

Study of Leaf Anatomy and Pollen of *Lilium Mackliniae* (SEALY) Shirui Lily of Manipur

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Abstract: *Lilium mackliniae* (Shirui lily) of the family Liliaceae is one of the rare and endangered plant species. The plant is restricted only in Shirui of Temparate Hill of Ukhrul district, Manipur. The plant has specific character of a flowering plant and well adapted to cool hilly region of the state. For anatomical studies, cross-sections of the leaves, and morphological properties of the pollen grains were investigated. The pollen grains are monosulcate, heteropolar, elliptical in polar view and oblate. Transverse sections of the leaves, nature of stomata were examined. The numerical value showed that *Lilium mackliniae* differs from the other *Lilium*. This study is the first reporting with anatomical and palynological features of Shirui lily of Manipur.

Keywords: Lily; anatomy; pollen; Shirui Hill; Manipur

The identified of leaf anatomy and pollen structure of (sealy) Shirui lily endemic lily species of Manipur showed the specific character of endemic lily plant.

1. Introduction

Lilium mackliniae (Shirui lily) is the rarest lily, very attractive and perennial plant. The liliaceae genus comprises 19 species of 11 genera distributed throughout the hill and valley of Manipur (R.gogoi, 2011). All the species consisting of fragrance, bulbous and perennial herb, form an important and unique flowering ornamental plant. Among the lilies species, *Lilium mackliniae*, commonly known as (Sealy) Shirui lily lovely pink lily is natively growing during the season of May to June in the hill slope and top of shirui hill of Ukhrul District and Dzuko valley of Senapati District Manipur. The shirui lily is Manipur's unique gift for the floral wealth of the world and was discovered by F.Kingdom ward in the 1948. The shirui hill and Dzuko valley area during flowering season is a paradise for tourist visiting side. In the taxonomic and anatomical point of view pollen have a great value. Thus the present study aims to explore the palynological and anatomical properties of *Lilium Mackliniae* of Manipur. The long-standing popularity of *Lilium* as ornamental plants are due to their large, showy flowers that often have a strong fragrance (Woodcock & Stearn 1950). Recently, many taxonomic viewpoints regarding the members and infra generic classification of genus *Lilium* have been put forward. Traditionally, it has been subdivided into 5–11 sections (Endlicher 1840; Baker 1871; Wilson 1925; Baranova 1988) based on the morphological characters such as flower shape and position. Shirui lily has 3-5 pale purple coloured petals with 4-5 cm long, peduncle 3 cm, ovary 4×2 cm with lobe 2.5 cm, style 3.5 cm, stamens 6, in two rows, anther 0.9mm long basifixed brownish red. But a more detailed and acceptable classification was proposed by Comber (1949) with seven sections based on combination of 13 morphological characteristics and two germination types. (The infra generic treatment of Comber (1949) has been supported by some molecular studies of Nishikawa et al. 2001, İkinci 2005, 2011, İkinci et al. 2006, Ronsted et al. 2005, Resetnik et al.

2007, Muratovic et al. 2010b, Lee et al. 2011 and Gao et al. 2012) (It is well known that pollen features have a great taxonomic value, and have been used in the classification of different genera (Troia et al. 2012; Ceter et al. 2013) and also closely related to Liliaceae taxa (Tek, sen et al. 2010; Kameshwari 2011; Masoumi 2012). Palynological studies showed that several features (Kosenko 1999; Muratović et al. 2010a; Pupuleku et al. 2010) and carbohydrate content of pollens in the genus *Lilium* (Clement & Audran 1995) provide important information, there are no records on pollen morphology and contents of lilies in Manipur. Similarly, as stated by Kim & Lee (1990), Kaviani et al. (2008), Dhyani et al. (2009) and Muratović et al. (2010a), the features related to stem and leaf anatomy have considerable taxonomic value in the systematic of *Lilium*. Since then, *L. mackliniae* has been recorded as a new species from Manipur (F.Kingdom ward 1948). These all preliminary studies do not provide detailed anatomical information for Shirui Lily species. Thus, the present study aims; (1) to explore the anatomical and palynological properties and (2) contribute systematic position of Shirui Lily taxa from Manipur. The study has been made to investigate the anatomical and morphological features of these endangered plants. The results reported in this paper is the first report so far made regarding this plant.

2. Material and Methods

The specimens, both pollen and anatomical material (stem and leaf) were collected from the habitat area during the flowering season in 2019. The specimens, was stored in the laboratory room. The materials were fixed in FAA (formaldehyde:acetic acid:alcohol) for 24hr and then preserved in ethanol (70%). Sections of the leaves were cut by free hand and were stained by saffranine for 30 minutes and mounted with Canada balsam/hematoxyline in order to obtain sections were photographed. All images were digitally captured using Fine Pix A350 camera attached to

light microscope. photographed with a camera from the slides. The polliniferous material was removed from anther of the flower and scraping the pollen style to the slide with a needle. A photo was taken with E- scope camera through the ocular lense with magnification of 40x Transverse section of the leaves were analyzed. Fig 3 . The microscope images were captured using an Digital camera attached to a microscope. The image resolution was set at 4800 × 3600 pixels, with a magnification of 8.6 pixels/μm.

Anatomical results

In the cross-sections of the leaves, the epidermis is found at the both of outsides (Fig 2.a,b). The upper and lower epidermis comprises 20μm and 10μm lengths in 10x uniseriate, almost square or oval cells. Both epidermises are covered with a thin cuticle. The size of stomatal pores 8 μm in length and 5 μm in diameter, contrary to the most monocots, occur only on the lower surface (hypostomatic leaf.) As seen in camera, the upper and lower surfaces consist of rectangular epidermal cells; the midrib is circular/triangular shaped. The vascular bundles are collateral type .A small vascular bundle is surrounded by thin wall parenchymatous cells. The mesophyll is homogenous bifacial and consist of single layered of palisade cells and 6-7 layers of isodiametric spongy parenchymatous cells. The midrib is poorly differentiated and it is surrounded by a parenchymatic bundle sheath that extends to the upper and lower epidermis the leaves have anomocytic stomata and found 12-16 in lower epidermis.

Palynological Result

According to our observation the colour of the pollen grain is yellow. The pollen size ranging is 70x50μm. The shape can be 1.μm long ellipsoidal/elliptical in polar view and oblate. The morphological features of pollen grains has been observed by Electronmicroscope The pollen type is monocolpate; pollen shape, boat like; apertures, colpi and long with an end of more or less pointed and pollen length, 700-1200 μm . All pollen grains are monosulcate, heteropolar, elliptical in pollar view and oblate. All Polar axis is (70) μm P/E ratio is 1.4μm. The sulcus width is 0.5.μm narrow deeply rounded ends almost as long as the equatorial axis and length is 0.4.μm. The exine thickness is 2 .04.μm and the thickness ranges from 2.37-2.74 μm. Based on the EM micrograph, the pollen size of *L. mackliniae* exhibited medium size because it has 50–80 μm in diameter. The influence of biological factors varies pollen size from habitat to another.

3. Discussion

Yentir (2003) indicated that the arrangement of mesophyll tissue consist of any uniform cells. In the present study the arm-shaped palisade parenchymatic cells are branched and lobed in the liliium. The leaves have anomocytic stomata. According Hodgson *et.al* (2010) stomata size is positively correlated to genome size across a wide range of major angiosperm taxa , so that the stomata characters are not so useful in the delimitation of the plant taxa, but *L.macklianae* growing in the hilly region of Manipur during the winter season. Most vascular bundle characters can be given some taxonomic significance, as they markedly differ from those of other Liliaceae, e.g., *Fritillaria* (Yembaturova and Gasilina,

2007). The peculiar traits of vascular strands in studied Asiatic hybrids (visually “bicollateral” type, bigger amount of phloem) can result from hybridization and and increased number and size of flowers. The study of pollen features was also examined. As a result of palynological study, it was found that pollen grains are monosulcate which is primitive trait in such plants and occur widely among the monocotyledons(Furness and Rudall 2001). .

The apertures of *L.Mackleneae* were monocolpate which is one Colpi and the exine was a reticulate. P/E is prominent character as reported in several palynological studies (Mackbul *et.al*) 2008. Some palynological characters such as P/E ratio, the sulcus length and the lumina width are more important than anatomical ones in explaining total variation. Pupuleku *et. al* (2010) indicated that pollen size influenced by biological factor varies from one habitat to another. Palynological studies performed on the liliaceae families indicated that some pollen character such as sulcus, muri and lumina are taxonomically useful (Kuprinova 1983, Kosenko 1990). Exine thickness is another important palynological trait of lilies. Kosenko(1999) reported that exine thickness varies 2.2 to 3.7 mμ in some members of liliium. In our observation the exine thickness of *lilium mackliniae* is 2.04mμ Pupuleku *et.al*(2010) determined that exine thickness ranges from 1.8 mμ to 2.3 mμ in *L. martagon*. The characters Anatomy and palynology character can upgrade morphological characters. There is an urgent need to take protection of natural habitats of, Shirui hill and Dzukou valley for conservation measures of these unique flowers.

4. Conclusion

Shirui lily was endemic of Shirui hills of Manipur which falls at Eastern Himalayas wet temperate forest types (Champion & Seth.1968) and its endemism for various ecological factors such as sunny slopes, adequate wind speed throughout the year, soil reach in chromium derived from the weathering of chromium rocks, frost and temperate climate (Sing *et.al*.2005).The liliae has been threatened by high biotic factor i.e grazing, burning & plucking of flowers by the tourist people and climate change. This lily plant is still remains unexplored anatomical aspects showed specific character of endemic lily plant if so far for karyosystematic information conservation. Shirui lily growing in the cold climate and high wind native habitat area of shirui hill of Manipur. The local people, tourist, climate change overexploiting this rare endangered lily flower and is suffering from negligence of government and researcher. It should be conserved with both ex. situ and in situ method of conservation strategies are urgently needed. This lily plant which remain unexplored cytologically. Further cytomorphological aspect mark out the better for further conservation. There has been no efforts made to preserved.

Table 1: EM micrograph of leaf anatomy and pollen grain of *Lilium mackliniae* in Manipur

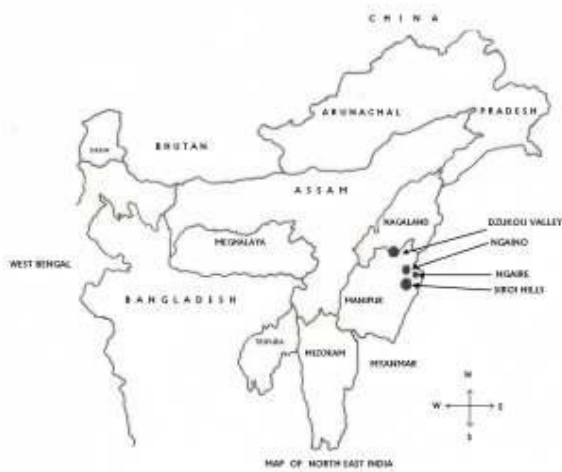
Anatomy of leaf Length of Lower epidermal cells:20.µm Length of upper epidermis:10.µm No.of stomata:12-16 Stomata pore length:8.µm Stomata pore diameter:5.µm Stomata type: Anomocytic
Pollen character P:70x50 µm E:50 µm P/E:1.4 µm Pollen shape:Boat like Pollen size:Medium Pollen type:Monocolpate Aperture:Colpi Exine:2.04.µm Sulcus length:0.5.µm

A photo of shirui lily



(A.A.Mao and R.Gogoi.2013)

Anatomical Result:



(A. A. Mao and R. Gogoi, 2013)

Figure 1: Distribution map of the Table: 1 locality information of Shirui lily

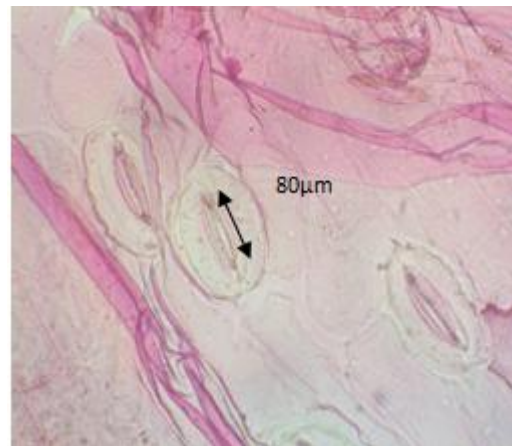


Figure 2 (a): The cross-section of a part of the Shirui Lily leaf containing the stomata (x10)



Figure 2 (b): A part of Leaf Epidermis of Shirui Lily

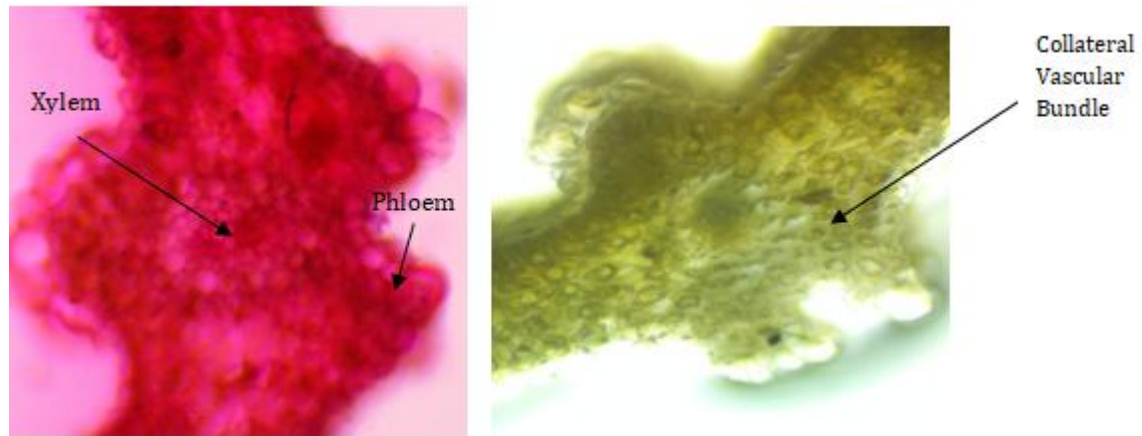


Figure 3: The cross-section of a part of the Shirui Lily leaf containing the various tissues (x10)

Pollen Result

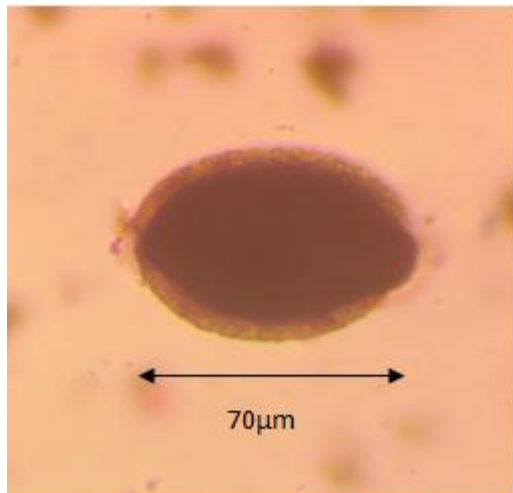


Figure 4 (a): Photo of Pollen of Shirui Lily of Manipur

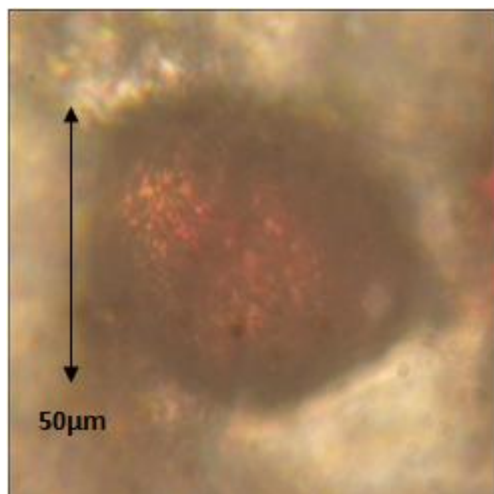


Figure 4 (b): Pollen structure of Shirui lily

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6. Declaration

Paper is containing original and has not been submitted / published earlier in any journal and is not being considered for publication elsewhere

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