Identification of Pollen Grains Morphology and Morphometry in Liliaceae

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Abstract. Palynology, the study of pollen, had a big role in paleontology, archeology, and forensics. Pollens from different plants had different morphology, such as in Liliaceae. Scanning electron microscopy (SEM) was applied to observe the morphology of Liliaceae pollens. This study aimed to determine the pollen grains morphology and morphometry in Liliaceae. The descriptive qualitative research was used five different species from three genera as the sample, namely Hemerocallis fulva, Hemerocallis lilioasphodelus, Aloe vera, Lilium longiflorum, and Lilium candidum. Parameters measured in this study were the types of pollen sizes, pollen shape, aperture characteristic, and ornamentation type of exine. The results showed that in Liliaceae had various shapes of pollen grains, i.e., prolate (1.33-2 μm) and perprolate (≥ 2 μm), as well as the pollen grains size, from minuta (10-25 μm in diameter) to medium (25-50 μm in diameter). Aperture characteristic of the pollen grains was monocolpate which has one colpus, and the ornamentation type of exine was reticulate patterns.

Keywords: Liliaceae, morphology, morphometric, palynology, pollen grains

1. Introduction

Liliaceae, commonly called “the Lily family”, consists of about 16 genera and about 700 species worldwide [1–5] of flowering plants. The family Liliaceae are cosmopolitan in distribution, and abundant in temperate and subtropical regions [6–9] which characterized by herbs perennual, with a rhizome, bulb, or corm, rarely shrubby or tree-like. The leaves of the family usually in the base of the plant and/or cauline, alternate, opposite, or whorled, parallel or rarely reticulate veined [7]. Members of the family usually have six-segmented flowers (bisexual) and three-chambered capsular fruits; occasionally the fruits are berries [2, 3, 7].

The members of Liliaceae are widely distributed. In Asia, Anthericum, Clintonia, Disporum, Reineckia, Tricyrtis and Zygadenus are found in East Asia, while Eremurus, Muscari and Puschkinia occur in Alpine - west and central Asia. Colchicum and Hyacinthella are found in central Asia. Temperate Asian genera are Erythronium and Tulipa. Besides these, Chlorophytum, Dianella, Gagea, Gloriosa, Hemerocallis, Merendera, Oligobotrya, Polygonatum, Puschkinia, Smilacina and Veratrum are also found in Asia [6, 10–12]. In Indonesia, some species of family Liliaceae are utilize for ornamental plants, such as Lilium longiflorum [13, 14]. Ophiopogon intermedias, Ophiopogon jaburan, Asparagus officinalis, Asparagus plumosus, Chlorophytum amaniense, Chlorophytum
comocum, Haworthia aloae aciae, Haworthia attenuata [14]. However, the study about Liliaceae pollen morphology (called palynology) in Indonesia is scarce.

Palynology studies include the nature and characteristics of pollen, the method of distribution, and its preservation. Pollen spread can occur through various intermediaries, such as wind, water, and animals. This spread is influenced by various factors, including air turbulence, wind direction and speed, weight and shape of pollen, as well as the height and strength of pollen sources. In terms of preservation, physical, chemical and biological processes can affect the durability pollen after being released from plants [15]. Pollen walls in angiosperms consist of two layers of exine and intine. The exine is composed of sporopollenin, while the intine is composed of cellulose. The exine is divided into two layers, namely sexine and nexine. Sexine is a layer that has ornamentation, while nexine is not. The pollen wall structure, especially the exine part, is one of the characters used in identification [15, 16]. The study of pollen is highly useful in paleoecology, paleontology, archaeology, and forensics [8, 17]. By employing electron microscopy we can observe the pollen morphology [8, 18–20]. Therefore, the study aimed to describe the pollen grains morphology and morphometry of Liliaceae in Indonesia.

2. Methods
This research was conducted using a descriptive qualitative method. There were five different species as the sample, namely Hemerocallis fulva, Hemerocallis lilioasphodelus, Aloe vera, Lilium longiflorum, and Lilium candidum. The pollen grain of Liliaceae was observed by using scanning electron microscopy (SEM). Parameters measured in this study were the types of pollen shape, pollen sizes, aperture characteristic, and ornamentation type of exine. The pollen shape classes are based on the ratio between the length of the polar axis (P) and equatorial diameter (E). P and E are measured from the equatorial view of a pollen grain and spore [15, 21]. The common shapes are presented in table 1. While the aperture morphology is an opening or thinning of the exine, physiologically it is a germination zone.

Table 1. The common shapes of the pollen grain

| No. | Shape          | Ration of P to E |
|-----|----------------|------------------|
| 1.  | Peroblate      | < 0.50           |
| 2.  | Oblate         | 0.50 ≤ x < 0.75  |
| 3.  | Subspheroidal  | 0.75 ≤ x < 1.33  |
|     | - Suboblate    | 0.75 ≤ x < 0.88  |
|     | - Oblate spherical | 0.88 ≤ x < 1.00 |
|     | - Prolate spherical | 1.00 ≤ x < 1.14 |
|     | - Subprolate   | 1.14 ≤ x < 1.33  |
| 4.  | Prolate        | 1.33 ≤ x < 2     |
| 5.  | Perprolate     | > 2              |

The exine ornamentation has two different types, the structure or texture and the sculpturing. The structure comprises of all the internal (infratectal) baculae of various form and arrangements. All the ektextine (including sexine and nexine) characters belong to the structural features, while the sculpturing comprises external (supratactel) geometric features without reference to their internal construction [21].

3. Results and Discussion
The observation result using SEM are presented in Table 2. The diameter of pollen grains was measured to determine the pollen size. Based on the SEM micrograph, the pollen size of H. fulva, H. lilioasphodelus, L. longiflorum, and L. candidum include medium size because they have 25–50 μm in diameter, while only A. vera has minuta size which diameter is 10–25 μm [21]. Pollen size influenced by biological factors, varies from one habitat to another, therefore important trait could vary
depending on the environmental conditions [22]. However, the pollen size can reflect the stigma depth, the distance a pollen tube has to reach the ovule [23].

Table 2. SEM micrograph of pollen grain in five species of Liliaceae in Indonesia

| Sample                  | SEM micrograph | Description                                      |
|-------------------------|----------------|--------------------------------------------------|
| *H. fulva* I            |                | P: 124 µm                                        |
|                         |                | E: 40.7 µm                                       |
|                         |                | P/E index: 3.05                                  |
|                         |                | Pollen size: medium                              |
|                         |                | Pollen shape: perprolate                         |
|                         |                | Aperture: monocolpate (one Colpi)                |
|                         |                | Exine ornamentation: reticulate                  |
| *H. fulva* II           |                | P: 122 µm                                        |
|                         |                | E: 43.2 µm                                       |
|                         |                | P/E index: 2.82                                  |
|                         |                | Pollen size: medium                              |
|                         |                | Pollen shape: perprolate                         |
|                         |                | Aperture: monocolpate (one Colpi)                |
|                         |                | Exine ornamentation: reticulate                  |
| *H. lilioasphodelus* I  |                | P: 115 µm                                        |
|                         |                | E: 37.1 µm                                       |
|                         |                | P/E index: 3.09                                  |
|                         |                | Pollen size: medium                              |
|                         |                | Pollen shape: perprolate                         |
|                         |                | Aperture: monocolpate (one Colpi)                |
|                         |                | Exine ornamentation: reticulate                  |
| *H. lilioasphodelus* II |                | P: 117 µm                                        |
|                         |                | E: 38.7 µm                                       |
|                         |                | P/E index: 3.02                                  |
|                         |                | Pollen size: medium                              |
|                         |                | Pollen shape: perprolate                         |
|                         |                | Aperture: monocolpate (one Colpi)                |
|                         |                | Exine ornamentation: reticulate                  |
| *A. vera* I             |                | P: 45.5 µm                                        |
|                         |                | E: 16.6 µm                                       |
|                         |                | P/E index: 2.74                                  |
|                         |                | Pollen size: minuta                              |
|                         |                | Pollen shape: perprolate                         |
|                         |                | Aperture: monocolpate (one Colpi)                |
|                         |                | Exine ornamentation: reticulate                  |
The ratio between the length of the polar axis (P) and equatorial diameter (E) significantly varied, namely: 2.82–3.05 µm found in *H. fulva*, 3.02–3.09 µm in *H. lilioasphodelus*, 2.35–2.74 µm in *A. vera*, 2.68–2.74 µm in *L. longiflorum*, and 1.93–1.98 µm in *L. candidum*. The pollen shape includes perprolate except *L. candidum* which has prolate shape [21, 24]. The differences in size and pollen shape cannot be a specific feature in each genus of Liliaceae, because it depends on the pollen’s
maturity and the different of focus in observation. Pollen maturity can be seen from the water content in it. Mature pollen will become dehydrated. Besides, pollen size depends on the shape, generally monocots have spheroidal or ellipsoidal in shape, sometimes pollen can also appear as a boat due to dehydration [25]. In the other hand, some studies showed that pollen size and shape related to environmental conditions [8, 22]. Pollen size and shape showed a significantly positive correlation with annual precipitation, and smaller pollen grains appear to adapt better in habitats with extreme conditions [8].

SEM micrograph of pollen grain in five species of Liliaceae showed that the exine ornamentation was the reticulate pattern, as showed in Figure 1. In subtectate or semitectate grains the tectum may be provided with minute perforations having a diameter of more or less 1.0 pm called punctum and the tectum is referred to as punctum, the diagram illustration can be seen in Figure 2 [21]. The width of such perforation is more and the sexine displays a net-like pattern usually forming a honey-comb like hexagonal meshes (Figure 1), it is called a reticulum [8, 18, 21, 24, 26]. Several investigations have shown positive correlations between pollen ornamentation and pollination mechanisms [27–29]. It is because this exine surface will stick to the stigma. However, the understanding of the controlling factor that determines the sculpturing pattern of the pollen exine remains unclear [30].

**Figure 1.** SEM micrographs of the exine ornamentation in (A) *L. longiflorum* (the result) and (B) *L. lancifolium* [8].

**Figure 2.** Illustrating diagram of the exine ornamentation structure. (A) Layers of exine. (B) Light and dark islands that appear from the high adjustment to low adjustment of the light microscope. 1, 2, 3, & 4 represent the first, second, third and fourth focus of microscope from the high to low adjustment [21].
At maturity, exine (outer) walls of pollen, multilayered, consist of chemical resistant sporopollenin polymers and are interrupted by openings called aperture [28, 31]. Magnoliids and monocots usually produce pollen with a single distal aperture [32] or called as monosulcate/monocolpate [33, 34]. The apertures characteristic of the Liliaceae pollen grains was monocolpate which has one Colpi. There are two types of apertures known as Pores (Porus, pl. Pori) and furrows (Colpus, pl. Colpi. or Sulcus, pl. Sulci) [18, 21]. As well as in L. kesselringianum pollen showed that the pollen was monosulcate or monocolpate, the surface of the aperture membrane was granular [35]. Although all five species in this study have a monocolpate aperture, the type of aperture in the genus of Liliaceae cannot be considered as a sectional feature nor a specific one. As in the case of genus Gagea (Liliaceae) which the aperture is distal-polar (ana-sulcate), elongated, and reaches the proximal side of the pollen with rounded or acute ends [24]. The type of aperture within Lilium spp. is, however, of value in establishing exine [28] and aperture evolution [18].

4. Conclusion

The pollen grains morphology and morphometry in five species of Liliaceae in Indonesia (H. fulva, H. lilioasphodelus, A. vera, L. longiflorum, and L. candidum) have various characteristics. Based on the pollen size, Liliaceae pollen is medium (25-50 µm), except A. vera which has minuta size (10-25 µm). Whereas the pollen shape L. candidum was prolate (1.33-2 µm), while the others were perprolate (≥ 2 µm). The apertures characteristic of the pollen grains was monocolpate which has one Colpi and the exine ornamentation was a reticulate pattern.

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