

# ***Rafflesia patma* (RAFFLESiaceae): NOTES ON ITS FIELD STUDY, CULTIVATION, SEED GERMINATION AND ANATOMY**

## ***Rafflesia patma* (Rafflesiaceae): Catatan Tentang Studi Lapangan, Budidaya, Perkecambahan Biji dan Anatominya**

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### **Abstrak**

*Rafflesia* spp. (Rafflesiaceae) mempunyai nilai strategis jika ditinjau dari segi ilmiah maupun konservasi. Sampai sekarang tidak banyak usaha yang telah berhasil untuk mengkonservasi jenis-jenisnya secara *ex situ* dan usaha konservasinya terutama dilakukan secara *in situ*. Penelitian yang lebih rinci diperlukan untuk mengetahui hubungan antara *Rafflesia* dengan tumbuhan inangnya untuk meningkatkan pengelolaan dan konservasinya. Studi anatomi, kultur *in vitro* dan perkecambahan biji untuk mengkonservasinya telah dilakukan di Kebun Raya Bogor. Usaha mengkonservasi *Rafflesia patma* di lokasi konservasi *ex situ* telah membuahkan hasil. Namun demikian masih dijumpai tantangan untuk dapat menjaga kehadiran *R. patma* di lokasi konservasi *ex situ* karena diperlukan individu dalam jumlah banyak untuk memperoleh populasi yang lestari.

**Kata kunci:** anatomi, konservasi *ex situ*, kultur *in vitro*, *Rafflesia patma*, studi lapangan

### **Abstract**

*Rafflesia* spp. (Rafflesiaceae) have a strategic value from both scientific and conservation viewpoints. To date only very few attempts have succeeded in growing the species *ex situ* and the main protection measures have been by *in situ* conservation. More detailed studies are required to understand the relationships between *Rafflesia* spp. and their host plants in order to improve their management and conservation. Studies on the anatomy, *in vitro* culture and seed germination in connection with conservation have been conducted in the Bogor Botanic Gardens. Effort to transfer *Rafflesia patma* to an *ex situ* conservation area has produced fruitful results. However, we encountered a bigger challenge to maintain the long term presence of *R. patma* in *ex situ* conservation, since a high number of individuals is required to make the viable population.

**Keywords:** anatomy, *ex situ* conservation, field study, *in vitro* culture, *Rafflesia patma*

### **INTRODUCTION**

*Rafflesia patma* (Blume) (Rafflesiaceae) population have been recorded at the Pangandaran Nature Reserve in the south-east corner of West Java

and Nusa Kambangan (Meijer, 1997). In both areas, they grow in association with the host that recently has been identified as *Tetrastigma scariosum* (Blume) Planch. (Veldkamp, 2009). Due to their restricted occurrence of *R. patma* (the species has been listed

in the Category 'EN' IUCN, 1994) and later under criteria EN 3B (Mogea *et al.*, 2001).

To date, only very few successful attempts have succeeded in growing the species *ex situ* and the main protection measures have been by conservation *in situ*. Continuing efforts, such as attempts to bring *Rafflesia* into cultivation have remained problematical as the species have complex biological requirements which are, as yet only partly understood. *Rafflesia* species are totally dependent on their hosts, *Tetrastigma* spp. (Vitaceae) for water and nutrition as they themselves have no photosynthetic capacities (Nais & Wilcock, 1999). Ultimately, factors affecting *Tetrastigma* will directly affect *Rafflesia*, however, the relationship between these two partners is poorly understood.

Meijer (1997) maintains that the total life cycle of *Rafflesia* is about 3-4.5 years, therefore more time is needed to finalise the whole experiment. Motley (1857) wrote that Binnendijk at Buitenzorg garden able to germinate *Rafflesia arnoldii* on *Cissus scariosa* only in 9 months, but Veldkamp (2007) observed that it took 18 months and 5 days.

Research on *Rafflesia patma* has been carried out since 2004, both in the field and in the laboratory. Field work at Pangandaran Nature Reserve was conducted for population and biological study of this species in its natural habitat. It was

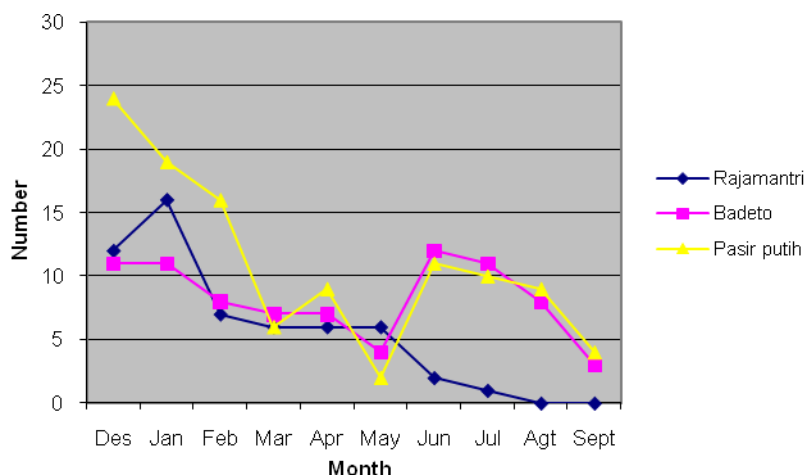
followed by trials on inoculation of *Tetrastigma scariosum* with *R. patma* seeds, both in its natural habitat and in Bogor Botanical Garden ground also in the laboratory. Laboratory investigation includes anatomical study of the early bud developmental growth on the host plant. *In vitro* studies of *Tetrastigma* and *R. patma* seeds were also undertaken to understand the development of seeds under controlled condition. Effort to cultivate *R. patma* was carried out also through grafting experiment of *T. scariosum* in the natural habitat in the field as well as in the Bogor Botanic Gardens.

## PROGRESS OF THE STUDY

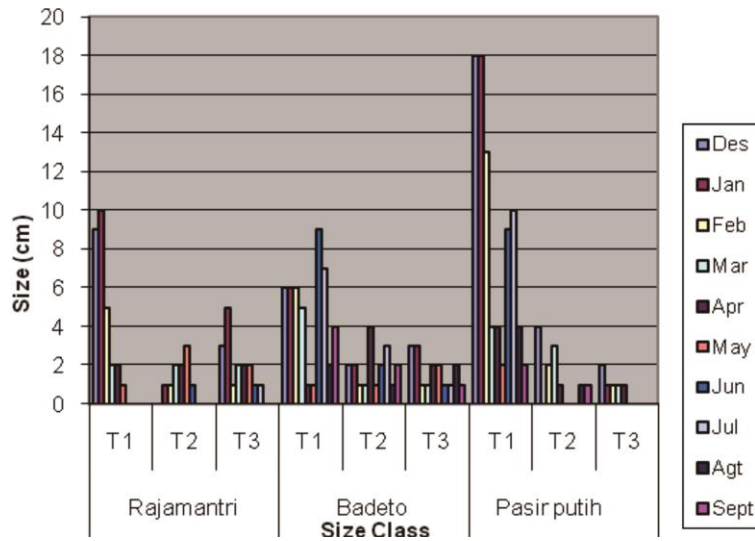
### a. Population Study and Field observation

A population study of *R. patma* at Pangandaran Nature Reserve in West Java was conducted in 2004-2005. The investigation had to be done very carefully, since the roots and stems of the host could easily be damaged by the investigators during observations. The number of buds found from Rajamantri, Badeto and Pasir Putih shown in Figure 1.

While the sizes of the buds at three stages (T1, T2 and T3) as shown in is shown in Figure 2. And Figure 3.



**Figure 1.** The number of buds in 3 colonies of *R. patma* in their habitat during observation (December 2004 – September 2005)



**Figure 2.** Size class of *R. patma* buds in 3 colonies during observation (December 2004 – September 2005)



**Figure 3.** Development of *R. patma* from bud to the beginning of flower opening during the period of December 2004 and September 2005 at the Pangandaran Nature Reserve.

The *Rafflesia* seems very sensitive to disturbance caused by the presence of investigators as indicated by many buds failed to grow further and died at different stages of bud development. New buds, mature buds or flowers of *R. patma* were found at any time of the year, but the natural mortality rate was observed to be very high also. It is implied that a demographic study in their natural habitat could be biased by presence of the researchers.

In its natural habitat, *R. patma* has been always found to be associated with more or less well-developed *Tetrastigma scariosum* plants, whose

stems or roots were more than 3 cm in diameter. In one field survey, young host plants naturally infected with *R. patma* were found and monitored. It was observed that they did not survive long as both *R. patma* and the host plants became dry and died.

The reason for their death was not clear. It could have been due to an unfavorable weather or other factors, but it is also likely that the host plant could no longer support the growth of the parasite. On July 2006, part of the habitat was severely damaged by the tsunami and wiped out the *R. patma* and its hosts.

**b. *In vitro* culture of *R. patma***

*In vitro* culture could be an answer to grow a difficult plant. *In vitro* culture has been tried for *Rafflesia patma*, but with limited success. Explants of *R. patma* were tried from different parts of the flower:

1. Parts of the disc tissue were cultured on Murashige & Skoog (1/2 MS) medium. No sign of growth was observed after 20 weeks.
2. Root pieces containing tiny buds of *R. patma* were cultured on ½ MS medium supplemented with 4 ppm BA and 0.5 ppm NAA. One of the explants enlarged, but did not develop further after 3 weeks

To date, there is no strong indication that the tissue culture technique is a suitable method to propagate *Rafflesia*. This is apparently due to the unreplaceable role of the host plant by the regular artificial media. Sterilization problem was one of the constraints in transferring the explants from the roots.

**c. *In vitro* culture of *R. patma* and *T. leucostaphyllum***

Seeds of *T. scariosum* (Tali koja) and *R. patma* were cultured separately in ½ MS medium. The seeds of *Tetrastigma* were easy to grow. When roots of *Tetrastigma* were formed, (after 2 weeks in culture) the seeds culture of *R. patma* were then introduced. Seeds of *Tetrastigma* developed vigorously into plantlets and already hit the lid of the jar (height 11 cm) within 3 months. However no further development was observed until the end of the experiment (24 weeks). In this case, the containers were too small to accommodate the growth of the host plant. Maintaining both host and parasite seeds by routine sub culturing would not be easy as it increases the risk of contamination especially when the tiny *Rafflesia* seeds had already established on the roots.

*In vivo* culture by inoculating the seeds of *R. patma* to the stems of the host plants have also been tried both in the field (Pangandaran) and in the Bogor Botanic Gardens. Infected and non-infected *T. scariosum* were chosen as hosts for the seeds. There was no sign of development after 652 days and 218 days both in Pangandaran and in Bogor Botanic Gardens respectively. The harvested seeds from both sites after 652 days (Pangandaran) and 218 days (Bogor Botanic Gardens) were observed under the microscope. All seeds from both sites were intact and remained turgid, green algae covered the surface of some seeds, especially those from the natural habitat. No interaction between the seed and the host plant was observed, even for the seeds inoculated in the infected host plant in the field (Pangandaran). Apparently, interactions between *R. patma* and its host plant are not easily understood, very complicated and involving specific physiological conditions. Environmental factors that directly affected the host as well as the *Rafflesia* may have added to the complexity of the interactions.

**d. Seed germination study of *R. patma***

The fresh seeds of *R. patma* were cultured in many different media, including those containing extracted bark of the host plant. Strigol (GR-24 = a germination stimulants for parasite in cotton) was added in low concentrations (0.05 – 1 ppm) to the media to stimulate germination, but no sign of growth until the end of the experiment. The experiment, therefore, still needs to be explored further.

Observation of the seeds cultured after 352 days showed that the seeds have imbibed water but did not grow further. An agar medium containing nutrition has induced growth and development of seeds of various plant species growing in the Bogor Botanical Garden, but it did not do so on seeds of *Rafflesia patma*. This indicates that growth of *Rafflesia* seeds needs very specific requirements. Supplementing the extract of *T. scariosum* bark and Strigol (GR-24)

did not give promising result. It is possible that, as a holoparasitic plant, *Rafflesia* can only be grown in a living host and depends on exudates it produces. Introduction of a mycorrhizal fungus to induce seeds germination should be explored as such technique has been successful in germinating many species with minutes seeds, such as Orchidaceae (Rasmussen, 1995).

#### e. Anatomical study of infected host tissue

An anatomical study of infected tissue was made to reveal early establishment and degree of invasion during its growth and development. The early stage of successful interaction with its host plant is critical for the establishment of *Rafflesia*. Anatomical samples of young buds of *R. patma* were prepared using the standard paraffin method. Serial images showed that early establishment of *R. patma* took place through different phases, starting with the penetration of the host plant by a thread-like cell, followed by the development of parasitic cells to establish the parasite inside the host plant. However, more samples are required to be able to follow the chronological sequence of the development. There are still missing gaps as the samples were selected only by morphological size and appearance.

#### f. Grafting

Cultivating *R. patma* on its host plant was carried out by several techniques, including removal of whole plants and grafting. Monitoring of both host and *R. patma* was made to assess their survival capacity in their new habitat in the Bogor Botanic Gardens. Both techniques were attempted from the beginning, but maintaining the balance between the host and the parasite became very difficult as environmental factors seemed to play an important role and uncontrollable. To date, relocation of *R. patma* from its natural habitat to the garden in Bogor was not successful.

The grafting experiment was more promising. This technique was applied to the host plants both in Bogor and in the natural habitat in Pangandaran. First attempt was, grafting of *Tetrastigma scariosum* from Bogor Botanic Gardens collection to an infected root stock of *T. scariosum* growing in natural habitat on August 2004. It was expected that in three months the new roots would have grown and the plant would have been then ready to be transported and transplanted in the new location while *R. patma* inside the host was not too affected by the treatment. After three months the grafted host plants containing *R. patma* bud (diameter of 1 cm) were removed and transported to Bogor. The root pieces of the host plant were carefully removed by digging its surroundings and a plastic bag was placed beneath the grafted point. The host plant was still alive, but the parasite persisted only for 3 weeks and did not survive further. After six years (August 2010), however, eight buds emerged from the new roots grown in Bogor Botanic Gardens and two of them have bloomed in the garden on June 2<sup>nd</sup> and July 21<sup>th</sup> respectively, both were female flowers.

The second successful experiment was carried out in Bogor on February 2007, where the (infected) roots of the host plant (*T. scariosum*) from the original habitat in Pangandaran was grafted on to the host plant (*T. scariosum*) in Bogor. Although the parasite did not survive, it lasted longer compared to the bud on the first experiment. It appeared that the grafted area united immediately and new roots started to grow and ramified, while the root diameter has grown larger than that at the original site. On June 2009 a new bud was detected on the stem and root right beside the grafting point on the root stock. The bud developed into a male flower, bloomed in the garden on June 20<sup>th</sup> 2010.

Grafting method gives a new hope for an effort of *ex situ* conservation of *Rafflesia*. The future challenge is, however, even bigger. The

unisexual flower of *R. patma*, either male or female, could not reach further the fruiting stage, since the pollination has not been observed to occur.

## CONCLUSION

The successful attempt to bring *R. patma* into *ex situ* cultivation after six years is a small step leading towards larger scenario to make an established population. Lesson from this initial successful attempt provides the foundation for more challenging undertaking to carry out human-induced and natural pollination experiments in an effort to make the population viable.

Studies in conservation biology of *Rafflesia* still need to be continued as many biological and ecological aspects of this taxon remain unknown, while the habitat is rapidly disappearing.

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## REFERENCES

- Meijer, W. 1997. Rafflesiaceae. *Flora Malesiana I*, 13: 1-42. Rijksherbarium/Hortus Botanicus, Leiden.
- Mogea, J.P., D. Gandawidjaya, H. Wiriadinata, R.E. Nasution & Irawati. 2001. *Tumbuhan Langka Indonesia*. Pusat Penelitian dan Pengembangan Biologi – LIPI. Bogor
- Motley, J. (1857). Botanical information. *Hooker's Journal of Botany and Kew Miscellany* 9: 148–153.
- Nais J and C.C. Wilcock 1999. The propagation of *Rafflesia* and its host plant: prospects for *ex-situ* conservation. *Sabah Parks Nature J.* 2. 111-120.
- Rasmussen, H.N. 1995. *From Seed to Mycotrophic Plant*. Cambridge Univ. Press. Cambridge
- Veldkamp, J.F. 2007. Some notes on the cultivation of *Rafflesia*. *Flora Malesiana Bulletin* 14: 50-53.
- Veldkamp, J.F. 2009. Notes on the names of the *Tetrastigma* (Vitaceae) host of *Rafflesia* (Rafflesiaceae). *Reinwardtia* 13 (1): 75-78.