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Submission date: 13-Jul-2022 09:21PM (UTC+0700)
Submission ID: 1870072556
File name: 7-Artikel-PudjiW-Bangladesh-Jur-Bot-Jun2022.pdf (486.75K)
Word count: 4906
Character count: 25364

Bangladesh J. Bot. **51**(2): 313-323, 2022 (June) DOI: https://doi.org/10.3329/bjb.v51i2.60429

MORPHOLOGICAL AND PHENOLOGICAL CHARACTERIZATION OF *HEDYCHIUM BORNEENSE* R.M.SM OF BORNEO AND ITS CONSERVATION MANAGEMENT

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Keywords: Hedychium borneense, Morphology, Phenology, Conservation and morphological management

Abstract

Morphological, phenological characterization and conservation management of *Hedychium borneense* R.M.Sm, a terrestrial-epiphytic herb and endemic to Kalimantan, Borneo are described. Results revealed that *H. bornensee* has a purple-brownish bractea, a terminal and raceme inflorescence, pale yellow flowers with a broad and pale-yellow labellum and orange stamen, a triocular fruit with an orange to red inner color, and brownish seeds with a dark red arillus. There are six phenology phases: initiation phase, flower development phase, pre-anthesis phase, anthesis phase, post-anthesis phase, and fruit development. Flower development lasts for 34 days, with a two-day anthesis phase in between. The ripe fruit occurs on day 94. It grows best in porous, moist environment; sphagnum moss and sand mix compost (1:1) is the best media for this plant. Since the plant is endemic, ex-situ conservation management through generative propagation is observed to be essential.

Introduction

The Indonesian people are well-versed in the benefits of the Zingiberaceae family, which includes medicinal, food spices, ornamental plants, and even natural dyes. *Hedychium* Koenig is a genus of Zingberaceae that has been shown to have therapeutic properties. *Hedychium borneense* is a Hedychium species having a limited natural distribution. It is a native species of Borneo that can be found in both high mountain and lowland forests (Lamb *et al*. 2013).

R.M. Smith was the first to introduce *Hedychium borneense* in 1990 (Smith 1990). This species has been found in Sabah (Smith 1990, Gobilik and Yosoff 2005), Center Kalimantan (Trimanto *et al*. 2019), and East Kalimantan (Fiqa *et al*. 2019), but the authenticity of the specimens has to be verified. Until now, research on *H. borneense* is limited. Several studies have been conducted, including those on morphology and anatomy (Benedict 2012, Gevu *et al*. 2017), propagation (Trimanto *et al*. 2019), and DNA- (Wood *et al*. 2000, Lin-Chun *et al*. 2011), and even then, not specific to *H. borneense*. The IUCN (2019) redlist notes that *H. borneense*'s status is Data Deficient, implying insufficient knowledge and data to estimate the risk of extinction based on population status and distribution. Meanwhile, the conversion of forest land in Kalimantan is causing more and more natural habitats to be affected. That is why a detailed investigation of *H. borneense* is required. Information of complete phenology on *H. borneense* are not available in the previous studies. This information is very essential to determine the fertilization time of *H. borneense*.

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Based on the studies of (Badeck *et al*. 2004), phenology itself refers to the study of the timing of recurrent biological events due to the biotic and abiotic forces, the interrelationship among phases of the same or different species. It also includes the events of leaf unfolding, bud-burst, full bloom, harvest and leaf fall (Van Vliet *et al*. 2003, Cleland *et al*. 2012). Rather than intrinsic controls, climatic variations linked with the changing of seasons drive the annual commencement of phenological phenomena (Badeck *et al*. 2004). Agronomic models, ecological forestry, and biogeoscience have all benefited from phenological models (Chuine and Regniere 2017). Phenological observation data can be used as a basis for determining the predominance of flowers observed from time to time and the length of blooming flowers. Phenological studies were conducted to determine the morphological changes of plant parts at each stage of their development.

Due to the scarcity of *H. bornensee* information, phenology research is urgently needed as part of a conservation effort. Thus study presents the complete morphological character of *H. Borneense* together with its phenology. The purpose of this research is to learn and describe *H. bornensee* as a terrestrial-epiphytic and endemic plant of Kalimantan based on its morphological, phenological characters and conservation management.

Materials and Methods

Hedychium borneense collected from Purwodadi Botanical Gardens with the access number or P2017040056 was studied in the laboratory and greenhouse of Purwodadi Botanical Garden - BRIN (National Research and Innovation Agency) in 2021. Seeds were characterized in the lab, while plants were grown in the greenhouse at a temperature of 24-29.9°C, humidity of 55-92% and light intensity of 180-17,500 Lux. The range of environmental factor values was obtained from measurements of the glass bulk area during morning, afternoon, and evening. *H. borneense* was grown using the media of sphagnum moss and nurtured in the greenhouse of Purwodadi Botanical Garden with an altitude of 300 m asl and rainfall of 2366 mm/year.

Morphological characterization was carried out both on vegetative and generative organs and conducted on several plant parts, starting from the stems, leaves, flowers, and fruits. Morphological observations were done using a digital caliper. Seeds morphological observations and documentation were done using a digital microscope (Dino-Lite AM3113T).

The phenological phases observed were flowering and fertilization from the formation of flower buds until the wilt of its flowers. Variables of flower growth and development included colour, flower length, and flower diameter. The flower development phase also counted the day's number used by the plants to develop the bud, bloom until fall. Observation of the flowering phase is divided into five stages which are: the initiation of flower buds phase (stage 1), the growth phase of the flowering phase (stage 2), the flower maturity phase (stage 3), the anthesis phase, in the full bloom flowers (stage 4), the flower begins to wither and fall out phase(stage 5)(Arteca 2013, Trimanto *et al*. 2020). Flower and fruit development are documented by digital camera.

The propagation research was carried out in the greenhouse of the Purwodadi Botanical Gardens. Seedlings are two weeks old. Secondary data from the results of previous research by Trimanto *et al*. (2019) were used and compared 4 type of growing media, namely pure sphagnum moss, compost mix sand (1:1), soil and soil mixed with compost (1: 1).

Morphological characterization and phenology of *Hedychium borneens*e were analyzed descriptively and qualitatively. Microsoft Excel 2016 and SPSS programs were used for the ANOVA test and present of the figure.

Results and Discussion

H. borneense is an endemic species from Borneo, with the main character being the brownish colour bractea that distinguishes it from the other species. A detailed account comprising taxonomic treatments, descriptions, habitat, and notes of *H. borneense* is provided hereunder.

Detailed characters of morphological *H. borneense* are described in Fig. 1. *H. borneense* is a perennial herb; *leafy shoots* reaching up to 0.6-0.8 m tall, erect; *pseudostem* green, 1,5 - 2 cm in diameter, glabrous; the *base of leafy shoot* reddish-green; young shoots reddish. *Rhizome* subterete, length 6 - 7 cm, diameter 2 - 4.5 cm, hard, fibrous, shiny, cylindrical, branched, 4 - 5

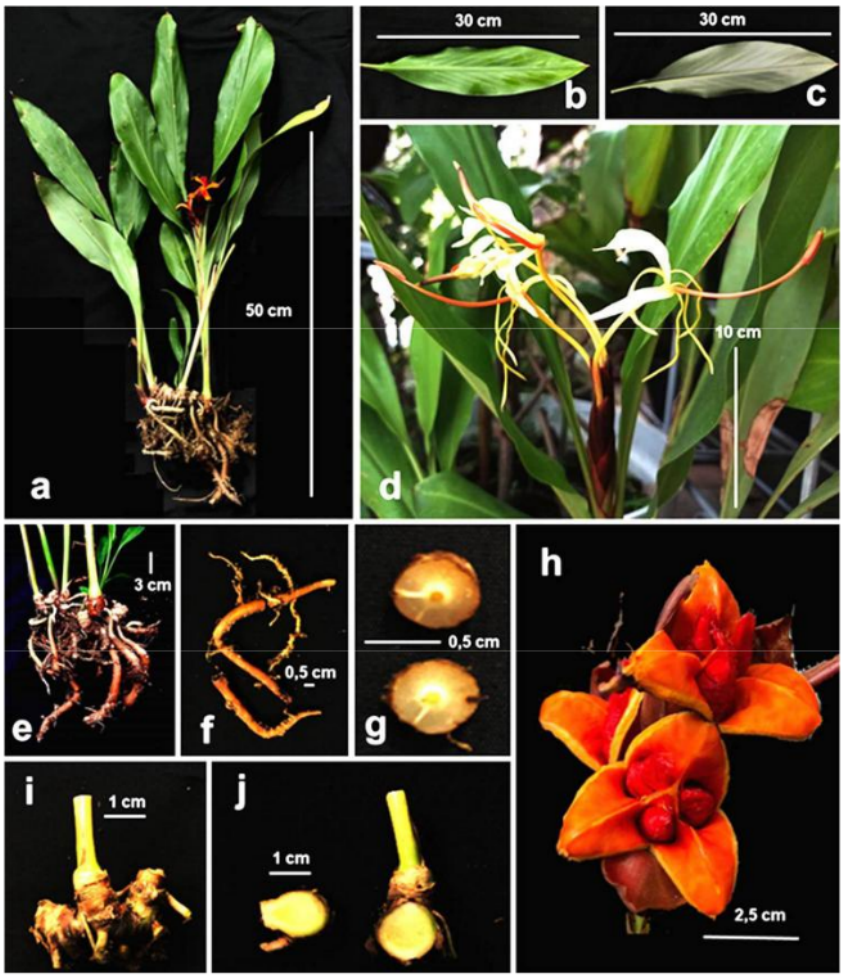

Fig. 1. Plant morphological characteristics of *H. borneense* a) Clump, b) and c) Leaves, d) Inflorescences, e & f) Root g) Slice of a root, h) Fruit and seed, i) Rhizome, j) Slice of a rhizome.

Table 1. Observation results of *H. borneense* flowering and fruiting phenology.

Days	Phenological observation result
Day-1	The flower bud is 2 cm long, 0.6 cm in diameter & there are 3 layers of bractea with a brownish color.
Day-3	The flower bud is 6 cm long, 0.8 cm in diameter & there are 4 layers of bractea with a brownish color.
Day-7	The flower bud is 7 cm long, 0.9 cm in diameter & there are 5 layers of bractea with darker brown colour.
Day-10	The flower bud is 7.5 cm long, 0.95 cm in diameter & there are 5 layers of bractea with dark brown colour.
Day-16	The flower bud is 8.5 cm long, 1.2 cm in diameter & there are 5 layers of bractea with brown colour.
Day-24	The flower bud is 10 cm long, 1.5 cm in diameter; there are 5 layers of bractea with brown colour & the tip of the bractea is ruptured.
Day-28	Flower buds are 10.5 cm long, 1.5 cm in diameter, 1 cm long flower buds have started to appear & the number of flower buds is 4.
Day-30	Flower buds are 11.5 cm long, 1.5 cm wide & the flower bud length increases to 1.9 cm
Day-31	Flower buds are 3-3.5 cm long, bracteole and corolla tube could be distinguished.
Day-32	Flower bud length increases to 7-8 cm.
Day-33	The flowers are fully bloomed. There are one flower blooms in the morning, 2 flowers during the day & 3 flowers in the afternoon. The part of the flower consists of the labellum, corolla lobe, lateral staminodes, stamen, anther, style, the stigma can already be distinguished.
Day-34	All flowers are in full bloom and show the fragments.
Day-35	Flowers begin to wilt, and at this period, the pollination process has occurred
Day-45	The flowers are very dry, and the bractea looks swollen, which indicates the beginning of fruit development, but it's not yet visible.
Day-51	The fruit begins to show, and it's pressing the bractea. The fruit's colour is brownish.
Day-57	The fruit begins to develop with a larger diameter.
Day-62	The fruit is developing and reaching the stage of maturity.
Day-82	The fruit begins to open, the flesh opens, and the seeds are visible with the red arils that are clearly visible.
Day-86	The fruit is fully open, the seeds are ripe, and can be harvested for planting.
Day-94	The fruit starts to dry out, and the arils are no longer fresh. The seeds have been harvested and planted.

segments, outer skin colour reddish brown, pale greenish-yellow when old, slice rhizome is white, hard-textured, strong aroma. *Root* fleshy, very pubescent, brownish, 5 mm in diameter; *Ligule* 2.5 cm long, apex rounded, greenish; *petiole* ca. 3.5 cm, green, glabrous; *leaf* lanceolate-oblong, number leaves 5-6, length 35 × wide 7.5 cm, adaxially green, abaxially pale green, leaf sheath green, glabrous on both surfaces, apex acuminate, base attenuate, margin entire, slightly undulating. *Inflorescence* terminal, raceme, white to orange, 10-12 cm; *bract* purple-brownish, shiny, tip acute; *peduncle* 2 cm long, green-brownish, shiny; *flowers* 11.0-12.2 cm long, 5-8 flowers; white- yellow; shiny; calyx tubular, ca. 1 cm long, white; *bracteoles* ca 2.2 cm, yellowish,

glabrous, tubular, margin membranous, non-ciliate. *Stamen* orange, shiny, erect; *filament* ca. 9,4 cm long, longer than labellum; *anther* incurved; dark orange, ca 1.75 cm long, anther crest absent; tip truncates; c*orolla lobes* oblanceolate, 4.05 x 0.45 cm, membranous, yellowish, glabrous, drooping from the flower, margins rolled inside. *Lateral staminodes* two at the base of the labellum, 3.80 x 0.70 cm, elliptic, white to yellowish, spreading on flower, margin slight undulate; *Style* slightly longer than stamen, greenish; *stigma* green, ca. 1 mm long, pubescent. *Labellum* ca 4-5.2 × 3-3.4 cm, white, pale yellow, spreading on flower, clawed at base; *Fruit* 2.5 cm in diameter, loculicidal capsule, three-angled, glabrous, trilocular, glabrous; fruit wall brown to reddish externally and orange internally; s*eeds* ca. 5.68 x 1.85 mm, brownish when dry; aril orange to red, brownish when dry.

Results showed that *H.borneense* seeds weighed 0.018 ± 0.001 g (mean and standard deviation). The seeds have a red or orange aril and are brown to black in colour. Seeds have a diameter of 1.85 ± 0.15 mm and a length of 5.68 ± 0.36 mm. The seeds of *H. borneense* have a hard skin with a thickness of 0.14 ± 0.04 mm. The embryo and endosperm are visible in the longitudinal section of the seed. The embryo is 0.73 ± 0.01 mm in length. The endosperm, which is white as flour and has a length of 4.8 ± 0.19 mm and a width of 0.45 ± 0.06 mm, surrounds the embryo as food reserves. The embryo will grow into the micropyle, which is found at the top of the embryo (Fig. 2).

Observing the phenology of *H. borneense* is useful for determining the reproductive term during a single period. The flowering phase is critical for propagation (Luna-Nieves *et al*. 2017, Yulistyarini and Hadiah 2021). This data may be utilized as a benchmark during the hybridization process. The phenological stage of H. borneense is divided into six stages. The first phase began with the emergence of flower buds, followed by the maturation of sex cells, flower blooming until the flowers wilted and fell off, and finally, fruit development. Figure 3 depicts the flowering and fruiting stages of *H. borneense*. A bloom matures and transforms into a ripe fruit in 86 days. On the other hand, the blooms took approximately 34 days to bloom. After flowering, the development stage leading up to fruit maturation lasts roughly 52 days.

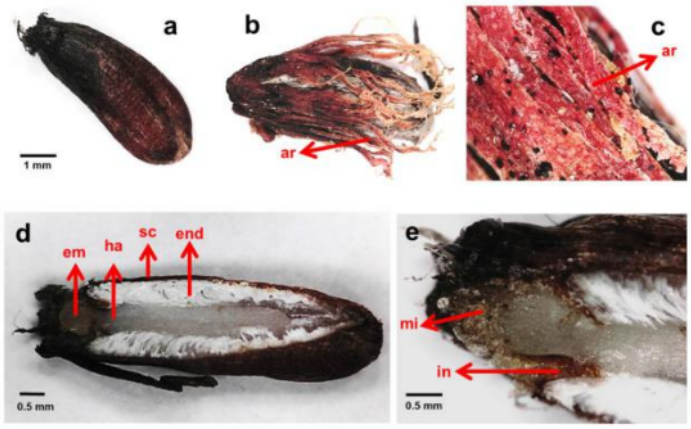

Fig. 2. Morphology of *Hedychium borneense* seed. a) seed, b) seed covered by aril, c) aril, d) longitudinal section of *H. borneense* seed. Note ar (aril), em (embryo), ha (haustorium), sc (seed coat), end (endosperm), e) in (integument), mi (micropyle cap).

The flowering process is influenced by external (temperature, light, humidity, rainfall, and nutrients) and internal factors (genetic hormones) (Yang *et al*. 2016, Navas-Lopez *et al*. 2019). Plants start flowering in optimal conditions. The flowering process will proceed to conception. The maturity of sexual reproduction can be determined when it is appropriate to hybridize. The flowers are in a reproductive period when they bloom, indicating the maturity of male sex cells (stamens) and female sex cells (pistils). The phenology of *H. borneense* is generally divided into six stages (Fig. 4), the initiation phase, the flower development phase, the pre-anthesis phase, the anthesis phase, the post-anthesis phase, and the fruit development phase.

Fig. 3. Phenological of *Hedychium borneense*. a) day 1, b) day 11, c) day 25, d) day 29, e) day 31, f) day 33, g) day 34, h) day 35, i) day 36, j) day 37, k) day 42, l) day 45, m) day 63, n) day 82, o) day 86.

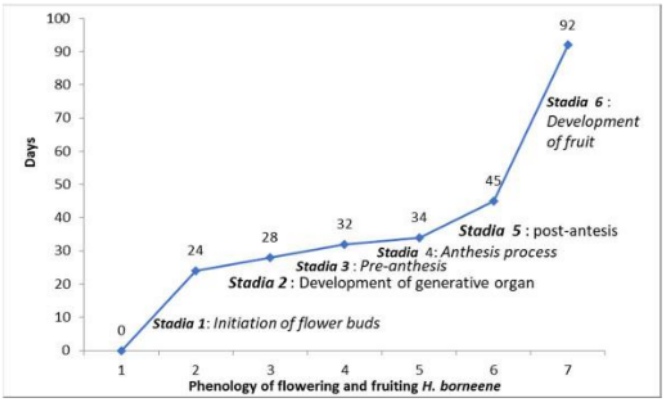

Fig. 4. Phenological stages of *Hedychium borneense*.

Bud initiation is the initial stage of flower formation. At this stage, cells in the flower's meristematic tissue begin to divide. Small brown inflorescence buds began to grow during this period. A layer of bractea (flower protection) forms the buds. This phase of flower bud development lasts up to 24 days. The exterior structure of the flower reveals five layers of brown bractea. The organs of the flower begin to form in this phase with a basic structure. The floral organs' structure is still intact. It demonstrates that the floral parts are still in the process of development at this stage. The flower bud is approximately 10 cm in length and 1.5 cm in diameter. Until the 24th day, there are five layers of brown bractea, with the bractea breaking at the ends.

In development of generative organ stage, small flower buds emerge inside the bractea of the inflorescence. Stamen(s), anther(s), and filament(s) are formed. The development of flower organs begins to appear. The floral bracteole is the most visible component of the flower. The corolla tube has begun to expand. Each inflorescence contains four flower buds. Flower buds measure approximately 1 cm in length and are brownish in color. This period lasts approximately 24 to 28 days.

In pre-anthesis phase the flower buds are getting longer. Flower buds are divided into 2 layers. The lower part of the flower is yellow, showing the corolla lobe and the upper part is brownishly showing the anther part, which is still covered by the labellum and lateral staminodes. This stage is known as the blooming stage. This stage lasts between days 29 and 32. The flower parts can be separated clearly. The flower part experiences maximum length growth, very long tube size, and corolla lobe that looks yellowish-white. At this stage, all flower parts have grown optimally with a very fast period, which is about 4 days after the flower buds leave the bractea. The colour of the older buds on the upper layer indicates that the male and female sex cells have begun to mature.

In anthesis process the *Hedychium borneense* flowers are in full bloom at this stage. This stage occurred between the 33rd and 34th days. This stage is identified by the complete opening of the floral components. The anther is extremely mature at this stage, as evidenced by the abundance of brownish pollen. Pollen is a brownish powder. Male and female sex cells may have matured at this state. The blooming phase is brief, lasting about three days. There are a total of four flowers that bloom. On the 33rd day, the first three flowers bloom, and one flower blooms on the 34th day. Pollination is possible during this phase of anthesis. Numerous ants were noticed walking about the flower at the time. As is well known, H. borneense pollinated with the assistance of insects.

In post-anthesis stage is marked by changes in numerous parts of the flower (starting to wither). The change is marked by a flower that was originally erect to be rolled up. The condition of the flowers is still fresh. This takes place on the 35th day or two days after the flowers have fully bloomed (anthesis). The style of labellum, corolla lobe, lateral staminodes, stamen were originally upright become bent. This condition indicates that the flowering period has ended. On the 36th day, all parts of the flower started to look wilted and turned paler. The more days the flower part dries, and on the 45th or 10th day after the anthesis, all the parts of the flower have dried but did not fall off. The flower part is still attached to the flower bractea.

In the development of fruit stage the fruit development starts for the first time after the flowers are fully bloomed. At this time, there is a pollination process. Fruit development lasts about two months or 60 days. Bractea is protecting fruit. On the 51st or 26th day after the fruit, anthesis was visible. The bractea is prominent, which indicates fruit development. By the 62nd day, the fruit is already looking big and pressing on the bractea. The dried part of the flower is still attached to the end of the fruit. The fruit is dark brown in colour. There were 3 successful fertilizers out of the 4 flowers produced (75%). Ripe fruit is shown by opening the pulp. Fruit

opening occurs on the 82nd day and fully opens on the 86th day. By this time, the seeds are ripe and ready to harvest. The seeds are wrapped in bright red arils. On the 92nd day, the fruit will begin to dry out, followed by the arils, which begin to wilt. This stage is the end of fertilization and the seeds can be harvested.

Study on the conservation efforts of *H. Borneense* was made. It is a species endemic to Sabah on Borneo (Lamb *et al*. 2013). Since it was noticed in the IUCN (2019) Red List as a species with the Data Deficient category (Oleander 2019), it remains unknown whether this species should be protected or unprotected in Kalimantan as its natural habitat. Moreover, Kalimantan has experienced significant forest degradation. The threat to the sustainability of species in the forests of Kalimantan is commonly happening because it is at constant risk of being logged and converted (WWF 2019). Land conversion, unsustainable use, invasive species, pollution and climate change are the causes of the threat to Indonesian plant species (Budiharta *et al*. 2011). *H. borneense* often grows as an epiphyte by attaching to trees or living on fallen trees. This habitat characteristic makes this plant species threatened in nature, as these plants require trees to survive in nature. If the host tree is cut off, these species will most certainly perish along with it. Purwodadi Botanical Gardens collected this species in 2017 from the East Kalimantan-Central Kalimantan region with the characteristics of wet lowland forest, which are high humidity (above 90%), low light intensity, and many trees with large diameters. *H. borneense* species are uncommon in Besiq Bermai Forest, East Kalimantan. This species is obtained from local residents who collect plants around the forest. Because the forest is located near the mining area, it is at risk of extinction because of forest land conversion.

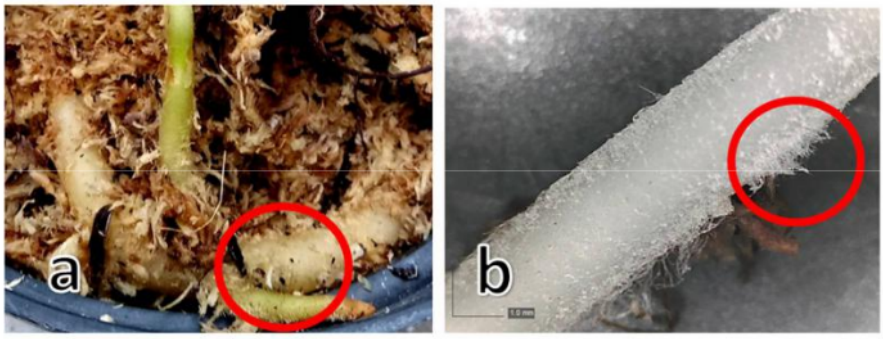

Fig. 5. a) The old roots of *Hedychium borneense* are thick, fleshy and have fine hairs on the surface of the root bark in spaghnum moss media. b) young root with fine hairs.

The success of *H. borneense ex-situ* conservation in Purwodadi Botanical Gardens provides a hope on its survival. According to (Mounce *et al*. 2017), ex situ conservation is a rational choice or even the only option for ensuring the future of plant species. Ex-situ collections serve as reserve material when natural populations experience degradation and extinction in nature (Brummitt *et al*. 2015). *H. borneense* has been adapting and growing well in the Purwodadi Botanical Garden greenhouse since it was collected in 2017. However, it requires a special condition since the plant cannot be planted in soil. *Hedychium borneense* is an epiphytic whose roots need a porous and moist growing media. It may be affected by growing media. Media that is too dense can cause root rot. The roots are fleshy, juicy, and covered with fine hairs (Fig. 5).

In Purwodadi Botanic Garden, *Hedychium borneense* can produce flowers regularly as well as its fruits. According to the previous research, the propagation process for this species is quite easy. *H. borneense* can be propagated either vegetatively using rhizomes or generatively using seeds. Seedlings will germinate after 14-20 DAP (Days After Planting). Pure sand or a mixture of soil: compost and burnt husk (1:1) are used for medium propagation. The germination character of *H. borneense* seeds is growing simultaneously and hypogeal. The plant germinated approximately 1 cm at 20 DAP, and by 44 DAP, the plant had grown to a height of around 7-14 cm with 2-3 leaves (Fig. 6).

Fig. 6 (a) Seed germination at 20 Days after Planting), (b) Seedling at 44 Days after Planting.

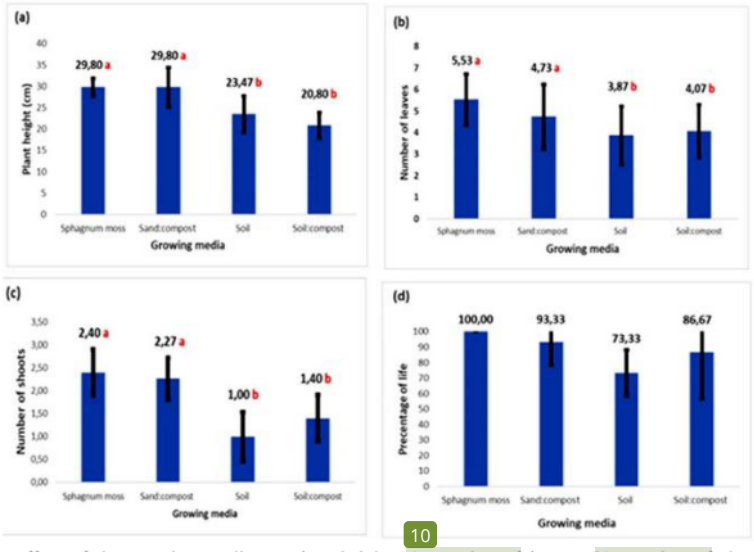

Fig.7. The effect of the growing media on plant height (a), number of leaves (b), number of shoots (c) and percentage of plant life (d) of *H. borneense* at the age of 3 months after planting (Trimanto *et a.l*, 2019). Different lowercase letters (red colour) represent significant differences between different growing media applications.

The growth of *H. borneense* was significantly different when sphagnum moss was used against soil media. Sphagnum moss generally provided the best growth in terms of plant height (a), leaf number (b), shoot number (c), and percentage of plant life (d) (Fig.7). Sphagnum moss is frequently used as a substrate for epiphytic orchids. This implies that the *H. borneense* type prefers epiphytic environment over terrestrial habitat. *H. borneense* prefers a porous, moist, well-aerated growth medium. Compost containing sphagnum moss and sand will promote the growth of *H. borneense* (Trimanto et al. 2019). Sphagnum moss, which has a great water-binding capacity of up to 80%, is an ideal medium for *H. borneense*. Moreover, it also has good aeration and drainage. Sphagnum moss, with an up to 80% water-binding capacity, is a suitable substrate for *H. borneense*. Additionally, it is well-aerated and well-drained. Sphagnum moss contains between 3% and 5% nitrogen (Nitrogen) and is excellent for root growth. By contrast, soils have a limited porosity, a high density, and a deficiency of nutrients. The soil will retain a great deal of water and make it harder for nutrients to be absorbed. That is why the roots of *H. borneense* have a tough time adapting to their new environment. It will not grow and decay (Trimanto *et al*. 2019). It is not recommended to use soil as a growth medium for *H. borneense* transplants.

Hedychium borneense is an endemic and terrestrial-epiphytic plant from Kalimantan. The prominent morphological characteristic of the plant is that it is a perennial herb that grows up to 1 meter tall with broad leaves and bractea that are purple-brownish in colour. This plant produces a terminal raceme with a purple, brownish bractea colour. Pale yellow flowers with a large and pale yellow labellum and orange stamens. The fruit is trilocular and its inner colour is orange to red. The seeds are brownish in colour with a dark red arillus. The phenology of *H. borneense* is commonly divided into six stages, the initiation phase, the flower development phase, the pre-anthesis phase, the anthesis phase, the post-anthesis phase and the fruit development phase. *H. borneense* is known to prefer a porous and moist growing medium. Ex-situ conservation by generative propagation is important for the survival of species.

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(Manuscript received on 27 December 2021; revised on 05 June 2022)

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