INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 7, Issue 4, October-December 2016: 1153-1160 www.ijcs.uaic.ro

# HAND-POLLINATION OF THE GIANT CORPSE FLOWER IN THE BOGOR BOTANIC GARDENS

SUDARMONO*, Dian LATIFAH, Sri HARTINI, Hary WAWANGNINGRUM

Centre for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences
(Lembaga Ilmu Pengetahuan Indonesia; LIPI),
Jl. Ir. H. Juanda No. 13, P.O. Box 309, 16003 Bogor, West Java, Indonesia

***Abstract***

*The remarkable inflorescence of Amorphophallus titanum (Becc.) Becc. ex Arcang (the giant corpse flower) has always attracted world-wide attention, especially from people involved in the plant sciences. However, the characteristics of its reproductive biology have challenged efforts to cultivate and domesticate the plant. The species rarely produces fruit/seeds because the male and female flowers do not mature simultaneously. The success of pollination, as indicated by subsequent fruit production, depends on the interaction between insects and the mature male and female flowers from different individual plants. Therefore this study on cross-hand pollination to produce seeds is very important to support the ex-situ conservation efforts of this species in collections. Based on observation, flowering of the species occurs at least once every three year. An inflorescence of A. titanum which opened in Bogor Botanic Gardens on 2 February 2012 was pollinated manually using (stored) pollen taken from another plant, which had bloomed on 29 November 2011. The hand cross-pollination was successful and the fruit (infructescence) produced on 22 February 2012 marked the first success for manual pollination of this giant aroid in Indonesia. In this research, the morphology of pollen of A. titanum was carefully observed and its quality of stored seeds was tested.*

***Keywords:*** *Amorphophallus titanum; Bogor Botanic Gardens; Giant inflorescence.*

## Introduction

Wild plants can be used as a source of food and medicine, and for many other purposes in life. Wild plants are generally no less rich in nutrients than are the plants that have entered into human cultivation. Sometimes the nutritional value of wild plants is higher than many well-known farmed vegetables and fruits. Because of this, in many cultures, wild plants are often a preferred food item.

People have recently realized the importance of such plants in rural economies and have recognized their strategic potential in tackling food in security. Thus, there has been a revival of interest in the survey, identification and documentation of wild edible plants during recent decades. Throughout the world, it has become an important scientific pursuit to determine the nutrient content of plants found in the wild and to assess the value of our forest genetic resources. *Amorphophallus titanum* (Becc.) Becc. *ex* Arcang, with its giant inflorescence is of potential economic interest because of its giant underground tuber [1].

* Corresponding author: s_darmono@yahoo.com

*Amorphophallus titanum* (from the Ancient Greek: *amorphos*, "without form, misshapen"; *phallos*, "phallus", and *titan*, "giant"), known as the Titan Arum/ Giant Aroid, is a flowering plant with the largest unbranched inflorescence, which is produced by the largest tuber in the world [2]. The spathe is approximately 3m in circumference, pale green and spotted white on the outside and rich dark crims on colour on the inside. The spadix is up to 2m tall, dull yellow in colour, hollow, and expanded at the base. The swollen underground storage stem (tuber) can weigh more than 75kg [3]. This species is an important plant but has become endangered in the wild as a result of deforestation. Many of its habitats have been destroyed to such an extent that they can no longer sustain the viability of many native species. The relatively small area of remaining habitats of the giant aroid requires protection efforts to preserve this rare and irreplaceable plant species.

The species has also an economic value, as people traditionally have used the giant aroid for food and medicinal purposes [4]. The tubers of the giant aroid contain more glucomannan than those of its relative, *Amorphophallus konjac* K. Koch (20%) which is very popular in Japan as a cooking supplement for soups and stew-like dishes. Clinical study indicates the glucomannan may assist in weight reduction, and in reducing cholesterol in consumers who have high cholesterol. It is eaten in Japan to clean the digestive tract of toxins. Glucomannan can also be used as a supplement to treat constipation, obesity and diabetes [4]. Unfortunately, in Indonesia, the cultivation of the giant corpse flower has not been developed, because the success of its pollination, on which fruit production depends, requires interaction between insects and the mature male and female flowers from different individual plants.

The success of the hand pollination on *Amorphophallus titanum* has been achieved in many countries worldwide. There are eight records of cultivated *A. titanum* that are hand pollinated successfully producing seeds previously in 1992 and 2000 (Palmengarten, Frankfurt), in 1996 (University Botanic Gardens, Bonn), in 1999 (Huntington Botanical Gardens, California), in 2003 (Fairchild Tropical Garden, Florida), in 2004 (Botanic Garden University of California, Davis and Botanic Garden University of Washington, Seattle) and in 2005 (Royal Botanic Gardens, Kew). But the more detail information on the productivity of fertile seeds in those countries were not available.

Many biological aspects of this plant need further study. A mature, bucket-shaped corpse flower emerges from a huge underground storage tuber once every one to three years. Based on the results of previous observations, the giant aroid begins to bloom ten years after growing from seed, by which stage its tubers have already reached 20-25kg in weight [5]. In a study by [6], a tuber increased in weight from 36kg (year 2000) to 78kg (2003) and then up to 117kg (2006). W. Lobin *et al*. [6] reported that the heaviest tuber was 72.6kg.

The flowering development in the giant aroid requires quite a long span of time, at least three to four years [7]. A critical phase in the life cycle of these plants is the dormancy phase, because the following phase can be either vegetative or generative.

The successful cross-pollination by hand, an outcome of there search described in this paper, is an important pre-requisite for further investigations into the inflorescence induction processes in the species; in particular into the processes that regulate the giant aroid's life cycle between vegetative, dormant, and generative phases within a three year period. Moreover, it is expected that plants sewn directly from seeds in cultivation can be manipulated to bloom at an age less than ten years.

The research reported here investigated the morphology and the quality of stored *A. titanum* pollen, and the factors influencing the success of hand pollination between two individual plants in Bogor Botanic Gardens and of subsequent fruit and seed development.

## Materials and Methods

An *A. titanium* plant (number: VI.C.484; collected from Lahat, South Sumatra) that bloomed on 2$^{nd}$ February 2012 in Bogor Botanic Gardens was pollinated by hand with pollen of VI.C. 485 (origin: Lahat, South Sumatra) that had previously bloomed in the Gardens on the 29$^{th}$ November 2011. Hand pollination was carried out when the opening of the spathe of VI.C.484 was completed in the night at 9:30 p.m.; at the time when the carrion smell was getting stronger and the female flowers were receptive as indicated by the sticky surfaces of the flower stigmas. The hand cross-pollination was successful, and the fruits (infructescence) produced here on 22$^{nd}$ February 2012 marked the first success for manual pollination of this giant aroid species in Indonesia. The pollen samples of VI.C.485 had been stored in a refrigerator at 5°C for 4 months after collection from its blooms in November 2011. The morphology of the pollen grains were identified using a scanning electron microscope (Hitachi SU3500). The pollen samples were mounted on a microscope slide and examined for their pollen quality (fertile or unfertile) using a light microscope (Olympus series BX53M).

The method used in the staining of pollen, was soaking in Anyline Blue dye for periods of various duration as treatments. The purpose of coloring with Anyline Blue dye dissolved in distilled water was to examine which pollen grains were fertile and which unfertile. Tested the quality of stored pollen grains by staining with Anyline Blue 0.1% (w/v) in 108 mM K3PO4 (pH ~11) [8].

This method helped to determine the feasibility of using this stored pollen for hand-pollination when fertile receptive female flowers became available in VI.C. 484.

This process of testing for pollen fertilty was performed on two replicates of four durations of submersion in the dye: 15, 45 and 60 minutes, and 90 minutes (after which, the pollen fertility did not vary anymore). After the pollen samples were placed on a preparation slide, they were covered with a glass cover attached by glycerin jelly as an adhesive and with paraffin applied to every corner of the cover glass. The final step was done carefully to minimize the possibility of bubbles arising. Data were analysed, utilizing the GLM (General Linear Model) procedure in STAR ('Statistical Tool for Agricultural Research', IRRI) to determine the significance between pollen-fertility test treatments.

## Results and Discussions

### *Pollen Staining and Fertility*

The highest percentage (98.91%±0.80) for observed fertility appeared to be after staining for 60 minutes (Fig. 1), although the difference from other staining treatments was not statistically significant (see Table 1). It suggests that the pollen can be stored at a temperature of 5°C for 4 months and still maintain high fertility. The pollen (extracted from VI.C. 485) had been stored for two months in the refrigerator (with 100% viability, tested by soaking in Anyline Blue dye for about 15 minutes).

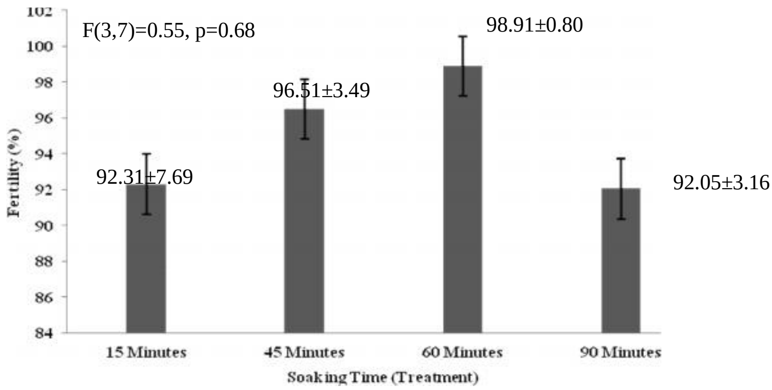


**Fig. 1**. The fertility of *A. titanum* pollen after four months, as assessed after four different durations of staining in an Anyline Blue Dye preparation.

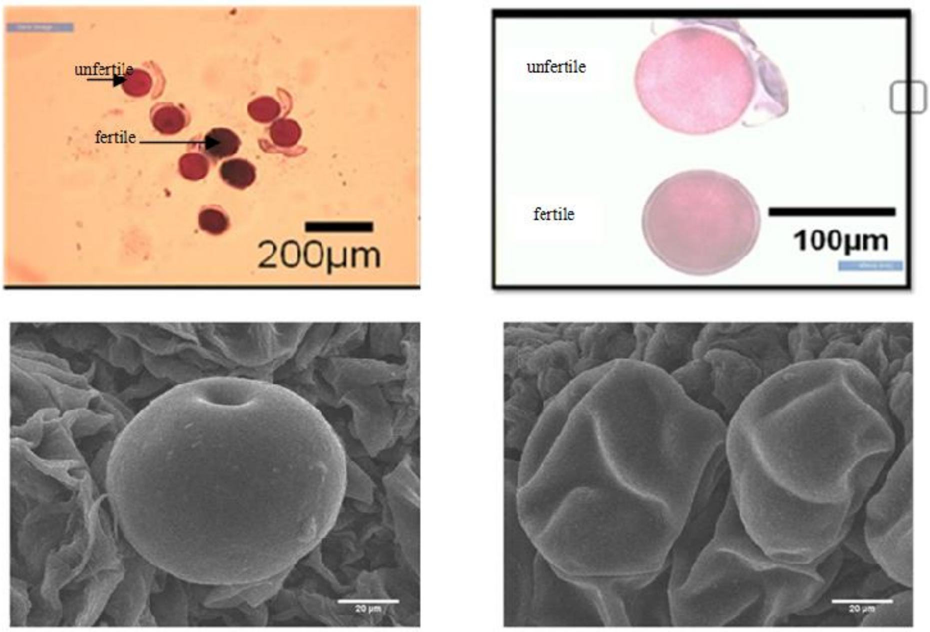


**Fig. 2**. Stained pollen of *A. titanum* showing light colored grains with collapsed exine, determined as 'unfertile', and dark pollen grains as 'fertile'(above); scanning electron of pollen *A. titanum* with 'fertile' (left) and collapsed 'sterile' (right) grains (below).

Pollen of *A. titanum* has a diameter of 70-100µm, while the shape is spheroid (Fig. 2). Pollen considered sterile is unable to complete the fertilization process.

The pollen of *A. titanum* is categorized as spheroidal with polar and equatorial size ratio approximately 1; i.e. 0.94-1.14 [9]. The pollen wall thickness affects the pollen's tolerance of the process of storage. The pollen walls consist of two layers: the outer layer (the exine) and the inner layer (the intine) [10]. In this species, the durability of the pollen is very high because it has a hard exine (although thin) and is chemically not easily destroyed by microbial activity, the level of salinity, wet conditions, low oxygen, or droughts [11].

***Hand- pollination and Fruit Development***

The success of our hand pollination resulted in approximately 50% fruit set, i.e. 110 fruits containing seeds. The fruit took six months to ripen from the time of pollination [12]. The form of the infructescence was bent over. This was due to the fact that manual cross-pollination produced a distorted fruit set compared to natural pollination in the wild, as a result of fungal attack from *Fusarium solani* [13].  It has been found by others that an *A. titanum* infructescence is able to produce more than 500 fruits in cultivation [6].

We did not expect the outcome of hand-pollination to be detectable within the first week. On 22nd February 2012, approximately three weeks after pollination, the infructescence was reddish-yellow, and by the next month the tips of the styles had dried and shrunk. One month later, as shown in Figure 3, the bases of the styles had expanded and the successfully pollinated pistils were orange in colour while those not successfully pollinated had decayed. In the second month after hand pollination, part of the infructescence was infected with a fungal rot.

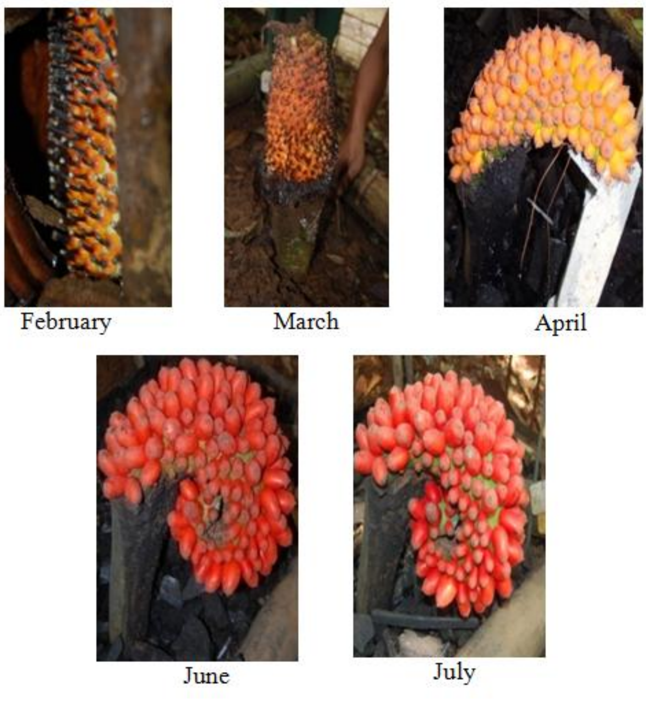


**Fig. 3**.The development of *A. titanum* fruit up until harvest in July 2013, after hand pollination in February 2013.

We smeared the rotten parts with charcoal but they remained moldy. Subsequent treatment, using a bio-pesticide, was applied by spraying every week. The Biopesticide was produced by the 'Centre for Research of Spice and Medicinal Plants' and containing lemongrass leaf essential oil and a neem leaf extract. This treatment was effective in inhibiting development of the fungal rot. Charcoal was also added to the soil surface around the plant (VI.C.484) to reduce excessive soil moisture. The charcoal application kept the peduncle green (fresh), and the infructescence grew bigger. The curved, decaying part of the infructescence was cleaned gently by applying 70% alcohol. The size of the seeds appeared to increase after three months (in May) and the peduncle curved more downwards. In the fourth month (June to July), the fruit color gradually became red and shiny. The fruit, ranging in dimensions, were harvested on 27 July 2012. There were only 110 fruit because more than 100 fruitlets failed to produce seeds. The smallest fruits were 1.5×2×1.5cm (diameter, length and width respectively) while the largest were 3x5x4 cm.

The fruit productivity was 51.16% as the number of the fruit was 215 at first recording but only 110 fruitlet remained until ripened (Figure 3). This contrasted with a plant observed in the rainforest margin at Sungai Talang Hills, Dusun Sungai Manau, Sungai Kalu Dua Village, Kerinci Seblat National Park, Resort Batang Suliti, Kota Parik Gadang di Ateh Sub District, Solok District, West Sumatera Province. That plant produced 762 fruit in total observed in 2000 by R.S. Purwantoro and team [13]. The length of the fruits in our hand-pollinated infructescence ranged from 0.77 to 3.41cm [12] and they were similar between three zones of the infructescence (lower, middle and upper) [$F(2, 21) = 0.44$, $p = 0.65$]. The diameter ranged from 0.64 to 2.29cm, and there were also no differences from lower to upper zones $F(2, 21) = 2.69$, $p = 0.07$). On the other hand, fruit weight was different between zones due to differences in their seed contents $F(2, 214) = 3.23$, $p = 0.04$). The weight ranged from 0.25 to 20.12g. The fruit contains 1-2 seed. A few fruit did not contain seed. The heavier fruit of the lower-middle zones indicated that they were closer to the tuber supplying nutrition [12].

There were only 110 fruit containing 1-2 seeds [12]. D. Latifah and R.S. Purwantoro [15] and M.M. Fayyaz [16] also recorded that the fruit usually contain 1-2 seeds. Ninety seeds were measured: the seed length ranged from 1.63 to 3.95cm, the diameter from 1.00 to 2.13 cm and the weight from 0.45 to 6.84g.The seed dimensions can reach 3.80 cm long and 2.42 cm wide [16]. The fruit containing seeds were usually large; approximately 3.07–4.51cm in length, 1.62–3.25cm in diameter and 4.40–20.12g in weight [12].

***Fruit and Seed Morphology***

The fruit is ovoid [17]. Pericarp or fruit skin is a thin layer approximately 1 mm, orange-red to darker orange-red in colour. The fruit parts may contain a volatilized essential oil or calcium oxalate crystals that may cause a mild-throat reaction or other irritation problems, and potentially causing a kidney problem; however, further studies are required [18]. Many parts of *Amorphophallus* spp. plants contain oxalic acid deposited as calcium oxalate crystal [18]. The first time for harvesting our fruit was on March 2015, ten months after pollination [12], whereas in Bonn Botanic Garden the growth and ripening of fruit took 7-8 month from pollination [6]. The ripened fruit in Bonn Botanic Garden was red, globose to ellipsoid; the endosperm was juicy and tasted sweet. Fruit size was 1.5×1.4 to 3.6×2.5 cm; the big sized fruit mostly set good quality of seed, while the small ones lacked seed [12]. The germination capability of the seeds produced will be published elsewhere.

The seed quality did not necessarily depend on the size of the fruit; the bigger fruit did not always produce bigger seeds; sometimes the pericarp was thicker. The seed shape is also ovoid. The seed coat is soft and smooth after cleaning off the remaining fleshy endocarp during seed extraction. Therefore the embryo is fragile. The seeds of *A. titanum* germinate after 42-56 days [19] or 12-91 days [15], with a germination capacity of 80%. This may suggest that the dormancy period varied from 42 to 91 days, caused perhaps by remnant fruit flesh on the seed testa [15]. The early growth stage, of the emergent seedling of *A. titanum* has a vegetative phase supporting photosynthesis, leading to the production of photosynthate such as starch that contributes to tuber enlargement [14].

## Conclusions

Storage in a refrigerator at a temperature of 5°C kept the pollen of *A. titanum* fertile with high percentage fertility, of 100% after two months, and 98.91% after four months. Hand pollination succeeded with approximately 50% fruit set, i.e. 110 fruits containing seeds. Fruit took six months to ripen (from the time of pollination). However, further investigations will be required to achieve consistent success in hand-pollination of *A. titanum* in the future.

## Acknowledgements

We would like to thank Harto, Aulia and Mimin for their assistance in this work. The authors are also thankful to Dr. Graham Eagleton (of Australian Volunteers for International Development) for English language correction of the manuscript. This research was funded by Inhouse Research PNBP Bogor Botanic Gardens, budget year 2011.

## References

[1] N. Sugiyama, E. Santosa, **Edible *Amorphophallus* in Indonesia – Potential Crops in Agroforestry,** Gajah Mada University Press, 2008, p.45.

[2] D. Gandawijaya, S. Idris, R. Nasution, L.P. Nyman, J. Arditti, *Amorphophallus titanum* (Bec.) Bec. : A historical review and some recent observations**, Annals of Botany**, **51,** 1983, pp. 269-278.

[3] R. Sholihin, R.S. Purwantoro, Pertumbuhan vegetative pada *Amorphophallus titanum* (*Becc*.) *Becc.di Kebun Raya Cibodas***, Biodiversitas**, **6(**3), 2005, pp. 190-193.

[4] W. Ananto, Potentials of Giant Corpse Flower Species (*Amorphophallus* Bl.) in Bengkulu Province as Glucomannan Source, **Research Report**, Faculty of Agriculture, University of Bengkulu, Bengkulu, 2000.

[5] W. Hetterscheid, S. Ittenbach, "*Everything you always wanted to know about Amorphophallus, but were afraid to stick your nose into*!!!!!", **Aroideana**, **19**, 1996, pp. 7-131.

[6] W. Lobin, M. Neumann, M. Radscheit, W. Barthlott, *The cultivation of Titan Arum (Amorphophallus titanum) – A flagship species for botanic gardens***, Sibbaldia, 5,** 2007, pp. 69-86.

[7] C. Giordano, *Observation on Amorphophallus titanum (Becc.) Becc. Ex Arcangeli in the forest of Sumatra***, Aroideana, 22**, 1999, pp. 10-19.

[8] T. Mori, H. Kuroiwa, T. Higashiyama, T. Kuroiwa, *Generative Cell Specific 1 is essential for angiosperm fertilization*, **Nature Cell Biology, 8**(1), 2006. pp. 64-71.

[9] W. Punt, S. Blackmore, S. Nilson, A. Le Thomas, **Glossary of Pollen and Spore Terminology**, LPP Foundation, Utrecht, 1994**.**

[10] D. Walker, **Studying Pollen,** Available at :http://www.geo.arizona.edu/palynology/pol_pix.html, [accessed on 27 January 2007].

[11] P.D. Moore, J.A. Webb, M.E. Collinson**, Pollen Analysis,** Blackwell Scientific Publication Oxford, 1991.

[12] D. Latifah, Sudarmono, H. Wawangningrum, S. Hartini, D.M. Puspitaningtyas, E. Munawaroh, *Fruit Productivity and Morphological of Giant Corpse Flower [Amorphophallus titanum(Becc.) Becc.ex Arcang.]*, **Proceedings International Conference on Tropical Horticulture,** Pusat Penelitian dan Pengembangan Hortikultura, Kementerian Pertanian, Jakarta, 2014.

[13] H. Wawangningrum, D. Latifah, S. Hartini, Sudarmono, *Identifikasi Patogen pada Bunga Bangkai (Amorphophallus titanum (Becc.)Becc.) Koleksi Kebun Raya Bogor dan Upaya Pengendaliannya*, **Prosiding Seminar Inovasi Florikultura Nasional 2013**, Pusat Penelitian dan Pengembangan Hortikultura, Jakarta, 2014 (Indonesian).

[14] D. Latifah, R.S. Purwantoro, *Seed Germination of The Corpse Giant Flower Amorphophallus titanum***, Berita Biologi, 14(**1), 2015, pp. 39-47.

[15] D. Latifah, R.S. Purwantoro, Solichin, *Perkecambahan biji Amorphophallus titanum Becc.: Pengaruh berbagai media dan konsentrasi $GA_3$*, **Widyariset, 2**, 2001, pp. 135-46. (Indonesian).

[16] M.M. Fayyaz, ,*Amorphophallus titanium***, Association of Education and Research Green House Curators Newsletter, 15**, 2003, p. 1.

[17] G.C. Kite., W.L.A. Hetterscheid, M.J. Lewis, P.C. Boyce, J. Ollerton, E. Cocklin, A. Diaz, M.S.J. Simmonds, *Inflorescence odours and pollinators of Arum and Amorphophallus (Araceae)*, **Reproductive Biology**, 1998, pp. 295-315.

[18] C.J. Prychid, R.S. Jabaily, P.J. Rudall, *Cellular ultrastructure and crystal development in Amorphophallus (Araceae)*, **Annals of Botany**, **101**, 2008, pp. 983-95.

[19] Roemantyo, *Konservasi ex-situ Amorphophallus titanum Becc. di Kebun Raya Bogor*, **Bulletin Kebun Raya Indonesia, 7(**2), 1991, pp. 45-49. (Indonesian).

*Received: March, 23, 2016*
*Accepted: November, 20, 2016*