *Ac*: P: 9 - 10 µm; E1: 11 - 12 µm; E2: 10 µm. ***UAc***: P: 9 µm; E1: 10 - 11 µm; E2: 10 µm. Expansion due to acetolysis was 1µm.

3.3. Palynological delineation of different species of *Piper*

The observations ascertained that the pollen grains of all the species of *Piper* and the cultivars were monosulcate, and the exine ornamentation pattern was either tuberculate or granulate with redundant features. The tuberculate form was characterized by alternate ridges and grooves. The tuberculate grains had secondary characteristics in the various species except for *P. galeatum*. Among other species with tuberculate surface, the secondary character was micro echinate in *P. chaba*, micro verrucate in *P. nigrum* (wild), and spinulate in *P.*

trioicum. In *P. hymenophyllum*, the ridges were segmented to give a tuberculate pattern, while in *P. mullesua* the segments were hemispherical (microverrucate). The secondary characters were present in granulate grains also, being fossulate in *P. argyrophyllum*, micro verrucate in *P. barberi* and agglutination of granules in *P. colubrinum*. Of the nine species studied, the smallest grain was that of *P. trioicum* measuring 9 µm (P) and the largest grain was encountered in *P. barberi* measuring 13 µm (P). Exine thickness was nearly 1 µm thick in most of the species, but was 2 µm in *P. mullesua*, *P. chaba*, *P. hymenophyllum* and *P. colubrinum*. The grains of most of the species were spheroidal with slight variation to sub-spheroidal, but this character was not of much significance in comparative morphology.

3.4. Palynological delineation of Pepper cultivars

The cultivars also possessed monosulcate pollen grains but with variations in the exine ornamentation, being tuberculate in Panniyur 2 and Panniyur 3, verrucate spinulate in Panniyur 1 and Panniyur 6, and pilate in Panniyur 4 and Panniyur 7. Among the tuberculate forms the segments of the ridges were variously sized in Panniyur 2 and tuberculate in Panniyur 3. Among the other forms, the exine surface is verrucate spinulate in Panniyur 1, pilate to sympilate in Panniyur 4 and verrucate in Panniyur 6. A secondary character of spinulate surface is found in Panniyur 7. The variations or additional characteristics, though minor, give an identification tool for the cultivar forms. The exine ornamentation is the only character of comparative value, on the basis of which the species studied may be deciphered.

3.5. Comparative pollen morphology

It may be noted that in the wild species of *Piper*, two basic lines of morphological differentiation, namely granulate and tuberculate are very significant. The secondary characters mark out the various species with tuberculate and granulate lines of differentiation. The above situation provides an idea about the association or dissociation among the species. The same features are significant in the cultivars too, the only difference being the surface is either tuberculate or variously patterned. It is therefore significant that the species and cultivars can be grouped. The tuberculate form appears to be primitive, although granulate form is of general significance.

3.6. Pollen key of *Piper* species

1. Grains tuberculate
    2. Segments heterogenous
        3. Tuberculae sinuate plate like .....*P. galeatum*
        3. Tuberculae not sinuous ……..……*P. trioicum*
    2. Segments homogenous
        4. Segments psilate/tuberculate without verrucate projection
            5. Tuberculate segments sinuous psilate……..*P. mullesua*
            5. Tuberculate segments with dense granules……..*P. chaba*
        4. Segments with microverrucate projections
            6. Segments smaller with dense microverrucae……..*P. nigrum*
            6. Segments larger with lax microverruca…....*P. hymenophyllum*
1. Grains faintly granulate
    7. Fossulate surface pattern ……*P. argyrophyllum*
    7. Granulate surface pattern
        8. Granulate with micro spinulate projection……...*P. colubrinum*
        8. Granulate with no projections……*P. barberi*

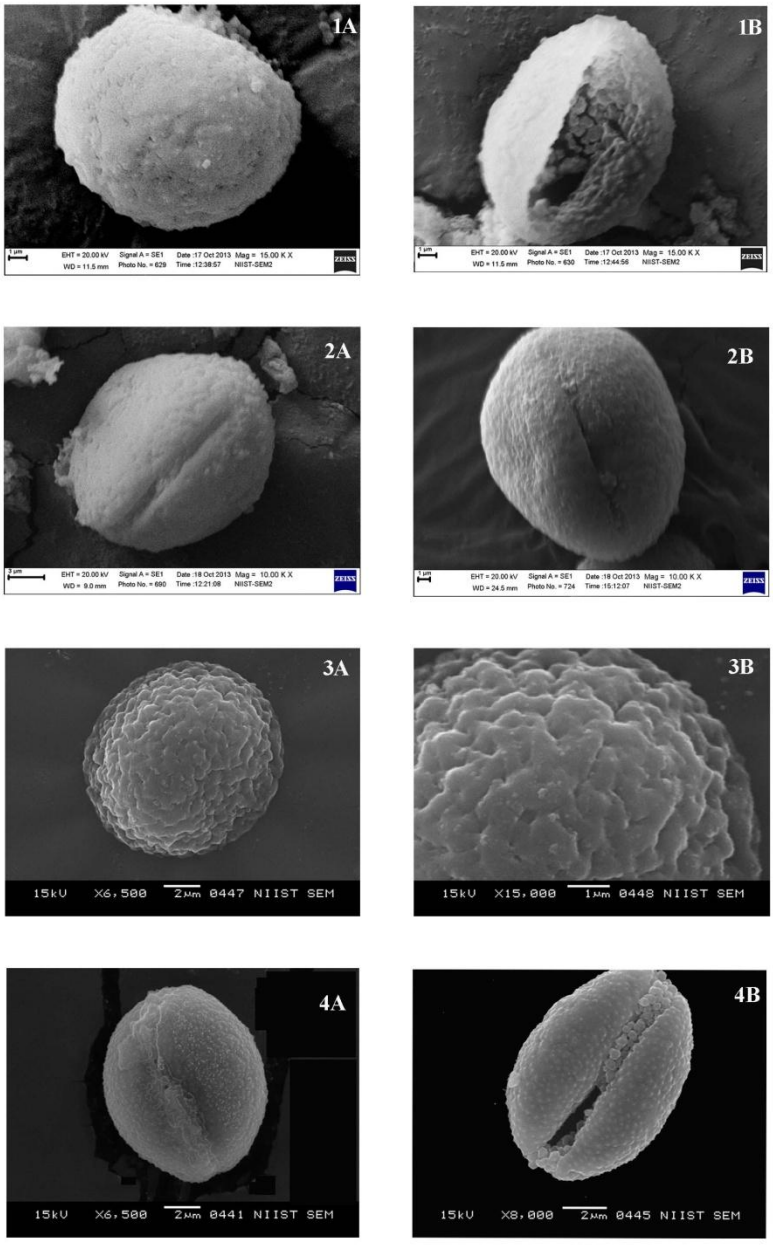

Figure 1. Scanning electron micrographs of pollen grains of *P.argyrophyllum* (1A, 1B), *P.barberi* (2A,2B), *P.chaba* (3A,3B), *P.colubrinum* (4A,4B).

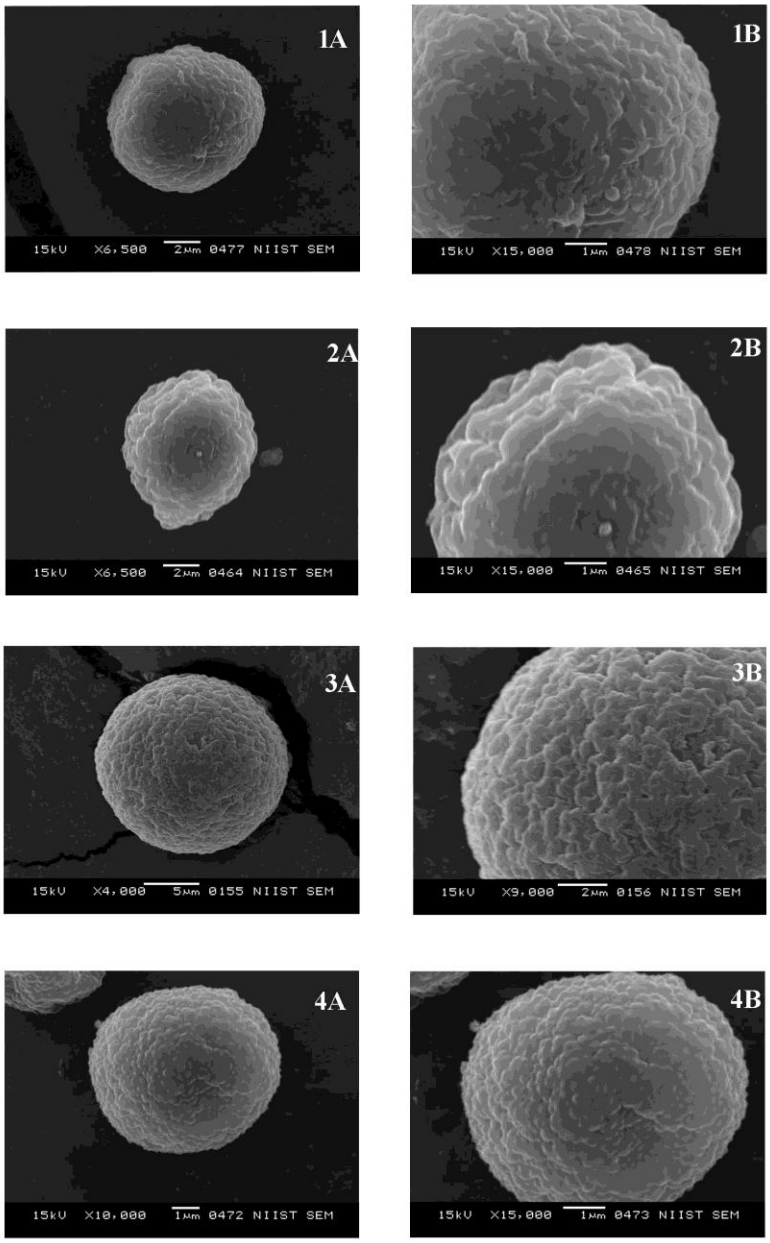

Figure 2. Scanning electron micrographs of pollen grains of *P.galeatum* (1A,1B), *P.hymenophyllum* (2A,2B), *P.mullesua* (3A,3B), *P. nigrum* var.*nigrum* (4A,4B).

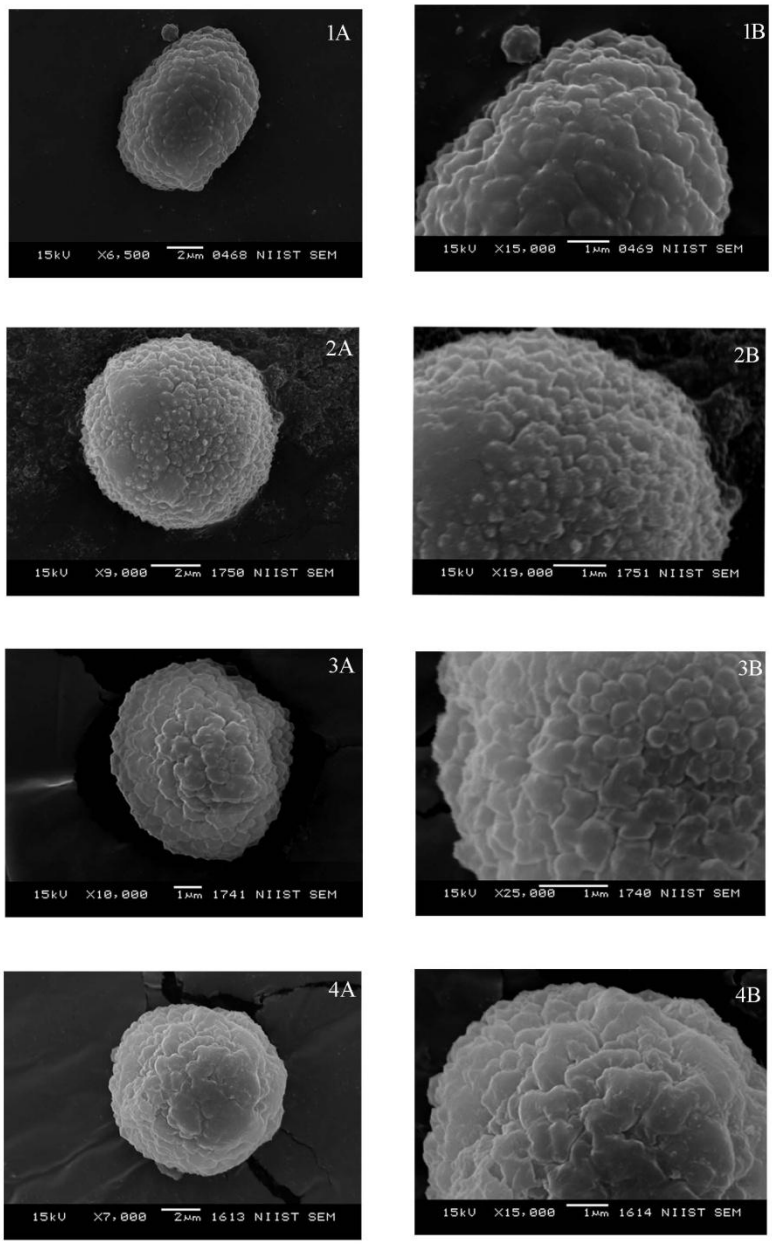

Figure 3. Scanning electron micrographs of pollen grains of *Piper trioicum* (1A, 1B), Panniyur 1 (2A,2B), Panniyur 2 (3A,3B), Panniyur 3 (4A, 4B).

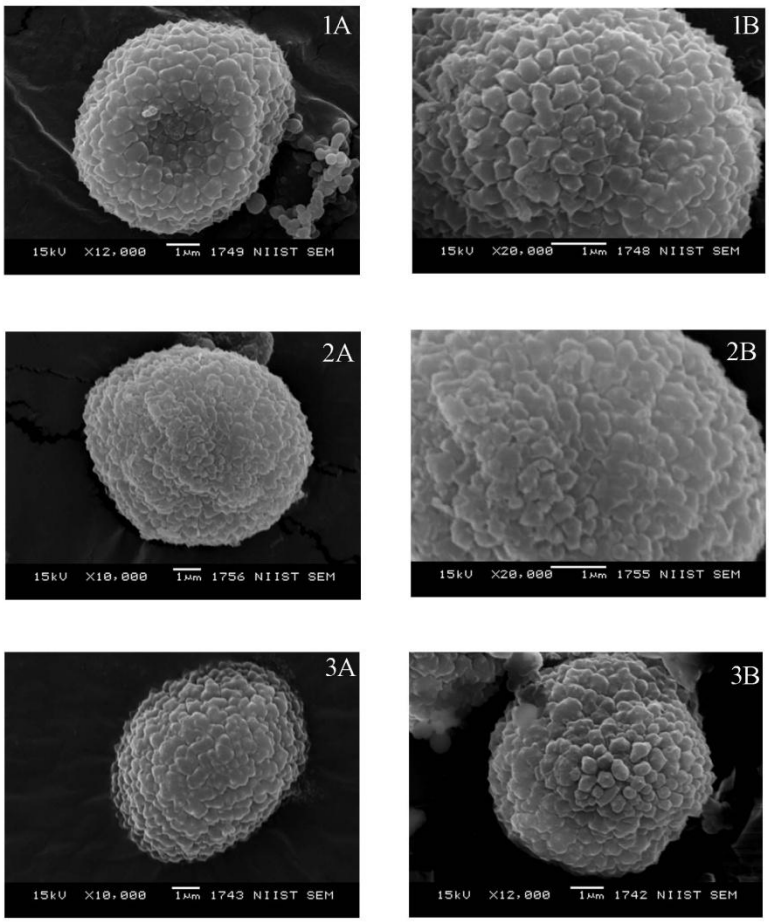

Figure 4. Scanning electron micrographs of pollen grains of Panniyur 4 (1A,1B), Panniyur 6 (2A, 2B), Panniyurv 7 (3A, 3B).

4. Discussion

The species and cultivars of Piper examined here are difficult to distinguish on the basis of plant morphology which makes development of an infrageneric classification challenging. However, the variation in pollen morphology between species offers another avenue for demarcating the taxa.

The first angiosperm pollen type which appeared in the early cretaceous fossil records as recognized by the paleopalynologists is monosulcate (Hochuli and Burkhardt 2013). The pollen grains of the entire *Piper* species in the present study are monosulcate and possess relatively thin exine (1-2 µm) providing evidence to the primitive character of the group with respect to earlier literatures on angiosperm classification (Thakthajan 1991, Cronquist 1968 and Nair 1970). Le Thomas (1980-81) and Sampson (2000) demonstrated that even the large *Magnolia* pollen grains with granulate structure also possess thin exine, which suggests the precision of grouping Piperales and Magnoliales in basal angiosperms.

Earlier studies on exine ornamentation in Chinese Pepper (Lei and Xing 1998) showed it to be varied between verrucate to psilate. The study on Thailand species of *Piper by* Suwanphakdee et al., (2008) found sculpturing varied from psilate to rugulate through scabrate and fossulate. Scabrate to psilate type of ornamentation was also recorded among the pollen of *Pipers* in the study of Tseng-Cheung Huang (1966).

The exine ornamentation pattern signifies the diversification of the species of *Piper* with in Piperales. It is therefore obvious that the exine pattern in *Piper* can be a reliable indicator of species relationship with perhaps two lines of divergence, namely the tuberculate line and the granulate line. The pollen morphology thus deciphered can be helpful in studying the species relationships at the morphological and genetic levels and will also help in rational selection, comparison and improvement of this important agronomic crop.

5. Acknowledgements

The authors are grateful to the Kerala State Council for Science, Technology and Environment (KSCSTE), Govt. of Kerala for funding support to the research programme of ERRC, *Palynological investigation of tropical spices and their wild allies*. We are also thankful to Dr. Prabhakar Rao and M.R. Chandran for providing the SEM facility at NIIST. The help and support rendered by Dr. V.P. Neema and Dr. C.R. Rini of Kerala Agricultural Uninversity-Pepper Research Station, Kannur in procuring authentic materials, and Dr. T. Shaju, JNTBGRI, Thiruvananthapuram in establishing taxonomic identity of the materials, are gratefully acknowledged.

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