PROCEEDING

LIPI

International Conference on
Tropical Plant Conservation and Utilization
Celebrating Bicentenary of Bogor Botanic Gardens
and The Golden Years of LIPI

"PLANTS AND PEOPLE IN HARMONY"

KEBUN RAYA BOGOR

PROCEEDING

International Conference on Tropical Conservation and Utilization
Celebrating Bicentenary of Bogor Botanic Gardens

"PLAN AND PEOPLE IN HARMONY"
Bogor, 28 December 2018

Center for Plant Conservation Botanic Gardens
Indonesian Institute of Sciences

PROCEEDING
International Conference on Tropical Conservation and Utilization
Celebrating Bicentenary of Bogor Botanic Gardens

"PLAN AND PEOPLE IN HARMONY"

Speakers	:	Dr. Tsukasa Iwashina
Steering Committee	:	Dr. Didik Widyatmoko, M.Sc. Dr. Made Sri Prana Dr. Usep Sutisna Dr. Suhirman Prof. Dr. Dedy Darnaedi Dr. Irawati Ir. Mustaid Siregar, M.Si.
Organizing Committee *Chairman of the Committee*	:	Dr. Joko R. Witono

Proceeding International Conference on Tropical Conservation and Utilization
Celebrating Bicentenary of Bogor Botanic Gardens

Published By:
Pusat Konservasi Tumbuhan Kebun Raya–*Indonesian Institute of Sciences*
Center for Plant Conservation Botanic Gardens–LIPI
Jl. Ir. H. Juanda 13, Bogor 16003, Indonesia

PREFACE

Proceeding of The International Conference on Tropical Plant Conservation and Utilization 'Plants and People in Harmony' is a compilation of papers presented in the conference which was held on 18-19 May 2017 in Bogor Botanic Gardens. The conference was part of the Golden year of LIPI (Indonesian Institute of Sciences) and the Anniversary and celebrating of Bicentenary of Bogor Botanic Gardens, and conducted by Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences.

The International Conference was intended to review and disseminate findings of research, activities, and development of flora conservation and utilization in tropical countries. The conference embraces for five subthemes, i.e. (1) Plant diversity and inventory, (2) Plant conservation, restoration, and climate change, (3) Economic botany and sustainable use, (4) Genetics and molecular research, and (5) Domestication of wild plants.

The conference was intended by 180 participants, including 8 invited speakers, 32 oral presentations, and 67 poster presentation. Keynote and invited speakers were from Indonesia, Singapore, China, Japan, Australia, Netherlands, and United Kingdom, covering researchers, academicians, government and non government institutions, and Business associations. Some scientific papers presented in the conference were delivered to publish in scientific journal, whereas 28 Papers were presented in this proceeding has gone through scientific reviewers.

At least, we would like to present our gratitude to all speakers, participants, reviewers, and communities for the success of the conference. We hope that this proceeding will be useful for further research. Should you have any constructive suggestions, please do not hesitate to contact us.

Bogor, 28 December 2018
Sign

Dr. Didik Widyatmoko, M.Sc.
Director
Center for Plant Conservation-Botanic Gardens
Indonesian Institute of Science

Proceeding

LIST OF CONTEN

Research and Display of Tropical Plants of Tsukuba Botanical Garden

Tsukasa Iwashina
The ex-prime Director, Tsukuba Botanical Garden, National Museum of Nature and Science
The President, Japan Association of Botanical Gardens
Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan
E-mail:iwashina@kahaku.go.jp

Organization of National Museum of Nature and Science

Tsukuba Botanical Garden belongs to National Museum of Nature and Science, Japan. Five departments are present in the museum, i.e., Department of Zoology, Department of Botany, Department of Geology and Paleontology, Department of Anthropology, and Department of Science and Engineering. These departments are located in Tsukuba City, Ibaraki Prefecture, central Honshu as Tsukuba District (Fig. 1). On the other hand, Ueno District for open to the public is in Tokyo (Fig. 2). Department of Botany conducts the collection and research of dried and live specimens of vascular plants, bryophytes, algae, lichens and fungi. Based on these specimens, the Department carries out research for their taxonomy, evolution and conservation on plants. The Department's aim is to gather the information on which a wide range of scientific inquiry is based, embracing plant lineage, biogeography, chemotaxonomy and resources, and organize these findings in a comprehensive database.

Figure. 1. Tsukuba District including

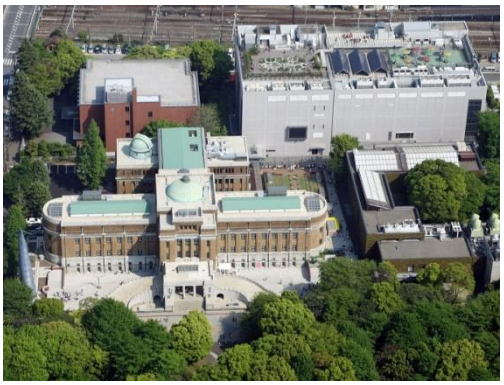


Figure. 4. 'Warm-temperate Deciduous Broad-leaved Forest'.

Department of Botany consists of three divisions, i.e., Division of Land Plants, Division of Fungi and Algae, and Division of Plant Diversity and Conservation. Four staffs assign in Division of Land Plants and study the taxonomy and evolution of bryophytes and vascular plants. On the other hand, five researchers belong to Division of Fungi and Algae, and study the taxonomy of fungi, lichens, myxomycetes and algae. Eight researches assign in Division of Plant Diversity and Conservation, and this division holds Tsukuba Botanical Garden. Tsukuba Botanical Garden collects and preserves the endangered and endemic plants in Japan as a base of biodiversity in East Asia. Moreover, the Garden aims to the establishment of living plant collection for the view of plant biodiversity in the world.

Display of Tropical Plants in Tsukuba Botanical Garden

Approximately 7000 species are grown in two major areas, "Japanese Vegetation Area" and "Human and Biodiversity Area". In the former areas, 'Highland Montane Grassland', 'Lowland Montane Grassland', 'Montane Riverside Sandy and Gravelly Area', 'Seashore Sandy and Gravelly Area', 'Montane Shrubs', 'Lowland Shrubs', 'Highland Rockery', 'Coastal Rockery', 'Evergreen Broad-leaved Forest' (Fig. 3), 'Temperate Coniferous Forest', 'Warm-temperate Deciduous Broad-

leaved Forest' (Fig. 4), 'Cool-temperate Deciduous Broad-leaved Forest' and 'Aquatic Plants Area' are included.

Figure. 3. 'Evergreen Broad-leaved Forest'.

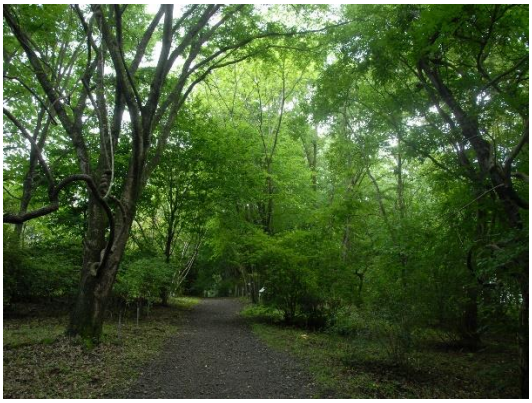


Figure. 4. 'Warm-temperate Deciduous Broad-leaved Forest'.

On the other hand, 'Useful Plants House', 'Savanna House' (Fig. 5), 'Useful Plants Area', 'Useful Plants House' (Fig.5), 'Endangered Plants Area', 'Tropical Rain Forest House' and 'Aquatic Plants House' are included in the latter area. Visitors can get in touch with world's plants diversity in compartmented major Japanese vegetation and 'Tropical Rain Forest House' and 'Savanna House'.

Plant resources that have been utilized in various ways by the human race are displayed in 'Human and Biodiversity Area' including 'Useful Plants House'. Specimens are labelled to indicate endangered plants and Japanese endemic species. The Garden is ideal for plant observation, enjoyment, recreation and relaxation, and for informal study by students. Almost tropical plants are growing in 'Tropical Rain Forest House' and 'Useful Plants House', e.g., *Ficus macrophylla*, *F. subpisocarpa*, *F. tinctoria*, *Garcinia xanthochymus*, *Quisqualis indica*, *Terminalia catappa*, *Melastoma sanguineum*, *Murraya paniculata*, *Theobroma cacao*, *Shorea guiso*, *S. multiflora*, *S. ovalis*, *Planchonella obovata* etc.

Figure. 5. Useful Plants House (right) and Savanna House (left).

Amorphophallus titanum is also grown in 'Tropical Rain Forest House', together with related *A. paeoniifolius*. This individual bloomed in 2012, and 2014, 2016 and then this year (2018). In 2016, 6000 peoples/day came to our garden for three days. When the inflorescence bloomed, author has isolated and identified the flower pigments and related compounds. The results are described below.

Research of Tropical Plants in Tsukuba Botanical Garden

Tsukuba Botanical Garden performed some Botanical Research to tropical areas. In 1996, 1997, 2000 and 2001, we surveyed the plants including angiosperms, gymnosperms, ferns, mosses, fungi and myxomycetes in South Pacific countries, such as Vanuatu and adjacent New Caledonia and Fiji (Fig. 6). As the results, we have published "Contributions to the Flora of Vanuatu" in 1998 and "Contributions to the Flora of Vanuatu, Vo. 2" in 2002 (Fig. 7).

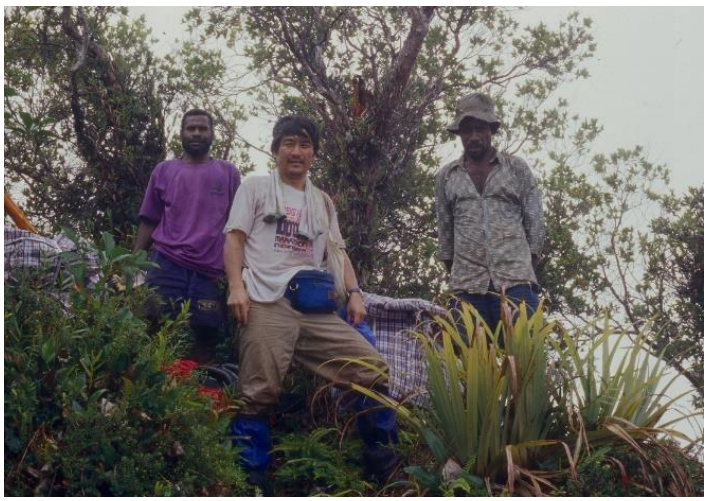


Figure. 6. On the top of Mt. Tabwemasana (1879 m), the highest mountain in Vanuatu.

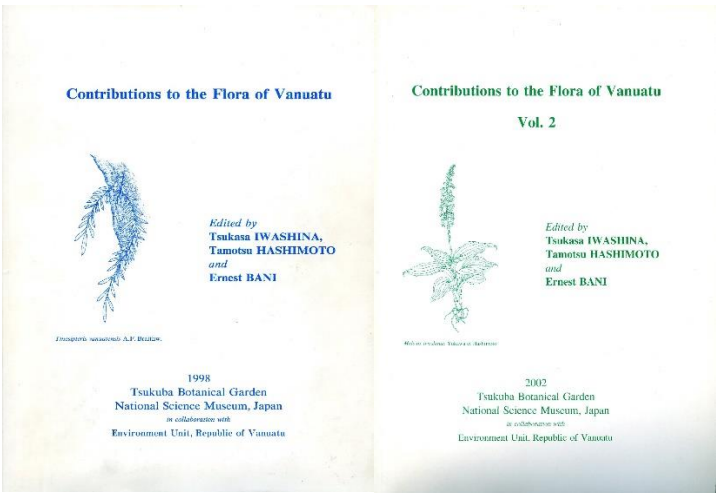


Figure. 7. "Contributions to the Flora of Vanuatu, Vol. 1 and 2".

We recently started the Botanical Research to Myanmar, especially southern parts by MoU with Ministry of Natural Resources and Environment Conservation, Myanmar. In this research, flora and fauna of Myanmar, not only flowering plants, ferns, mosses and fungi but also insects, fishes and mammals are surveyed (Fig. 8). In flowering plants, *Amherstia nobilis* (Leguminosae) is endemic to Myanmar and generally regarded as "one of the most beautiful of all flowering trees in the world". However, its wild population in Myanmar is not known until now. Flower color is orange-red and its pigments has been surveyed by us. In this research, we search the wild population of *A. nobilis*.

Figure. 8. Botanical research in Myanmar.

Survey of Pigments in the Inflorescence of *Amorphophallus titanum*

I study the chemical adaptation in plants, so we analyzed the secondary metabolites, especially flavonoids and related compounds in the leaves and flowers of *Amorphophallus. titanum*. In 2012, 2014, and 2016, the inflorescence of *A. titanum* come out in our botanical garden. In that time, we analyzed this inflorescence of the plant, together with *A. paeoniifolius* and *A. rivieri*.

Figure. 9. *Amorphophallus titanum* in 2012 (left) and 2016 (right).

As the results, we isolated and identified 16 flavonoids, i.e., five anthocyanins, cyanidin 3-*O*-glucoside and 3-*O*-rutinoside (Fig. 10), peonidin 3-*O*-glucoside and 3-*O*-rutinoside, and pelargonidin 3-*O*-rhamnosylglucoside, twelve *C*-glycosylflavones, vitexin (Fig. 11), vitexin 2′′-*O*-glucoside, isovitexin, isovitexin 2′′-*O*-glucoside, orientin, isoorientin, vicenin-2, lucenin-2, schaftoside, isoschaftoside, isovitexin X′′-*O*-rhamnoside and isoscoparin X′′-*O*-glucoside, and each one of flavone and flavonol, chrysoeriol 7-*O*-glucoside (Fig. 12) and kaempferol 3-*O*-robinobioside (Fig. 13). Of their flavonoids, dark red color of the spathe of *A. titanum* is mainly due to an anthocyanin, cyanidin 3-*O*-rutinoside (Iwashina *et al*., 2015). They may be use as invitation of pollinator, together with stink. On the other hand, other flavonoids were a little amounts. By contraries, in the spadix, although anthocyanins are a little, other flavonoids, especially *C*-glycosylflavones are rich. However, the function of these compounds are still uncertain.

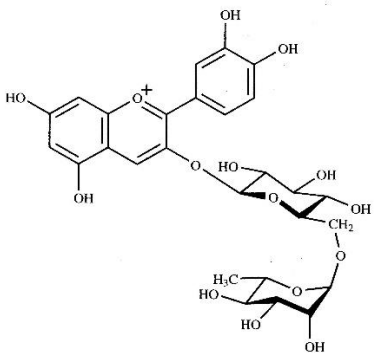


Figure. 10. Cyanidin 3-*O*-rutinoside.

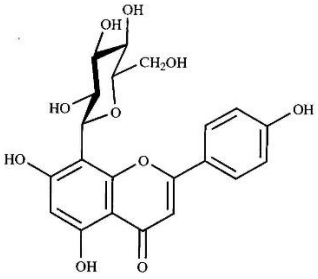


Figure. 11. Vitexin.

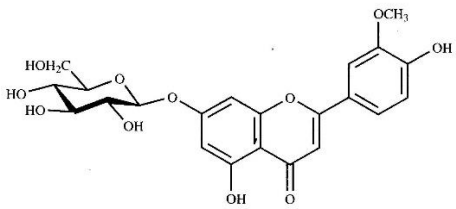


Figure. 12. Chrysoeriol 7-*O*-glucoside.

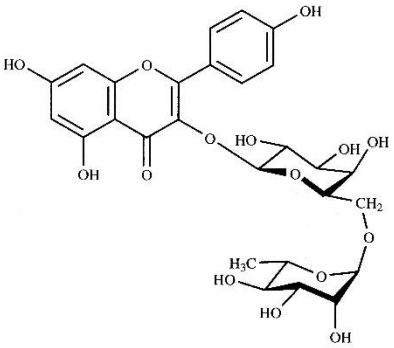


Figure. 13. Kaempferol 3-*O*-robinobioside.

We are now in progress the survey of the foliar flavonoids of *Amorphophallus titanum* and flower pigment of *Aeschynanthus* species (Gesneriaceae) in collaboration with Bogor Botanic Gardens and Cibodas Botanic Gardens, Indonesia.

Tropical plants are now very important resources for human being. On the other hand, many species are exposed to peril of the extinction. We must further progress co-existence with their plants.

REFERENCES

Iwashina, T., Hashimoto, T. and Bani, E. (eds.), 1998. Contributions to the Flora of Vanuatu. Tsukuba Botanical Garden, National Science Museum, Tsukuba, Japan.

Iwashina, T., Hashimoto, T. and Bani, E. (eds.), 2002. Contributions to the Flora of Vanuatu, Vol. 2. Tsukuba Botanical Garden, National Science Museum, Tsukuba, Japan.

Iwashina, T., Uehara, A., Kitajima, J. and Yukawa, T., 2015. Anthocyanins and other flavonoids from *Amorphophallus titanum* having largest inflorescence in plant kingdom, and other two species. Bulletin of the National Museum of Nature and Science, Series B 41: 33-44.

In commemorating 200 year of Bogor Botanic Garden (BBG): The tale of some novel fern species cultivated in the BBG

Wita Wardani
Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI)
Cibinong Science Center, Jl. Raya Jakarta-Bogor Km. 46, Cibinong, 16911
Email: wita.wardani@lipi.go.id; wt.wardani@gmail.com

ABSTRACT

Bogor Botanic Gardens is one of historical key institution in the development of science in Indonesia. One of its important role is in the establishment of new taxa based on its living plant collection. There were ten pteridophyte taxa newly described based on the garden' collection, of which type specimen housed in the Herbarium Bogoriense. Most of them were established by Capt. C.R.W.K. van Alderwerelt van Rosenburgh who once were conservator and assistant of Bogor Herbarium. Among these are *Platycerium wilhelminae-reginae* Alderw, *Angiopteris subfurfuracea* Alderw. and *Acrostichum smithii* Racib. Findings on these taxa are presented, consist of related names, taxonomical status and some key characters.

Keyword: Bogor Botanic Gardens, Herbarium Bogoriense, Pteridophyte, type specimen

INTRODUCTION

Bogor Botanical Garden was established on 1817 with the name 's Lands Plantentuinte Buitenzorg. It was decided following the approval of proposal of C.G.C. Reinwardt by the Dutch-colonial government on the need of a garden that plant economic importance species from around the archipelago. Research are expanding from this collection and dried material of plant were piling up. It was the idea of J.E. Teijsman in 1837 to house these herbarium in Bogor instead of keep sending the specimen to Leiden. As the idea was approved, a building inside the garden were then operated as such in 1844. Its main collection was plant that grown in the garden but it also sheltered collected specimen since botanical exploration were escalating. Many botanist visited Bogor to study both living and preserved plant since and produced new finding to science, especially new taxa (Sihotang & Royani, 2007).

Among many taxa newly described from plant that cultivated in the BBG, some of them are pteridophyte. Although not many taxa of this group known to have direct economic value, their variety are highly attractive especially for further botanical investigation. Some of the dried material of considered novel species is housed in the Herbarium Bogoriense (BO) of which building to this moment expanding and located further away from the garden. As science is advancing, some of the established names were then considered to be synonym for other species while some remain unresolved. This paper present some of the name of pteridophyte species that were established based on living specimen grown in BBG and its current taxonomical status based on most recent finding.

MATERIAL AND METHODS

The collection in the type section of Herbarium Bogoriense and documented those that was collected form Bogor Botanic Garden is investigated. This finding is not presenting every novel species of pteridophyte that were described from BBG since many of type specimens from the colonial time also sent to herbaria abroad. All specimens were re-examined and validated for their correct identification and type status by referring back to their related literatures. Some other aspects as found through reference tracking along with the key morphological characters are also presented to show future potential work.

RESULT AND DISCUSSION

The research found ten species of fern described from plant collection of BBG that all but two were published by Alderwerlt. Other newly described taxa were published by Raciborski. The domination by Alderwerlt was largely pampered by his position as the assistant in the herbarium (1905-1908) in T. Valeton time that later also conservator of the Herbarium (1910-1922) in directorship of both T. Valeton and J.J. Smith (Steenis-Kruseman 1950). Whereas, as also many other botanist, M. Raciborski was an assistant to the BBG earlier (1896-1897) at M. Treub time when the head of Herbarium was J.G. Boerlage (1896-1900) who also an assistant to the garden. Both were working mostly on fern and its allies which specimen available either in the herbarium or cultivated in the garden. In their time, the type specimen of newly described taxa was not always indicated in the protologue.

This following section present, in alphabetical sequence based on family and species, name, publication, location of the cultivated plant of the type in the garden and notes regarding relationship with other taxa or on other more general aspect.

Aspleniaceae

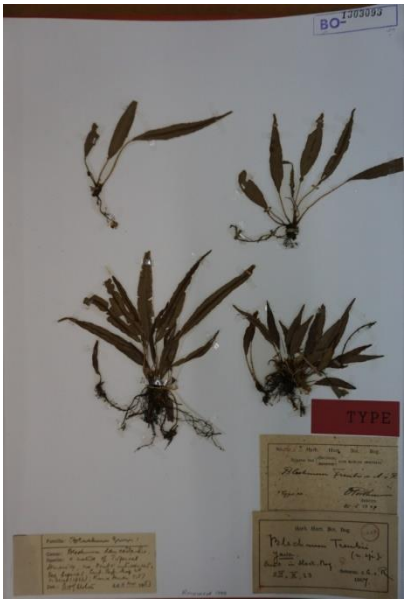
1. *Asplenium nidiforme* Alderw. – Bull. Jard. Bot. Buitenzorg Ser. 2, VII. 6. 1907. ; Cult. In Hort. Bog. II K XIV 39

Alderwerlt mentioned it in its protologue that the plant was brought from New Guinea. The main distinctiveness of this simple frond *Asplenium* according to Alderwerlt's view was on its spathulate form (Fig.1). Baker & Posthumus (1939) included this name under *A. nidus*. This species most likely part of *A. nidus* complex species. As Yatabe (2001) suggested, morphology does not provide good qualitative taxonomic characters for species recognition. Therefor this name must be included in further study of *A. nidus* complex. However, currently location of the plant that was labeled with this name in the garden is unknown.

Figure 1. Type specimen of *Asplenium nidiforme*

Blechnaceae

2. *Blechnum treubii* Alderw. – Bull. Dépt. Agric. IndesNéerl. 18: 13, 27, t. 5. 1908 Cult. In Hort. Bot. 2 K X 23



There was a note on the specimen dated 1953 that indicate this species is a typical tropical American plant, *B. lanceola*, an eublechnoid plant with simple blade (Fig. 2). This was also mentioned by Morton (1967). It seems that the locality of its origin on the label of the living collection was a mistake and misled Alderwert to think the specimen before him was a novel one. This view seems to be adopted by Nooteboom (2012) in Flora Malesiana account for Blechnaceae which exclude the name from revision. *B. treubii* Alderw. should be listed as synonym for *B. lanceola* unless a study that includes specimen collected from the wilderness in Java confirms it as a distinct species

Figure 2. Type specimen of *Blechnum treubii*

Cyahtheaceae

3. *Alsophila annae* Alderw. – Bull. Buit. II no. 23. 3. 1916; Cult. In Hort. Bog. II K XIII 10

In the revision, Holttum (1963) recognized this species under the genus *Cyathea* with known distribution only Moluccas-Ambon. Lehnert (2013) mentioned this name in a key to the Australian species of the *Gymnosphaera* Clade with notes inside bracket referring its distribution is Sulawesi and Moluccas. This species being somewhat distinctive on its reduced-skeletonized pinnae at the base of stipe (Fig. 3) which character only possessed by very few *Alsophila* species. However, the living collection is no longer exist. Hovenkamp and Ciccuza (2013) have reported a specimen collected by M. Kessler in 1999 from Lore Lindu National Park in Central Sulawesi under this name. During my own trip, have found this species in Gandangdewata Mountain in Western Sulawesi at about 1700 m asl. Therefore, it is unlikely the plant would survive in much lower elevation such Bogor and its recorded locality of Ambon need to be clarified.

Figure 3. Part oftype specimens of *Alsophila annae* showing the skeletonized basal pinnae

Lomariopsidaceae

4. *Acrostichumsmithii* Racib. – Bull. Int. Acad. Sci. Cracovie 1902. 59. 1902; Cult. In Hort. Bog. II K V 23 ----- synonym for *Lomariopsisspectabilis* (Kunze) Mett. Fil. Hort. Bot. Lips. 22. 1856 as in Fl. Males., Ser. 2, Pterid. 1(4): 262. 1978 [Lomariopsis Group; Holttum]

In the protologue, Raciborski mentioned that this species probably part of variable Stenochlaenoid group, that includes species now recognized in several different genera, which easily distinguished through its small number of pinnae. Holttum in his note (1978) also mentioned that specimen of this name, known to be brought from Moluccas, had very few pinnae that indicated it might be a distinct taxon. However, no other specimens were known from this area. He included it into a species with wider distribution.

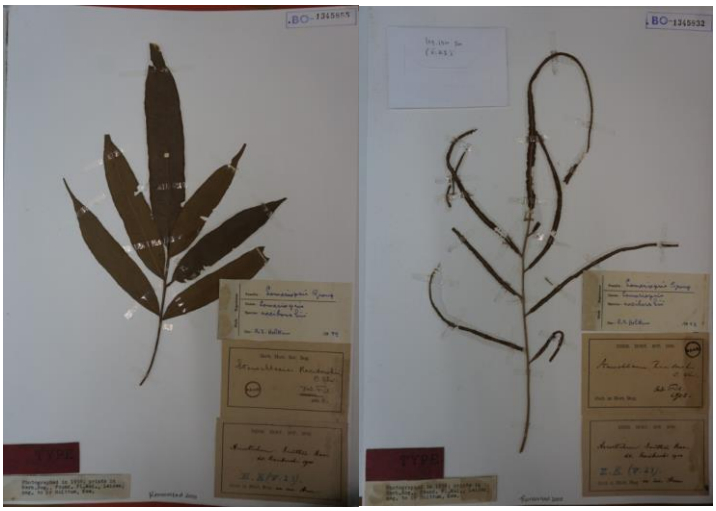


Figure 4. Type specimen of *Acrostichum smithii*: steril (left) and fertile (right) frond

Marattiaceae

5. *Angiopteris subfurfuracea* Alderw. – Bull. Jard. Bot. Buitenzorg Ser. 2, XI. 1. 1913. ; Cult. In Hot. Bog. II K XI 15; II K X 32

This taxon differs from typical *A. evecta* in its scurfy appearance of the rachis and "moderately long" false vein instead of "long". This name was not included in the last comprehensive study on *Angiopteris* by Rolleri (2003) although she did mention that combination of venuloid characters which include length are diagnostic character at species level. Further investigation on its relationship especially to *A. evecta* would be worthwhile.

Figure 5. Part of type specimen of *Angiopteris subfurfuracea*

6. *Angiopteris smithii Racib.* – Bull. Int. Acad. Sci. Cracovie 1902. 54. 1902Cult. In Hort. Bog. II K X 13 ----- synonym *Macroglossum smithi*i (Racib.) Campb. – Philipp. J. Sci., C 9: 209, t. 1, f. B, C. 1914.

Douglas Houghton Campbell visited Buitenzorg Botanic Garden in 1914 as part of his second excursion to the region. He examined this Raciborski species and transferred it to another genus. It is likely that Alderwerlt noticed Campbell's work, realized that there were no specimen of the mentioned species in the Herbarium so then he re-collected the plant from BBG but labeled the specimen with Campbell's combination instead of original Raciborski's name. It is suspected that this kind of practice were common and possibly misleading whenever complete authorship of the taxon were not inscribed.

New classification (Christenhusz et. al., 2011) has included *Macroglossum* into *Angiopteris*. This taxon is being peculiar in the absent of false vein and significantly larger pinnae (Fig.6). Origin not known but Sumatera and Borneo mentioned as probable source.

Figure 6. Part of type specimen of *Macroglossum smithii*, frond apex (left) and larger pinnae (right)

Polypodiaceae

7. *Platycerium wilhelminae-reginae* Alderw. – Bull. Dépt. Agric. IndesNéerl. 18: 24, t. 6-7. 1908 ; Cult. In Hort. Bot. II K V 2 ----- Synonym for *Platyceriumwandae* Racib. Bull. Int. Acad. Sci. Cracovie 1902. 58. 1902 as in Fl. Males., Ser. 2, Pterid. 3: 142. 1998 [Polypodiaceae, Hovenkamp].

The origin is not known, but suspected as had grown cultivated in BBG for years before described. Long after the publication, P. Hovenkamp included the name as synonym of *P. wandae* that was published by Raciborski (1902) based on pictures of nearly dead plant that sent from New Guinea to Bogor in 1899, which thought would not be survived. There were some expeditions to the Dutch New Guinea between 1902 to 1908 that made botanical collection for BBG (Steenis 1950) although no information whether this particular species was included in the collection.

Figure 7. Type specimen of *Platycerium wilhelminae-reginae*

8. *Leptochilus trifidus* Alderw. – Bull. Dépt. Agric. IndesNéerl. 18: 26. 1908 ; Cult. In Hort. Bog. II K V 20 ------ synonym for *Leptochilus decurrens* Blume Enum. Pl. Javae 2: 206. 1828 as in Fl. Males., Ser. 2, Pterid. 3: 83. 1998 [Polypodiaceae, Hovenkamp]

Unknown origin. Alderwerlt thought that this species might close to *L. harlandii* (now *Tectariahar landii*(Hooker) C.M. Kuo of Taiwan-China-Indochina). Long after his publication, P. Hovenkamp (1998) included this species into the highly variable *Leptochilus decurrens* with note that the *trifidus* form might indicate its hybrid status.

Figure 8. Type specimen of *Leptochilus trifidus*

Pteridaceae

9. *Pteris salakensis*Alderw. – Bull. Jard. Bot. Buitenzorg Ser. 2, VII. 26. 1912. ; Cult. In Hort. Bot. II K XIV 38

The species was said being collected from Mt. Salak and probably a local derivative of *Pteris quadriaurita* Retz. with some discrepancies (Alderwerlt, 1912). However, the occurrence of the living specimen is unknown.

Figure 9. Type specimen of *Pteris salakensis*

Thelypteridaceae

10. *Dryopteris piloso-squamata* Alderw. – Bull. Dépt. Agric. IndesNéerl. 21: 4. 1908; Cult. In Hort. Bog. II K II 8 ------ synonym for *Sphaerostephanos pilososquamatus* (Alderw.) HolttumFl. Males., Ser. 2, Pterid. 1(5): 481. 1982

The name based on a specimen collected from New Guinea. Alderwerlt (1908) explained that this species close to a more common species *Mesochlaena polycarpa* (*Sphaerostephanos polycarpus* (Blume) Copel.) but with some discrepancies. Holttum (1982) included this species into *Sphaerostephanos* with distinct character on pinnae incision and nearly dimorphic appearance.

Figure 10. Type specimen of *Dryopteris piloso-squamata*

CONCLUSION

The existence of cultivated plant in the garden are proven to be valuable for science around the beginning of 20th century with the publication of new taxa. In the group of fern only, at least 10 species were known to be newly described from plant cultivated in the garden. The existence of this living collection also aided re-confirmation of their taxonomic status which emphasized the crucial role of accurate labeling. Their survival will be even more important as more investigation is needed for science to understand relationship and morphological evolution of plants. Experiment and examination of living material might enlighten on the questions that was not answered with observation of the preserved one.

REFERENCES

Alderwerlt van Rosenburg CRWK van. 1908. New or Interesting Malayan Fern. Bull. Dept. Agric. IndesNéerl. 18:24

Alderwerlt van Rosenburg CRWK van. 1908. New or Interesting Malayan Fern 2.Bull. Dept. Agric. IndesNéerl. 21:4

Alderwerlt vanRosenburg CRWK van. 1912. New or Interesting Malayan Fern 4.*Bull. Jard. Bot. Buitenzorg Ser. 2*, VII. 26

BakerCA. &PosthumusO. 1939. *Varenfloravoor Java, overzicht der op Java voorkomindevarensenvarenachtigen, hare verspreiding, oekologieentoepassingen*. Uitgave van's lands plantentuin, Buitenzorg.

Christenhusz MJM, Zhang X, & Schneider H. 2011. A Linear Sequence of extant families and genera of lycophytes and ferns. *Phytotaxa*. 19: 7-54

Holttum RE. 1963. Cyatheaceae. *Flora Malesiana Ser. II Fern and Fern Allies* 1(2): 65-176 Rijksherbarium/HortusBotanicus. Leiden

Holttum RE. 1978. Lomariopsis Group. *Flora Malesiana Ser. II Fern and Fern Allies* 1(4): 255-330. Rijksherbarium/HortusBotanicus. Leiden

Holttum RE. 1982. Thelypteridaceae. *Flora Malesiana Ser. II Fern and Fern Allies* 1(5): 331-560. Rijksherbarium/HortusBotanicus. Leiden

Hovenkamp PH, Bosman MTM, Hennipman E, Nooteboom HP, Rödl-Linder G &Roos MC. 1998. Polypodiaceae. In: Kalkman C. *et al.* (eds). *Flora Malesiana, Ser. II, Ferns and Fern Allies* 3: 1–234. Rijksherbarium/HortusBotanicus, Leiden

Lehnert M., Coritico FP, Darnaedi D, Hidayat A, Kluge J. KargerDN, Kessler M. 2013. Taxonomic and Ecological Notes on *Alsophilahornei* Complex (Cyatheaceae-Polypodiopsida) with the description of the new species *A. phlebodes* from New Guinea. *Systematic Botany*. 38(4): 875-886

Morton CV. 1967. Studies of fern types. *Contribution from the United States National Herbarium* 38(2): 29-83

Raciborski M. 1902. O Kilkunieznanychpaprociachaarchipelagomalajskiego. *Bull. Int. Acad. Sci. Cracovie* 54-65.

Rolleri C H. 2003. Caracteresdiagnosticos y taxonomiaen el genero*Angiopteris*Hoffm. (MarattiaceaeBercht. &J.S. Presl) II, Sinopsis de las species. *Revista Del Museo de La Plata, Botanica* 16 (116): 1-23

Sihotang VB &Royani MF. 2007. Herbarium Bogoriensedalamlipatansejarah Indonesia (Unpublished). BidangBotani, PuslitBiologiLIPI

Steenis-Kruseman MJ van. 1950. Illustrated Alphabetical List of the Collectors. *Flora Malesiana Ser. I Vol.1*. Noordhoff-Kolff, Jakarta.

Yatabe Y, MatsuyamaS, Darnaedi D, Murakami N. 2001. Molecular systematics of the *Asplenium nidus* complex from Mt. Halimun National Park, Indonesia: evidence for reproductive isolation among three sympatric *rbc*Lsequence types.*Amer. J. Bot.*88: 1517-1522

Tree species composition of lowland hill forest on volcanic rock in Waigeo Island, Raja Ampat district, West Papua

Arief Hamidi[1], Jefry Tibalia[2], Fazlurrahman Shomat[1], Sirijan Lunga[2]
[1]Fauna&Flora International – Indonesia Programme.
Komplek Margasatwa Baru, Jl. Margasatwa Baru No. 7A, Pondok Labu, Jakarta 12450, Indonesia.
[2]Balai Konservasi Sumber Daya Alam Papua Barat.
Jl. Jendral Sudirman No. 40 Sorong, Papua barat 98412, Indonesia.
*Email: Arief.Hamidi@fauna-flora.org

ABSTRACT

Waigeo Island possess an intact forest with various unique ecosystems.This forest is under serious threat from infrastructure development, but the baseline data on forest ecosystems are limited. This study aims to provide baseline information on tree composition and diversity, particularly on hilly volcanic rock. We surveyed nine sample plots (125 X 20 m) that were purposively placed next to threatened areas in three trails. On each plot, tree species were recorded and its DBH (Diameter at Breast Height) was measured. A total of 119 species were recorded from this study. The species diversity in the study area is considered high with Shannon-Diversity Index equal to 3.8. The richest families was Fabaceae, followed by Myrtaceae, Anacardiaceae, and Burseraceae. The general vegetation type is *Vatica rassak*Bl. with an Important Value Index (IV) of 55.3, in association with *Teijsmanniodendron bogoriense* Koord. (IV 17.3), and *Pimelodendron amboinicum* Hassk. (IV 9.9). Pseudomontane vegetation was observed in the study area due to Massenerhebung effect on hills, where *V. rassak* is absentand it is replaced by *Gymnostoma rumphianum* (Miq.) L.A.S.Johnson, *Manilkara fasciculata* (Warb.) H.J.Lam & M.Geest and *Kjellbergiodendron celebicum* (Koord.) Merr. This result can be used to help in develop better forest management in response to infrastructure development.

Keywords: lowland hill forest, pseudomontane, species composition, tree diversity, Waigeo Island

INTRODUCTION

Waigeo Island which has a wide terrestrial area about 3,155 Km^2, is the largest among other in Raja Ampat archipelago. Its position is at the northernmost of the acrhipelago and ranged off the western coast of Vogelkop Peninsula (Arifiani, 2010; Beehler, 2007 *in* Marshall & Beehler, 2007). Even relatively close to Vogelkop in Mainland of Papua, Waigeo along with Halmahera and Batanta has different origin where was part of the Tosen Micro-Plate (Pacific), while Vogelkop Peninsula was part of Australian Plate along with Misool and Salawati(Webb, 2005; Pholemus, 2007).This origin and the adjacent location to Vogelkop Peninsula has resulted a unique ecosystems and species assemblages in this island. This is because the island acquired flora and fauna both from Pacific and Australian Regions (Marshall & Beehler, 2007). Prior studies shows that Waigeo Island has at least nine vegetation types i.e: mangrove, freshwater swamp woodland, beach, lowland volcanic forest, lowland ultrabasic forest, lowland limestone forest, savanna, other secondary forest, and submontane forest (Darnaedi *et al*., 2006; Takeuchi, 2002; Webb, 2005). Botanical expedition in eastern Waigeothat was conducted by Indonesia Center of Science (LIPI) in 2007collected 650 specimensfrom 70 families. Those specimens consist of more than 400 vascular plants and several of them are new record to Waigeo or Papua (Arifiani, 2010; Widyatmoko, 2010). It has proved that despite Waigeo is well-known for the worlds' richest coral diversity, its terrestrial ecosystem and biodiversity are undoubtfuly remarkable yet lesser explored.

About 89% of total area of Waigeo Island is classified as forest area, which it consists of 80% primary forest and 9% secondary forest (Ministry of Forestry, 2013). About 56% of primary forest is classified as hill forest on volcanic rock (figure 1). Waigeo island is a habitat for several endemic species, such as Waigeo Cuscus, Waigeo Brush-turkey, Wilson's Bird of Paradise, Red Bird of Paradise, and Danser's pitcher plant (Beehler, 2007; Helgen, *et al*., 2016; Mauro, 2006; Sudarmono, 2011). Webb (2005) suggests that the plant diversity of Raja Ampat per unit area is considered as the

highest in the world. Because itsdiverse ecosystem and endemicity, Ministry of Forestry designated 85% of Waigeo area as Nature Reserve (NR) under the management of *Balai Konservasi Sumber Daya Alam* (BKSDA) Sorong, West Papua.

As the capitalof Raja Ampat district and a highly popular of tourim destination, the development pressure in Waigeo are inevitable, including the road development. Recently, government has planned a ring-road in this island thatis encircling Mayalibit Gulf, and extent to eastern-western most part of the island (figure 1). Several parts of the ring-roadencompassthe NR which is mostly a pristine lowland forest particularly on volcanic rock. In-depth ecological study are required prior to implementation of the ring-road development planning. Fauna-Flora International – Indonesia Programme (FFI-IP) and BKSDA of Sorong conduct biodiversity survey in both western and eastern parts of Waigeo NR. As part of biodiversity survey, this study aims to collect detail baseline information of tree composition in three trails next to the planned ring-road area, especially in volcanic rock area.

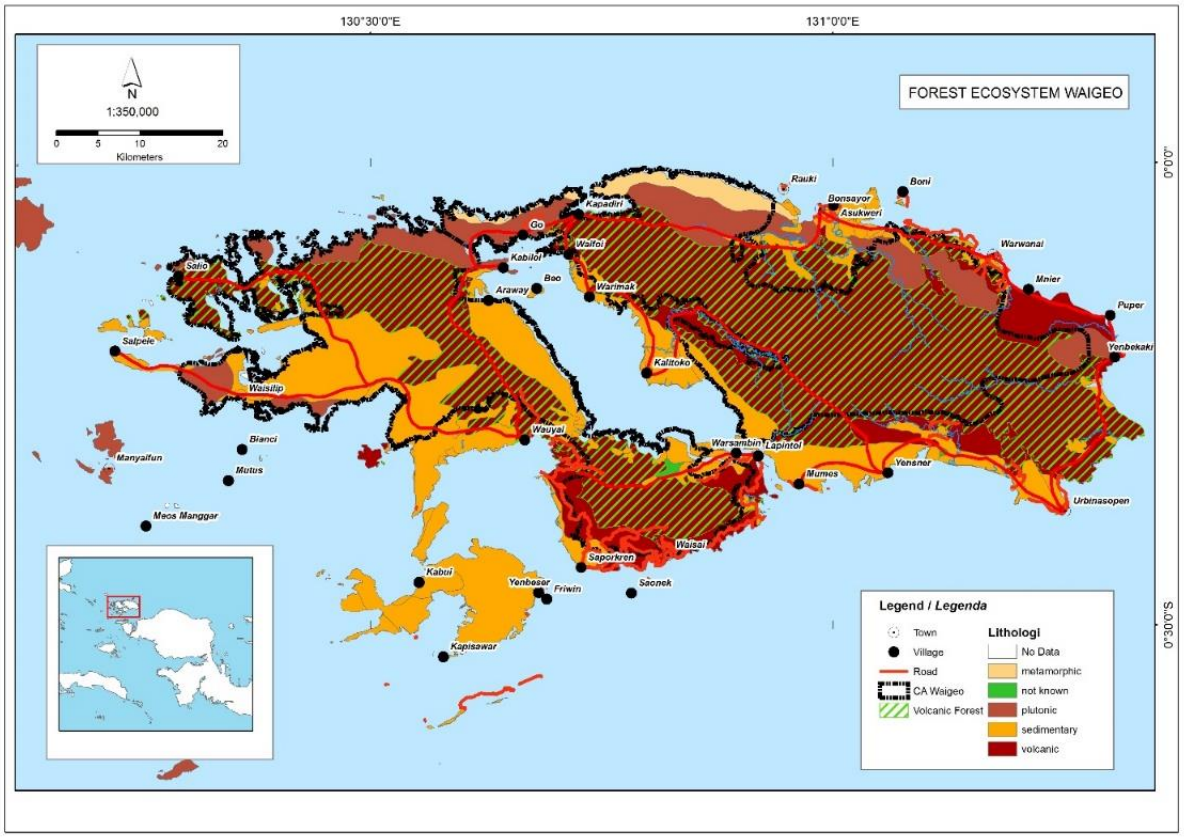


Figure 1. Waigeo Island. Black line is the boundary of the Waigeo Nature Reserve. Red line is the planned Waigeo ring-road. Brown color is the volcanic lithology area. (map sources from *Balai Besar Pelaksana Jalan Nasional X Direktorat Bina Marga – Kementrian Pekerjaan Umum*, 2015 and *Kementrian Kehutanan*, 2015)

MATERIAL AND METHODS

Time and study area

The study was conducted from September to October 2016, in western and eastern parts of Waigeo Nature Reserve, Raja Ampat district, West Papua. Data collection focused in three forest trails (figure 2) that can be accessed from Warimak, Warsambin, and Saporken Villages. All the forest trails are located in undulating of hill forest on volcanic rock ecosystem type. The slope are ranging from 15 to 65 % or undulating to steep. Following Schmidt-Fergusson category, Raja Ampat has a very wet

climate with the Q-value: 0.07, according to the mean rainfall on wet and dry season which is 20 and 254 mm/month respectively (BPS Raja Ampat, 2016). The highest mean monthly rainfall occurs in May to July i.e 339.5 mm while the driest in September i.e 129.3 mm.

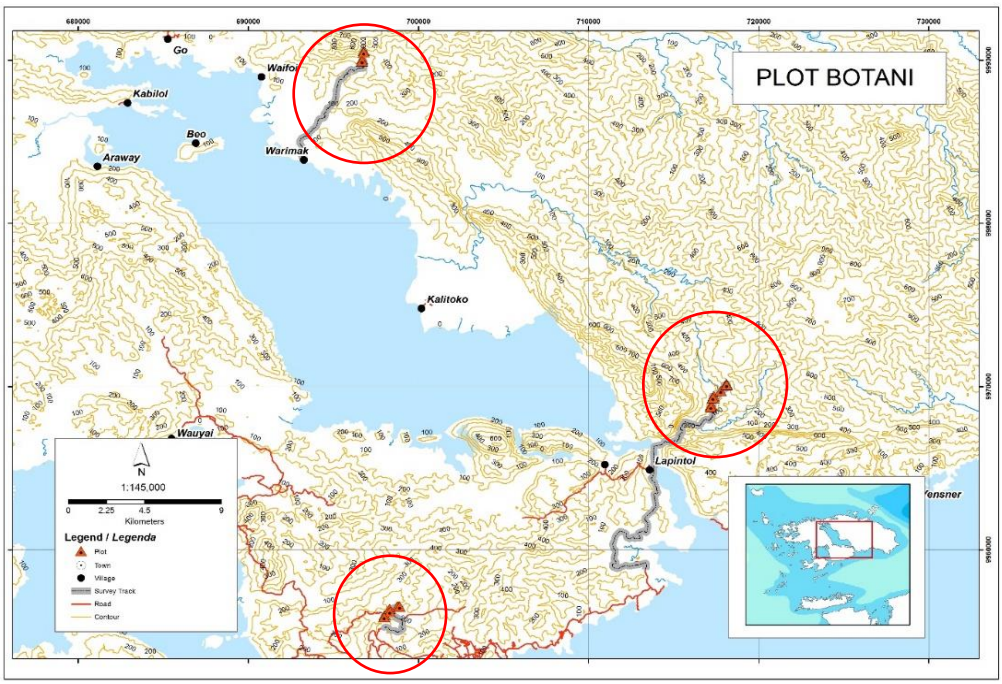


Figure 2. Sampling locations (red circles). Grey line is forest/observation trail and red triangle is plot location.

Sampling methods and analyses

Along three trails, nine nested plots were placed puposively next to the planned ring-roads in different elevation (Table 1). Each nested plot are consist of three subplots with size 125 x 20 m (subplot A), 20 X 20 m (subplot B), and 10 X 10 m (subplot C). Subplot A is used to collect data for tree with DBH $\geq$ 30 cm, subplot B for tree with DBH 15 – 29.99 cm, and subplot C for tree with DBH 5 – 14.99 cm. All trees inside sub-plot are recorded complete with its DBH and height measurement. Palms, cycads, and pandans were included due to their contribution in vegetation structure. Leaf, fruit, and flower (if available) were collected for voucher herbarium. In addition, field characters, such as bark, sapwood, resin, and exudate are observed. Species identification and determination are conducted using herbarium observations, digital image comparison, books of Flora Malesiana and Flora of Papua New Guinea, as well as experts discussion.

Table 1. Description of sampling locations.

Trail	Direction	Plot ID	Description
Warimak (eastern Waigeo)	From village to Mt Nok	WARI1	Hillside at elevation 316-342 m asl
		WARI2	Hillside at elevation 238-255 m asl
Warsambim (eastern Waigeo)	From village (crossing Orobiai river) to Mt Danai	WARS1	Top hill at elevation 234-245 m asl
		WARS2	Plain at elevation 41-62 m asl
		WARS3	Plain at elevation 56-77 m asl
		WARS4	Hillside at elevation 123-129 m asl
Saporkren (western Waigeo)	Adjacent to existing local airport and abandoned road	SAP1	Hillside at elevation 129-132 m asl
		SAP2	Top hill at elevation 205-206 m asl
		SAP_3	Hillside at elevation 148-162 m asl

Species Important Value (IV) are calcuted to analyze tree composition and to examine the ecological importance of each species in a community(Mueller-Dombois & Ellenberg, 1974). Species diversity indices (e.g. Shannon-diversity index (H'), Simpson and Bray-Curtis similarity)) are analysed to compare communities, using PAST software (Hammer *et al*., 2001). In addition, Principal Component Analysis (PCA) are used to investigate altitudinal zonation and similarity of community among plots.

RESULTS AND DISCUSSIONS

Tree Diversity

Forest condition in the study area are a near-pristine to pristine forests with intact canopy stratum, i.e subcanopy (mean 10.4 m), mid-canopy (mean 21.1 m), upper canopy (mean 28.6 m) and some emergent (mean 37.2 m and max 39.2 m). The total species recorded from this study is 119 species from at least 41 families. Of those species,thirteen species arefailed toidentifiedin family level. In general, Fabaceae is the richest family in the study area (8 species), followed by Myrtaceae (7), Anacardiaceae and Burseraceae (6), Elaeocarpaceae, Myristicaceae and Sapotaceae (5) (figure 3).

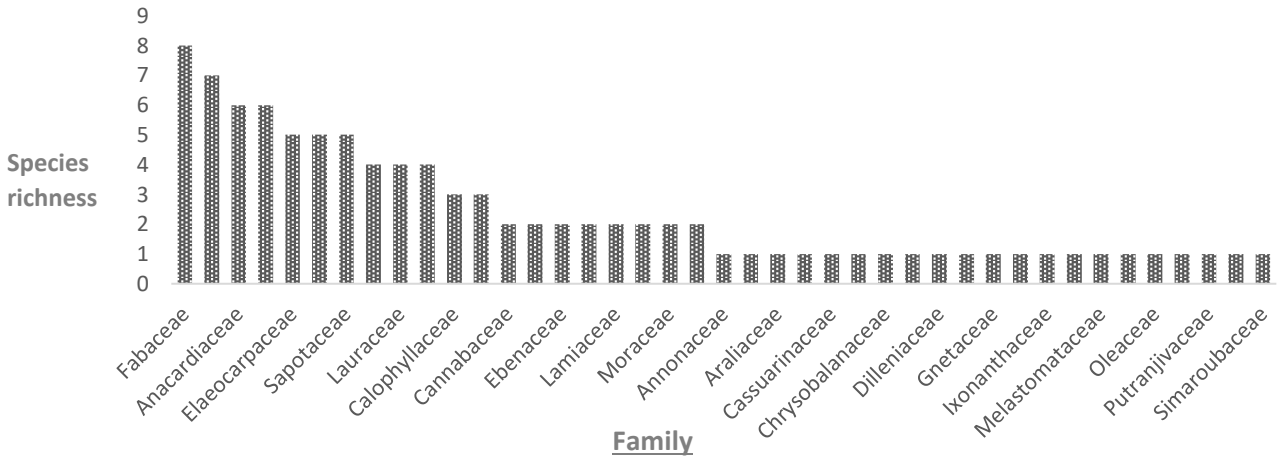


Figure 3. Species richness on each family

Species diversity in the study area is considered high with Shannon-diversity index (H') equal to 3.86. About 51% of total species occur as a single individual only. It is about 7.4% species that represented by 7 or more individuals. This indicates that its floristic richness are well above the average recorded in tropical rain forest, equivalent to the richest rain forest of Borneo and Malay Peninsula (Clunie, 2007). The distribution of H' are shown in figure 4. Plots in Saporkren has lower H' than others. It is due to the higher domination by single species while species richness is low (12-19 species), *Vatica rassak* Bl. was dominant by 29% abundance among other. It was happen as well in WARS 3 where *V. rassak* very dominant by its abundance exceeds 40% of 16 species. The highest diversity occurs in Warimak where domination by little species was low and species richness was high (up to 32 species). Plots of Warimak situated in higher elevation at 238-344 m asl. It appears that at this range of elevation the compositional change was happening, whereas species was mix between the lower and upper community. Thus, this mix composition resulting the higher species diversity. This result was in line with Webb's (2005) notes where he found that the vegetation composition was change in 300 m asl elevation zone.

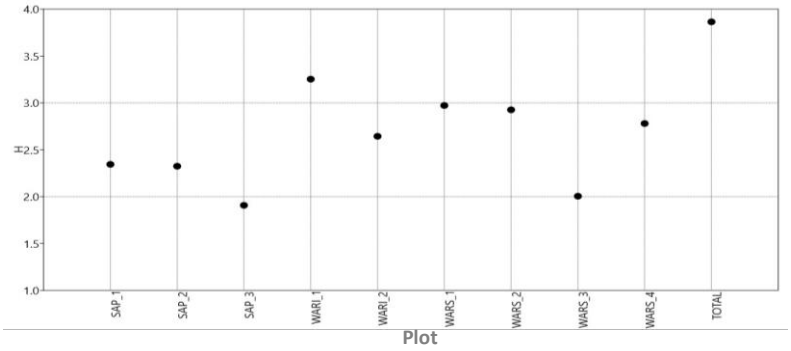


Figure 4. The distribution of Shannon-Diversity Index (H') among plots

The indication that formed a highly tree diversity upon all Waigeo forest, are the occurences of mixing species element between western and eastern Malesia. Such common genera like*Intsia*, *Vatica*, *Teijsmanniodendron*, *Celtis*, *Homalium*, *Calophyllum*, *Garcinia*, *Ixonanthes* and *Canarium* are western Malesia element. This notes are recognized as well by Takeuchi (2003) and Webb (2005) with additional notes 'the lack of Dipterocaps'. Whilst, Papuan element which has a great influence to Waigeotree community are come from, in particular,the family of Elaeocarpaceae, Arecaceae, Combretaceae and Lythraceae (Clunie, 2007). Besides, many western Malesia family are also consisting Papuan species (Maluku to New Guinea), such like*Canarium maluense* (Burseraceae), *Hopea papuana*, *Hopea novoguineensis*, *Anisoptera thurifera* (Dipterocarpaceae), *Serianthes minahassae* (Fabaceae), *Chisocheton ceramicus* (Meliaceae), *Manilkara fasciculata* (Sapotaceae), *Virola surinamensis* (Myristicaeae), and *Kjellbergiodendron celebicum* (Myrtaceae).Despite 'the lack of Dipterocaps', which had recorded consists of five species only (including *Hopea iriana* Slooten found outside plot), *V. rassak* had shown a high domination upon forest community. This high domination are contribute to the low H' (usually H'<2.5) in several plots, particularly SAP_3 and WARS_3.

Tree communities

According to the species IV of all plots, the most dominant species is *Vatica rassak* with IV of 55.3 or 18.4% of total species IV. Its basal area (BA) reaching 70.7 m^2 alone (see annex). Other highest IV species following *V. rassak* is *Teijsmanniodendron bogoriense* and *Pimelodendron amboinicum* with IV of 17.3 (5%) and 9.9 (3.3%) respectively. By the far different IV between *V. rassak* and the second highest IV, it is obvious that *V. rassak* is the major composition of forest community. Meanwhile, the community of each habits are majorly composed by (with IV):

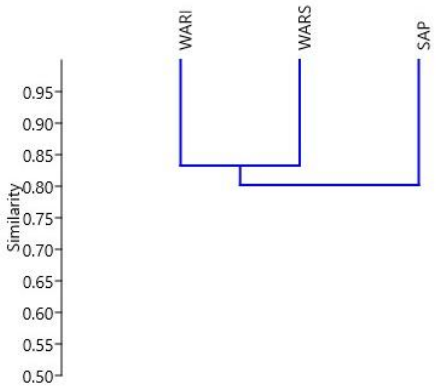
Large trees: *Vatica rassak* (59,9), *Teijsmanniodendron bogoriense* (23,9), *Pometia pinnata* (11,7), *Pimeleodendron amboinicum* (8,7), *Palaquium* cf *obovatum* (8,4), *Hopea novoguineensis* (7.9), *Hopea papuana* (7.6), *Pertusadina eurhyncha* (6.5), etc.;

Small trees: *Vatica rassak* (56,6), *Pimelodendron amboinicum* (17,8), *Horsfieldia sylvestris* (12), *Syzygium sp*. (11,9), *Hopea novoguineensis* (11,6), *Gymnacranthera paniculata* (10.2), *Garcinia* cf *archboldiana* (8.8), *Parastemon versteghii* (8.7), *Elaeocarpus* sp (8.6), *Maniltoa plurijuga* (6.6), etc.;

Poles: *Vatica rassak* (43,2), *Garcinia* cf*archboldiana*(23,7), *Pimelodendron amboinicum* (16,3), *Calophyllum parvifolium* (15,2), *Canarium acutifolium* (13,5), *Elaeocarpus* cf *multiflorus* (12,9), *Decaspermum* (12,3), *Hopea novoguineensis* (8.3), etc.

According to species IV per habit above, *V. rassak* clearly very dominant in all stages followed by *P. amboinicum*,while *T. bogoriense* only common in tree form. The abundance of those species at each stages indicates that its growth and regenerationis at optimum level which made their population remain viable. In term of canopy layer, the upper canopy is majorly composed by large trees and some small trees which also composing the mid-canopy. The subcanopy is composed mainly by poles.

Beside a total community, the comparison of tree community among three trails had been investigated. The Bray-curtis dendrogram shown the similarity of community among trails exceeding 80% (figure 5). This indicates that the forest comunities are fairly even among trails given the different location, including between western versus eastern parts of the Waigeo NR. Meanwhile, differ from community, the species diversity between two sides has significantly different based on t-test analysis using Shannon-diversity index ($\rho < 0.05$). It means that though the community was about to even, the specific species composition are not similar. Two sides of Waigeo NR was disjuncted by Mayalibit Gulf which has 38 Km long and 12 Km wide (Hamidy and Mulyadi, 2007). This wide of Gulf may effectively play a role as barrier between these two community, with exceptional for *V. rassak*. This species can disperse thorough salt water and stream river up to hills (Gazoul, 2016). However, it is required further investigation to reveal this ecological phenomenon.



trails t-test of Simpson index (H'):

Warsambin (H' 3.7) vs Sapokren (H'3.2)
ρ 0.0000001 (<0.05)

Warimak (H'3.6) vs Sapokren (H'3.2)
ρ 0.0003 (<0.05)

Warimak vs Warsambin
ρ 0.029 (>0.05)

Figure 5. Bray-curtis similarity (left) and t-test Shannon-diversity analysis (right) among trails

There is the differences among sides in term of species composition (Table 2). Despite *V. rassak* remains the major composition in both side, its associate species is changing. The IV of Papuan Hopea i.e. *H. papuana* and *H. novoguineensis* along with *Manilkara fasciculata* is higher in the west than in the east, and thus become associate species instead of *T. bogoriense* and *P. amboinicum* as in the west. The domination of *V. rassak* is incredibly high in the west, leaving much smaller space for other species. Moreover, the highest of 50%-total-IV is owned only by 7 species, while in the east it has owned by 11 to 13 species. This is leading to smaller diversity in the west due to smaller species number and higher domination by fewer species.

Table 2. The most common species that compose the forest community in each trail

Saporkren[w]	Warimak[e]	Warsambin[e]
Vatica rassak (60.1)	*Vatica rassak* (49.3)	*Vatica rassak* (55.2)
Hopea papuana (22.3)	*Teijsmanniodendron bogoriense* (16.1)	*Teijsmanniodendron bogoriense* (16.7)
Manilkara fasciculata (20.6)	*Myristica subalulata* (12.91)	*Pimelodendron amboinicum* (12.8)
Teijsmanniodendron bogoriense (19.6)	*Pimelodendron amboinicum* (11.7)	*Pometia pinnata* (11.9)
Hopea novoguineensis (15.4)	*Inocarpus fagiferus* (11.4)	*Horsfieldia sylvestris* (8.9)
Gymnostoma rumphianum (9)	*Syzygium* sp (9.6)	*Intsia palembanica* (7.9)
Ixonanthes reticulata (8.9)*	*Pometia pinnata* (9.5)	*Pertusadina eurhyncha* (7.5)
Anisoptera thurifera (7.9)	*Palaquium lobianum* (8.8)	*Garcinia* cf *archboldiana* (6.6)
Homalium foetidum (7.6)	*Dillenia papuana* (8.6)	*Hopea novoguineensis* (6.2)
Etc.	*Myristica inutilis* var. *papuana* (7.7)	*Celtis philiphinensis* (5.6)
	*Celtis philiphinensis (*7.7)*	*Duabanga moluccana* (5.5)
	Etc.	*Syzygium* sp.2 (5.3)
		Kjellbergiodendron celebicum (5.2)*
		Etc.

Notes: 'w' for western and 'e' for eastern of Waigeo; the number inside parentheses showing its IV; species list up to '*' means that the cummulative IV reaching 50% of total species

Besides similarity among trails, we investigated the forest community among plots. PCA was employed using 25 most abundance species of all plots. We use PC1 and PC2 as it has highest total variance of eigenvalues that reach 55.6%. The result show that plots has divided into two different axis (figure 6 left). This indicates that the study area has two major communities. The first group (left axis) comprised by seven plots where all situated in the plain or hill-side area, while the other two (right axis) are plots where placed at the top of the hills. First group is clustered by the high abundace of *Vatica rassak*, *Pimeleodendron amboinicum*, *Pertusadina eurhyncha* and *Teijsmanniodendron bogoriense* (figure 6 right). The second group has divided as well due to its speci es characters. One plot (SAP_2) was separated due to the high abundance of *Manilkara fasciculata*, *Gymnostoma rumphianum*, *Ixonanthes reticulata* while WARS_1 by *Garcinia* cf *archboldiana*, *Syzygium rosaceum*, and *Kjellbergiodendron celebicum*.

The first group has a major community of *Vatica rassak* forest and slightly even among group (table 3). However, its association is different following the gradual increase of elevation per 100 m. Association of *V. rassak* and *Teijsmanniodendron bogoriense* always happen in all elevation except at 200-300 m asl. *Gymnacranthera paniculata*, *Dillenia papuana*, and both Papuan Hopea become common from 100 to 300 m asl. At elevation of 300 m asl and above, the dominance of *V. rassak* was decreased and its abundance are closer to *T. bogoriense* and *Pometia pinnata* as the associate species. In addition, Fagaceae occuring in this elevation represented by *Lithocarpus* cf *megacarpus*, and Lauraceae become more common. Many species e.g *Maniltoa plurijuga*, *Homalium foetidum*, *Myristica lancifolia*, *Mangifera inocarpoides* and *Inocarpus fagiferus* always present in all elevation but in smaller IV (minor element).

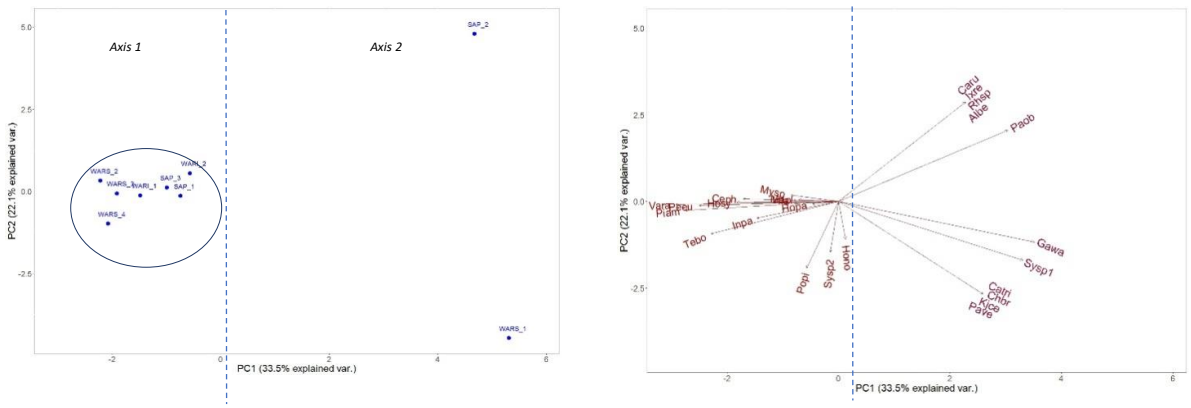


Figure 6. PCA among plots. Acronyme of species i.e. VARA – *Vatica rassak*; PIAM – *Pimelodendron amboinicum*; TEBO – *Teijsmanniodendron bogoriense*; MYSP – *Gymnacranthera paniculata*; INFA – *Inocarpus fagiferus*; MAPL – *Maniltoa plurijuga*; PEEU – *Pertusadina eurhynca*; CEPH – *Celtis Philiphinensis*; HOSY – *Horsfieldia sylvestris*; INPA – *Intsia palembanica*; HOPA – *Hopea papuana*; POPI – *Pometia pinnata*; SYSP2 – *Syzygium* sp.2; HONO – *Hopea novoguineensis*; CARU – *Gymnostoma rumphianum*; IXRE – *Ixonanthes reticulata*; RHSP – *Rhodamnia cinerea*; ALBE – *Alstonia beatricis*; PAOB – *Manilkara fasciculata*; GAWA – *Garcinia* cf *archboldiana*; SYSP1 – *Syzygium rosaceum*; CATRI – *Canarium asperum* f. *triphyllum*; CHBR – *Chionanthus* cf *brassii*; KJCE – *Kjellbergiodendron celebicum*; PAVE – *Parastemon versteeghii*;

Table 3. Tree composition along the increse of 100-m-asl elevation

Elevation	Vegetation type	Important species (highest IV)	Minor Element (low IV)
300-400 m	Association of *Vatica rassak-Teijsmanniodendron bogoriense-Pometia pinnata*	*V. rassak* (34.1), *T. bogoriense* (21.3), *Pometia pinnata* (17.4), *Inocarpus fagiferus* (13.5), *Celtis philiphinensis* (11.7), *Sloanea* sp., *Pimelodendron amboinicum*, *Gironniera subaequalis*, *Haplolobus floribundus*, *Palaquium supfianum*, etc.	*Koordersiodendron pinnatum*, *Myristica inutilis*, *Maniltoa plurijuga*, *Homalium foetidum*, *Aceratium* sp, *Alseodaphne archboldiana*, *Lithocarpus* sp, *Myristica lancifolia*

Elevation	Vegetation type	Important species (highest IV)	Minor Element (low IV)
200-300 m	*Vatica rassak* forest association with *Gymnacranthera paniculata - Dillenia papuana*	*V. rassak*(67), *G. paniculata*(21.4), *D. papuana* (19), *Syzygium rosaceum* (14), *P. amboinicum* (14), *Intsia bijuga*, *T. bogoriense*, *Hopea papuana*, *H. novoguineesis*	*Maniltoa plurijuga, Myristica lancifolia, Palaquium lobianum, Campnosperma brevipetiolatus, Ficus subulata, Mangifera inocarpoides, Dillenia papuana, Anisoptera thurifera, Inocarpus fagiferus, Cynometra sp, Calophyllum persimile, Crytocarya infectoria*
100-200 m	*Vatica rassak* forest association with *Teijsmanniodendron bogoriense – Manilkara fasciculata*	*V. rassak* (45), *T. bogoriense* (23), *Palaquium* sp (18.5), *H. papuana* (15), *H. novoguineensis* (9), *P. amboinicum*, *Canarium acutifolium*, *Pometia pinnata*,*Intsia palembanica*	
0-100 m	*Vatica rassak* forest association with *Pimelodendron amboinicum-Teijsmanniodendron bogoriense*	*V.rassak* (78), *P. amboinicum* (18), *T. bogoriense*(14.5), *Horsfieldia sylvestris, Pertusadina erhyncha, Celtis philipinensis, Diospyros pilosanthera, Intsia palembanica.*	

notes: number in parentheses show the highest IV of the species

The second group consist of two plots where were situated at the top of the hills. Based on result, it is interesting that *V. rassak* is completely disppear in both plots, and replace by species that almost absent in other area. Moreover, its vegetation structure is also distinct where poles is dense and the closed canopy is shorter at around 10-12 m height (figure 7). The circumstances seem more humid as moss are common, yet the soil are much-drained due to its steep countur of the top. The forest majorly constructed by small-leaves species such like *Gymnostoma rumphianum*, *Calophyllum parvifolium*, small-leaves Myrtaceae, etc., which made the vegetation brighter. This vegetation are resemble to upper-mountainous than lowland forest, but located at much lower elevation (from 200 m asl). Webb (2005) recognized it as pseudomontane, a result of 'Massenerhebung' effect occurs at much lower elevations since the proximity of their tops to the ocean are close. This pseudomontane seems scatered along Waigeo landscape as patches wherever meets the steep of top hills. Van Royen (1960) found it at the top of Mt. Nok and Widyatmoko (2010) at Manitalu hill.

Figure 7 the interior vegetation of pseudomontane (left) and lowland forest (right)

Two pseudomontane plots are located in separate location, one in Sapokren trail (205 m asl) and other in Warsambin trail (234 m asl). The community between two plots are different as shown by figure 6 above. In Sapokren, the community was the associaton of *Manilkara fasciculata* –

Gymnostoma rumphianum along with the abundance of *Alstonia beatricis*, *Calophyllum parvifolium* and *Rhodamnia cinerea*. In Warsambin, the community is association of *Garcinia* cf *archboldiana* – *Kjellbergiodendron celebicum* along with the abundance of *Manilkara fasciculata*, *Parastemon versteghii*, and *Syzygium rosaceum*. All of these species are small leaves species, especially *G. rumphianum* (Cassuarinaceae) which has needle-like 'leaves'. *K. celebicum* particularly has a distinct bark that peeling as in *Eucalyptus* and showing a white-smooth inner bark, the leaf lamina are resemble to *Tristaniopsis*. Both *G. rumphianum* and *K. celebicum* are only found in this pseudomontane along with the Waigeo endemic *Alstonia beatricis*.

Species findings and taxonomic notes

Besides 119 tree species that recorded inside all plots, other species occurs outside plots were also recorded to complete the species list. The species richness then higher exceeding 170 species of trees from at least 52 family. The species identification is challenging, plus with the absence of generative characters made some species remains unidentified. However, we believe that many species is newly record to Waigeo Island and several may possible to new species. Among identified specimen to the level of genus, we highlight two interesting genera that is newly record for Waigeo. One of them is *Sympetalandra* (Fabaceae) that is newly record even for Papua region. *Sympetalandra* has bipinnate leaf and differ with *Archidendron* in the absence of glands along rachis (fig 8 left). Its distribution are recorded only in west Malesia up to Philiphine and Lesser Sunda Islands (van Steenis, 1975). According to the revision of genus *Sympetalandra* by van Steenis (1975), it has consist of five species only i.e. *S. densiflora*, *S. unijuga*, *S. borneensis*, *S. hildebrandii*, and *S. schmutzii*. Based on its description, *Sympetalandra* found in this study may resemble to *S. densiflora* from the Philiphine, but with the more pairs of pinnae as well as jugate.

Other genera is *Aceratium* (Elaeocarpaceae) which is newly record for Waigeo Island. *Aceratium* are well-recognized by the opposite-leaves of Elaeocarpaceae which also exstipulate and has petioles less than 1 cm (fig 8 right) (Coode, 1995). This genera mostly occurs in highland of Papua, only several species present in lowland. Since the flowering specimen are lacking, the identification are hard to the species level. However, according to The Handbook of Flora of Papua New Guinea vol. 2 (Coode, 1995), this *Aceratium* is similar to *A. sphaerocarpum*, *A. oppositifolium*, and *A. parvifolium*. *A. oppsotifolium* have a higher possibility to be refered since it has wider distribution from Celebes, Moluccan Islands and Papuasia up to Santa Cruz group, where Waigeo may included in. It has also similar habitat with the study area where is primary or secondary forest from sea level to 300 m asl.

Figure 8. left : *Sympetalandra* leaf and its absence of gland (inset); right: *Aceratium* leaves

Human impact and forestry management

Since most of the Waigeo forest area were designated as Nature Reserve, under the management unit of BKSDA of Sorong, West Papua, the sustainability of forest quality are protected under the law. Besides, low population and well concern of local people in conserving forest regarding their influential customary, made the forest has lesser threats (Webb, 2005; Widyatmoko, 2010). Nevertheless, Waigeo has historically experienced illegal logging by outsider about two decades ago and seems remain there but in lower number. To alter this threats, BKSDA was intensely conducting protection and monitoring in colaboration with local Police Department, Indonesian army, and local attorney.

Recently, the serious threats might come from the infrastructure development plan following provincial spatial palnningupon Waigeo. Effective and well conservation planning are then required. One method may apply using this baseline information that is arranging an effective block on forest communities which protect also the rare, threatened, endangered and endemic (RTEE) species native to Waigeo (table 4). In response to infrastucture planning, BKSDA shall propose a valuable input for sustainable development with minimize destruction impact. The habitat for such RTEE species are recommended to be included in the core of conservation zone. This would include the pseudomontane habitat and lowland pristine forest. This core of conservation block then also recommended to be avoided from the infrastructure development.

Table 4. The rare, threatened, endangered and endemic species found in volcanic-rock forest of Waigeo

Species	Family	RTE status	Habitat	Study
Intsia bijuga	Fabaceae	IUCN VU A1cd	Lowland forest	This study
Intsia palembanica	Fabaceae	P.92/2018	Lowland forest	This Study
Agathis labillardierei	Araucariaceae	P.92/2018	Hill forest	This study
Alstonia beatricis	Apocynaceae	IUCN VU D2 Endemic waigeo	Pseudomontane forest	This study, Sidiyasa, 1996
Calophyllum parvifolium	Calophyllaceae	IUCN VU D2	Pseudomontane forest	This study
Anisoptera thurifera	Dipterocarpaceae	IUCN VU A3cd	Lowland hill forest	This study
Aquilaria filaria	Thymelaeaceae	IUCN VU A2cd CITES App. II	Lowland hill forest	This study
Gyrinops versteeghii	Thymelaeaceae	CITES App. II	Lowland forest	This study
Gonystylus cf *macrophyllus*	Thymelaeaceae	CITES App. II	Lowland hilll forest	This study
Many species of orchids	Orchidaceae	CITES App. II	Lowland hill forest, pseudomontane forest	This study
Many species of pitcher plants	Nepenthaceae	CITES App. II	Pseudomontane, hill forest	This study
Guioa waigeoensis	Sapindaceae	Endemic Waigeo	N/A possibly on volcanic-rock	Widyatmoko (2010)
Rhododendron cornu-bovis	Ericaceae	Endemic Waigeo	Sub-montane on volcanic (Mt. Nok)	Van Royen (1960) in Webb (2005)

Notes: IUCN - International Union for Conservation of Nature, VU = Vulnerable; CITES – Convention on International Trade in Endangered Species, P.92/2018 – Regulation of Ministry of Environment and Forestry No. P.20/MenLHK/SetJen/Kum.1/6/2018.

CONCLUSION

The major community in Waigeo lowland forest on volcanic rock are *Vatica rassak* forest with various associate species, such like *Teijsmanniodendron bogoriense*, *Manilkara fasciculata*, and *Pimelodendron amboinicum*. Other communities are identified at each top of hills namely pseudomontane, that resulted from Massenerhebung effect on lowland hills. Since the infrastructure development seems inevitable in Waigeo, many of these area are under threats along with its existing

RTEE species. Therefore, using this baseline information we strongly recommended that these roads development should avoid some specific areas, especially inside Waigeo NR and RTEE habitat. Further studies are required to enforce ecological information in order to construct well-sustainable forest management responding the development threats. It is include population and habitat for RTEE species, pseudomontane mapping, in-depth species community for other ecosystem type, and impact of development to biodiversity.

ACKNOWLEDGEMENT

We would like to thanks to Oroverde for kindly funding support; management of FFI-Raja Ampat program i.e Ririn Fitria, Setija Budiawan, Andhy P. Sayogo, Maurits Kafiar, Otto Wanma and Salmon Weyai for technical and logistic support; and BKSDA Sorong for permit and field support. We are appreciate for Hariyo T. Wibisono, Angga rachmansah, and Andri Irawan for review and valuable discussion. At last, we greatly appreciate for team survey in the field: Mudin, Stanley, Godlif and Lamek.

REFERENCES

Arifiani, D. 2010. Newly recorded Endiandra R.Br. (Lauraceae) from Waigeo Island, Raja Ampat, Papua, Indonesia. *Gardens' Bulletin Singapore*62(1): 23–30.

BPS Raja Ampat.2013-2016. Kabupaten Raja Ampat dalam angka2013-2016. Badan Pusat Statistik Kabupaten Raja Ampat.

Beehler, B. M. 2007. *The Ecology of Papua Part One*. (A. J. Marshall & B. M. Beehler, Eds.). Singapore: Periplus Editions (HK) Ltd.

Coode, M. J. . 1995. Elaeocarpaceae. In: E. E. Henty (Ed.), *Handbooks of the Flora of Papua New Guinea Vol. II*. Victoria: Melbourne University Press.

Darnaedi, D., Y. de Fretes, J. Mogea, & B. Iraningrum. 2006. Keanekaragaman biota Kepulauan Raja Ampat, Papua. In:*Workshop Nominansi Kawasan Raja Ampat sebagai Warisan Alam & Budaya Dunia*.

Hammer, Ø., D.A. Harper & P.D. Ryan. 2001. Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*4(1): 9–18.

Helgen, K., K. Aplin & C.D. 2016. *Spilocuscus papuensis*, Waigeo Cuscus. *The IUCN Red List of Threatened Species 2016, 8235*. Retrieved from http://www.iucnredlist.org/details/20638/0 (accessed 20 Auguts 2017).

Mauro, I. 2006. Habitat, microdistribution and conservation status of the enigmatic Bruijn's Brush-turkey Aepypodius bruijnii. *Bird Conservation International*16(4): 279–292.

Mueller-Dombois, D., & H. Ellenberg. 1974. The count-plot methods and plotless sampling techniques. In:*Aims and Methods of Vegetation Ecology* (pp. 110–120). New York: John Wiley and Sons.

Sidiyasa, K. 1996. *Alstonia beatricis* (Apocynaceae), a new species from Irian Jaya, Indonesia. *Blumea* 41: 29-31.

Sudarmono. 2011. An Inventory of Endemic Plant in Waigeo Island, Raja Ampat Regency, West Papua Province. *Globë*13(1): 78–84.

Takeuchi, W. 2002. A community-level floristic reconnaissance of the Raja Ampat Islands in New Guinea. *Sida, Contributions to Botany*20(3): 1093–1138.

van Royen, P. 1960. The vegetation of some parts of Waigeo Island. *Novo Guinea, Botany*5.

van Steenis, C.G.G.J. 1975. A review of the genus Sympetalandra Stapf and its position in Caesalpinioideae. *Blumea*22: 159–167.

Webb, C.O. 2005. Vegetation of the Raja Ampat Island, Papua, Indonesia. A report ot The Nature Conservancy Rev.1.5.

Widyatmoko, D. 2010. Plant- â Diversity and Composition in Mount Nok and the Waifoi Forest of the Waigeo Raja Ampat Islands: with Special Reference to The Threatened Species. *Jurnal Biologi Indonesia*6(2): 195–209.

ANNEX

Composition of tree-species recorded on plot in Waigeo lowland hill forest on volcanic rock with Important Value (IV) Index and Basal Area (BA). The list are sorted descending from highest to lowest IV. *A total species list (including species outside plot) are available upon request.*

Family	Species	IV	IV %	BA (m^2)
Dipterocarpaceae	*Vatica rassak* Blume	55.27	18.42	70.73
Lamiaceae	*Teijsmanniodendron bogoriense* Koord.	17.32	5.77	16.09
Euphorbiaceae	*Pimelodendron amboinicum* Hassk.	9.91	3.30	15.06
Sapindaceae	*Pometia pinnata* J.R.Frost&G.Frost	8.84	2.95	9.38
Sapotaceae	*Manilkara fasciculata* (Warb.)H.J.Lam&M.Geest	8.11	2.70	9.06
Dipterocarpaceae	*Hopea novoguineensis* Slooten	7.24	2.41	8.95
Dipterocarpaceae	*Hopea papuana* Diels	6.36	2.12	6.87
Rubiaceae	*Pertusadina eurhyncha* (Miq.)Ridsdale	5.53	1.84	3.83
Clusiaceae	*Garcinia* cf *archboldiana* A.C.Sm.	4.98	1.66	8.69
Myristicaceae	*Gymnacranthera paniculata* (A.DC)Warb.	4.82	1.61	7.24
Myristicaceae	*Horsfieldia sylvestris* Warb.	4.68	1.56	3.85
Cannabaceae	*Celtis philippensis* Blanco	4.21	1.40	4.21
Fabaceae	*Intsia palembanica* Miq.	3.69	1.23	4.64
Dilleniaceae	*Dillenia papuana* Martelli	3.61	1.20	7.56
Unidentified	Unidentified	3.57	1.19	1.82
Salicaceae	*Homalium foetidum* Benth.	3.02	1.01	0.80
Myristicaceae	*Myristica inutilis* var *papuana* (Markgr.)W.J.de Wilde	2.89	0.96	2.69
Fabaceae	*Inocarpus fagifer* (Parkinson)Fosberg	2.82	0.94	2.45
Ebenaceae	*Diospyros pilosanthera* Blanco	2.77	0.92	2.14
Elaeocarpaceae	*Elaeocarpus* cf *multiflorus* (Turcz.)Fern.-Vill.	2.72	0.91	0.78
Myristicaceae	*Virola venosa* (Benth.)Warb.	2.61	0.87	1.59
Lythraceae	*Duabanga moluccana* Blume	2.59	0.86	1.48
Cassuarinaceae	*Gymnostoma rumphianum* (Miq.)L.A.S.Johnson	2.54	0.85	1.85
Ebenaceae	*Diospyros* sp.	2.52	0.84	2.12
Ixonanthaceae	*Ixonanthes reticulata* Jack	2.52	0.84	1.78
Myrtaceae	*Syzygium* sp.2	2.49	0.83	3.60
Elaeocarpaceae	*Sloanea* sp. (cf *pullei*)	2.46	0.82	2.98
Myrtaceae	*Kjellbergiodendron celebicum* (Koord.)Merr	2.43	0.81	3.45
Fabaceae	*Serianthes grandiflora* Benth.	2.42	0.81	9.15
Myrtaceae	*Syzygium decipiens* (Koord.&Valeton)Merr.&L.M.Perry	2.42	0.81	1.73
Oleaceae	*Chionanthus* cf *brassii* (Kobuski)Kiew	2.41	0.80	2.67
Myrtaceae	*Syzygium rosaceum* Diels	2.38	0.79	2.97
Anacardiaceae	*Campnosperma brevipetiolatum* Volkens	2.30	0.77	1.95
Sapotaceae	*Palaquium lobbianum* Burck.	2.26	0.75	1.73
Fabaceae	*Intsia bijuga* (Colebr.)Kuntze	2.23	0.74	1.70

Chrysobalanaceae	*Parastemon versteeghii* Merr. & L.M.Perry	2.18	0.73	3.24
Unidentified	indet_WR2A04	2.16	0.72	1.93
Fabaceae	*Maniltoa plurijuga* Merr. & L.M.Perry	2.13	0.71	2.93
Dipterocarpaceae	*Anisoptera thurifera* (Blanco)Blume	2.11	0.70	1.09
Calophyllaceae	*Calophyllum celebicum* P.F.Stevens	2.10	0.70	1.39
Moraceae	*Artocarpus altilis* (Parkinson ex F.A.Zorn)Fosberg	2.07	0.69	2.05
Calophyllaceae	*Calophyllum persimile* P.F.Stevens	2.05	0.68	0.92
Clusiaceae	*Garcinia latissima* Miq.	2.03	0.68	1.55
Calophyllaceae	*Calophyllum parvifolium* Choisy	1.95	0.65	2.24
Burseraceae	*Canarium acutifolium* (DC.)Merr.	1.91	0.92	2.10
Family	*Species*	IV	IV %	BA (m2)
Apocynaceae	*Alstonia beatricis* Sidiyasa	1.80	0.60	2.17
Myrtaceae	*Rhodamnia cinerea* Jack	1.69	0.56	2.34
Fabaceae	*Maniltoa schefferi* K.Schum.&Hollrung	1.65	0.55	1.33
Anacardiaceae	*Mangifera inocarpoides* Merr. & L.M.Perry	1.50	0.50	2.11
Myrtaceae	*Syzygium synaptoneurum* (K.Schum.&Lauterb.) Merr. & L.M.Perry	1.47	0.49	1.97
Unidentified	indet_WR4A29	1.42	0.47	1.80
Burseraceae	*Canarium maluense* Lauterb.	1.40	0.47	1.80
Sapindaceae	*Harpullia cupanioides* Roxb.	1.39	0.46	2.48
Fabaceae	*Cynometra* sp.	1.37	0.46	2.89
Myrtaceae	*Decaspermum bracteatum* (Roxb.)A.J.Scott.	1.37	0.46	1.15
Unidentified	indet_WA1A08	1.36	0.45	2.29
Fabaceae	*Serianthes minahassae* (Koord.) Merr. & L.M.Perry	1.29	0.43	1.75
Cannabaceae	*Gironniera subaequalis* Planch.	1.27	0.42	1.53
Burseraceae	*Haplolobus floribundus* (K.Schum.)H.J.Lam	1.24	0.41	1.46
Unidentified	indet_SA2B5	1.24	0.41	1.20
Sapotaceae	*Palaquium supfianum* Schltr.	1.23	0.41	1.41
Celastraceae	*Lopopetalum* cf *javanum* Turcz.	1.22	0.41	1.91
Sapotaceae	*Madhuca* cf *magnifolia* (King ex S.Moore)S.Moore	1.21	0.40	1.26
Lamiaceae	*Teijsmanniodendron hollrungii* (Warb.)Kosterm.	1.21	0.40	1.25
Elaeocarpaceae	*Elaeocarpus* cf *chartaceous*	1.14	0.38	3.11
Unidentified	indet_WR4A03	1.11	0.37	0.79
Anacardiaceae	*Buchanania* sp.	1.11	0.37	0.75
Burseraceae	*Canarium oleosum* (Lam.)Engl.	1.11	0.37	0.75
Burseraceae	*Canarium sylvestre* Gaertn.	1.10	0.37	0.73
Moraceae	*Ficus subulata* Blume	1.10	0.37	0.73
Meliaceae	*Chisocheton ceramicus* Miq.	1.08	0.36	0.66
Unidentified	indet_WR2C05	1.08	0.36	0.68
Rutaceae	*Melicope exuta* T.G.Hartley	1.07	0.36	0.61
Putranjivaceae	*Drypetes* cf *neglecta* (Koord.)Pax&K.Hoffm.	1.05	0.35	0.55
Unidentified	indet_WR3A11	1.05	0.35	0.52
Unidentified	indet_WR2A20	1.04	0.35	0.48
Elaeocarpaceae	*Elaeocarpus nouhuysii* Koord.	1.04	0.35	0.48
Anacardiaceae	*Campnosperma* sp.	1.04	0.35	0.48
Melastomataceae	*Pternandra* sp.	1.03	0.34	0.48

Family	*Species*	IV	IV %	BA (m2)
Fabaceae	*Sympetalandra* sp.	1.03	0.34	0.47
Unidentified	indet_WR1A18	1.03	0.34	0.46
Unidentified	indet_WR1A5	1.03	0.34	0.45
Anacardiaceae	*Koordersiodendron pinnatum* Merr.	1.03	0.34	0.44
Unidentified	indet_WR4A06	1.02	0.34	0.43
Fagaceae	*Lithocarpus* cf *megacarpus* Soepadmo	1.02	0.34	0.43
Lauraceae	*Alseodaphne archboldiana* (C.K.Allen)Ksterm.	1.02	0.34	0.42
Anacardiaceae	*Mangifera mucronulata* Blume	1.02	0.34	0.42
Burseraceae	*Canarium asperum* Benth. ssp. *papuanum* (H.J.Lam)Leenh.	1.01	0.34	0.40
Simaroubaceae	*Ailanthus integrifolia* Lam.	1.01	0.34	0.38
Calophyllaceae	*Calophyllum* cf *tacamahaca* Willd.	1.01	0.34	0.38
Unidentified	indet_WR2A10	1.00	0.33	0.35
Gnetaceae	*Gnetum gnemon* L.	0.99	0.33	0.33
Araliaceae	*Schefflera* sp.	0.98	0.33	0.32
Rubiaceae	*Psychotria leptothyrsa* Miq.	0.98	0.33	0.31
Rubiaceae	*Mastixiodendron pachyclados* (K.Schum.)Melch.	0.98	0.33	0.31
Lauraceae	*Cryptocarya infectoria* (Blume)Miq.	0.98	0.33	0.30
Araucariaceae	*Agathis labillardierei* Warb.	0.95	0.32	1.49
Burseraceae	*Dacryodes* sp	0.95	0.32	1.49
Lamiaceae	*Teijsmanniodendron* sp	0.93	0.31	1.22
Anacardiaceae	*Buchanania nitida* Engl.	0.93	0.31	1.13
Elaeocarpaceae	*Elaeocarpus*sp.	0.92	0.31	1.05
Lauraceae	*Endiandra* sp.	0.91	0.30	0.89
Burseraceae	*Canarium hirsutum* Willd.	0.91	0.30	0.85
Euphorbiaceae	*Ptychopyxis chrysantha* (K.Schum.)Airy Shaw	0.90	0.30	0.76
Malvaceae	*Pterygota horsfieldii* (R.Br.)Kosterm.	0.87	0.29	0.52
Anacardiaceae	Indet. (Anacardiaceae)	0.87	0.29	0.47
Combretaceae	*Terminalia copelandi* Elmer	0.87	0.29	0.45
Malvaceae	*Microcos* sp.	0.86	0.29	1.74
Burseraceae	*Canarium* sp.	0.86	0.29	1.49
Sapindaceae	*Nephelium* sp.	0.84	0.28	1.17
Lauraceae	*Litsea guppyi* (F.Muell.)F.Muell. ex Forman	0.84	0.28	0.93
Elaeocarpaceae	*Aceratium* sp.	0.83	0.28	0.82
Phyllanthaceae	*Cleistanthus papuanus* (Lauterb.)Jabl.	0.83	0.28	0.82
Sapindaceae	*Pometia* cf *coreacea*	0.82	0.27	0.61
Unidentified	indet_WR3C05	0.82	0.27	0.57
Clusiaceae	*Garcinia celebica* L.	0.81	0.27	0.43
Cardiopteridaceae	*Gonocaryum litorale* (Blume)Sleumer	0.80	0.27	0.33
Myristicaceae	*Myristica* cf *subalulata* Miq.	0.80	0.27	0.31
Annonaceae	*Polyalthia rumphii* (Blume Ex. Hensch.)Merr.	0.80	0.27	0.25

Mycoheterotrophic Plants of Gunung Halimun-Salak National Park, West Java, Indonesia

Muhamad Muhaimin[1], Destario Metusala[2], Wendy Achmmad Mustaqim[3]

1. Cibodas Botanic Garden, Indonesian Institute of Sciences (LIPI), Cianjur, Indonesia. Telp. (0263) 520448, (0263) 511385. e-mail correspondence: mh.muhaimin91@gmail.com
2. Purwodadi Botanic Garden, Indonesian Institute of Sciences (LIPI), Pasuruan, Indonesia. Telp. (0343) 615033. e-mail: metusala.destario@gmail.com
3. Plant Biology Program, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Dramaga, Bogor, West Java. e-mail: wendyachmmadm@gmail.com

ABSTRACT

Gunung Halimun Salak National Park (GHSNP) is the largest protected tropical forest in West Java and has high plant diversity, including high diversity of mycoheterotrophic plants. Mycoheterotrophic plants (MHPs) are a unique group of the plants that have no chlorophyll and their sources of carbon are obtained through a symbiotic relationship with fungi. However, the information on the diversity of MHPs species in the GHSNP region is limited. In this paper, the description, distribution, and ecological preferences of MHPs species of GHSNP are presented. There are 33 MHPs species recorded in GHSNP region so far, consisting of 14 species of ferns and lycophytes, 16 species of monocotyledons, and 3 species of dicotyledons. From all of these species, one species (*Gastrodia crispa* J.J.Sm.) is endemic to Java. Therefore, the forest of GHSNP is important for the conservation of many MHPs species.

Keywords: Gunung Halimun Salak National Park, Mycoheterotrophic, Species Diversity

INTRODUCTION

Mycoheterotrophic plants (MHPs) are a unique group of plants. Unlike the majority of plants which perform photosynthesis to obtain their source of carbon (C), MHPs get a carbon source from a symbiotic relationship with a fungus (Leake 1994, Cameron & Leake 2007, Merckx 2013). Similar to parasitic plants, MHPs also lack chlorophyll or have very low level of chlorophylls, extremely reduced leaves, and the production of many seeds (Cameron & Leake 2007). In the past, MHPs were commonly referred to as 'saprophytes', but this term is inappropriate because there is no evidence that the plants get a direct carbon source from organic material in the soil. In addition, the definition of 'saprophyte' for plants is often inconsistently applied (Leake 2005, Merckx 2013). In some recent literature, MHPs can be also called holomycotrophic plants (ex. Campbell 2014).

Currently MHPs occur in 17 families, 101 genera and 880 species (Merckx *et al*. 2013a). In particular, MHPs species are monocotyledons especially orchids (Leake 1994, Merckx *et al*. 2013a). The majority of MHPs species live in forest habitat that is shaded and damp. Therefore, MHPs species are very abundant in the tropics (Leake 1994).

Java is a tropical area in the Sundaland region rich in MHPs species, especially *Didymoplexis* and *Gastrodia* species (Merckx *et al* 2013b). Some of these taxa are endemic to Java. In addition, new MHPs species have recently been found in the region (Tsukaya & Hidayat 2016, Metusala & Supriatna 2017). Nevertheless, MHPs species in Java are poorly known and little studied. On the other hand, the existence of MHPs species has become more threatened due to habitat destruction as the extent of Javan rainforest decreases. Therefore, an inventory, as well as a list of species are needed to know the latest condition of the Javan MHPs species.

One of the areas that are assumed as an important location for the MHPs species in Java is Gunung Halimun Salak National Park (GHSNP). GHSNP region which has an area of 113.357 ha is an important protected area for the remaining tropical rainforest in West Java. This National Park occurs at elevation of 500–2211 meter above sea level and very high rainfall ranging between 4000–6000 mm/year (Mirmanto *et al*. 2008). GHSNP also has considerable habitat preferred by tropical MHPs species, such as wet primary forests, from submontane to upper montane zones and bamboo forest. Until now, information on MHPs species in the region of GHSNP are scattered throughout the literature

and no one has completely documented these taxa. In this paper, for the first time, we provide information on all MHPs species recorded in GHSNP.

MATERIAL AND METHODS

This study is based on an extensive survey of the literature, examination of herbarium specimen held at BO, and a few recent field explorations. The field explorations were carried out from 2010 until 2017 in Cikaniki-Citalahab trail and Cikudapaeh, especially in locations suspected to represent the habitat for tropical MHPs species, such as bamboo forest or dense forest with thick accumulated of litter (Leake 1994, Merckx *et al*. 2013b). The literature used to determine the species recorded in this paper was based from Jonker (1948), Backer & Bakh.f. (1963, 1965, 1968), Comber (1990), Uji (2002), Hidayat & Wiriadinata (2003), Mahyar & Sadili (2003), Mirmanto *et al*. (2008), and Suetsugu *et al*. (2018).

The species included in this paper that are categorized as fully mycoheterotrophic. That is, only those species that depend solely on the carbon source from the fungus, in all phases of its life. These species can be characterized by the absence or lack of chlorophyll or by the absence of green leaves. Species of lycophytes and ferns that have mycoheterotrophic gametophyte are also included in the list, given the body of the gametophyte has a mode of life similar to full mycoheterotrophy species in general.That is, the source of carbon is dependent on the fungus (Leake 1994, Merckx *et al*. 2013a). Meanwhile, partial mycoheterotrophy species (living as autotrophy and mycoheterotrophy) and initial mycoheterotrophy species (mycoheterotrophy only at the stage of germination like most orchids) (Merckx 2013, Merckx *et al*. 2013a) are not included in the list.

List of MHPs species presented in this paper consists of scientific name, synonyms, species description, distribution, ecological information, and additional notes of related species. The species descriptions are adopted from various litterature, such as Alderwerelt (1915), Backer & Bakh.f. (1965), Backer & Posthumus (1939), Boonkerd *et al*. (2005), Comber (1990), Jonker (1948), Lindsay & Middleton (2012), Verdcourt (2005), Zhang & Iwatsuki (2013), and Zhang *et al*. (2013). The classification for the group of lycophytes and ferns is based on PPG I (2016), whereas flowering plants are based on APG IV (2016). Classification of taxa in flowering plants is divided into monocotyledons and dicotyledons. Monocotyledons are the group that the seeds have one embryonic leaf or cotyledon, whereas dicotyledons have two cotyledons. In APG IV (2016), dicotyledons include basal angiosperms and eudicots group. For information on the family and genus included in the category of full mycoheterotrophy, referred to Leake (1994) and Merckx *et al*. (2013a).

RESULT AND DISCUSSIONS

A total of 33 MHPs species belonging to 22 genera in 7 families has been recorded in GHSNP. Monocotyledons are a group of flowering plants that have the highest number of species in GHSNP (16 species). This result agrees with previous research, that the majority of MHPs species belong to monocotyledons, which is about 88% (Imhof 2010). Ferns and lycophytes consist of 14 species, with remainder of species belonging to dicotyledons (3 species). Considering the plant families, Orchidaceae has the highest number of species (13 species), followed by Lycopodiaceae (10 species). Seven out of 33 species were relocated during field exploration in 2010 to 2017. One species still needs to be further identified, that is *Didymoplexis* sp. (Orchidaceae) which was found on Cikaniki Trail (Fig. 1C.).

From the 33 species of MHPs in GHSNP, 13 species have a limited distribution in the western part of Java (see Appendix). From these species, *Didymoplexis striata* and *Gastrodia crispa* were regarded as endemic to Java (Comber 1990), but now only *G. crispa* is still considered endemic because *D. striata* was eventually also found in Borneo and Solomon Islands (Hsu *et al*. 2016). One species, *Gastrodia spathulata*, was recently discovered in GHSNP based on the *Sadili & Komara 623* specimen that previously identified as *G. javanica*. *Gastrodia spathulata* occurs in Borneo (Mt. Kinabalu, Sabah) and Java (Cepak Datar-Leuwi Jamang, Res. Cigudeg, GHSNP) (Suetsugu *et al*. 2018).

[LYCOPHYTES AND FERNS]

Lycopodiaceae

1. ***Diphasiastrum complanatum*** (L.) Holub, *Preslia* 47 (1975) 108
   Lycopodium complanatum L., *Sp. Pl.* 2 (1753) 1104; Backer & Posthumus, *Varenfl. voor Jav*. (1939) 274; Zhang & Iwatsuki, *Fl. China* vol. 2–3 (2013) 30–31
   Main stems (stolon) completely or mostly subterranean; leaves on stolon spatulate or ovate; aerial shoots creeping, up to 100 cm. Lateral branches suberect, up to 15 cm tall, dichotomously branched with well-differentiated lateral branchlets much branched, branchlets obviously flattened; sterile branchlets light green or grayish green abaxially. Leaves arranged in 4 rows, dense, triangular, 1–2 × c. 1 mm, herbaceous, midrib indistinct, base appressed on branches, sessile, margin slightly involute, entire, apex acute. Strobili (1–)3–5(or 6), 1.5–3 cm long, terminal on peduncles (10–20 cm long), pale yellow, terete; sporophylls broadly ovate, imbricate, c. 2.5 × 1.5 mm, with irregular teeth, apex acute, caudate.
   Distribution: Cosmopolitan in northern temperate regions and alpine regions in the tropics.
   Ecology: Terrestrial on mountain slope, dry ground to marshy places, usually in open areas above 2000 m elevation.
   Specimen examined: BO186075 (*De Voogd s.n.*); BO186076 (*De Voogd s.n.*).

2. ***Huperzia serrata*** (Thunb. ex Murray) Trevis., *Atti Soc. Ital. Sci. Nat.* 17 (1875) 248; Zhang & Iwatsuki, *Fl. China* vol. 2–3 (2013) 18
   Lycopodium serratum Thunb. ex Murray, *Syst. Veg.* ed. 14 (1784) 944; Thunb, *Fl. Jap*. (1784) 341, t. 38; Backer & Posthumus, *Varenfl. voor Jav*. (1939) 276–277
   Stems ascending with several erect branches, 8–20 cm tall, slender, 1–3 times dichotomously forked. Leaves elliptic to oblanceolate, 1.5–2.5 × 0.3–0.5 cm, patent, thin but firm in texture, deep green, margin irregularly serrate, midrib normally distinct. Strobilus indistinct. Sporophylls lanceolate, usually below apex of vegetative branches. Sporangia reniform, in the axils of sporophylls.
   Distribution: Widely distributed in South to East Asia, Malesia except New Guinea, Australia (NE Queensland), Pacific Islands including Hawaii, Mexico and Carribean Islands (Cuba and Haiti).
   Ecology: Terrestrial on humus-rich ground in evergreen forest above 1000 m elevation.
   Specimen examined: BO1456166 (*W.S. Hoover* 30546, *A. Sadali, & J. Hunter*); BO1522659 (*Afriastini* 23); BO1533660 (*MMJ van Balgooy* 2863 *& H. Wiriadinata*); BO187091 (*van Steenis* 132); BO187118 (*Raciborski s.n.*); BO187138 (*Backer* 9233); BO187139 (*Backer* 10741); BO1831613 (*Arief Hidayat* 508).
   Notes: Hidayat & Wiriadinata (2003) record this species as occurring in GHSNP.

3. ***Lycopodium clavatum*** L., Sp. Pl. 2 (1753) 1101; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 275; Zhang & Iwatsuki, *Fl. China* vol. 2–3 (2013) 30 – 31
   Stem subterranean, slender and creeping, 1 or 2 times forked, green, with sparse entire leaves; lateral branches erect, 20–25 cm tall, 3–5 times forked, sparse, flattened (young branches terete). Leaves spirally arranged, dense, angled upward, lanceolate, 4–6 × c. 1 mm long, leathery, with transparent hairs, midrib visible on both surfaces, base cuneate, decurrent, sessile, margin entire, apex acuminate. Strobili 2 or 3 together on a peduncle, peduncle up to 12 cm long, bracts of peduncle spirally arranged, sparse, herbaceous, narrowly lanceolate; strobili erect, terete, 3.5–4.5 × c. 0.4 cm, subsessile or with short, equally long pedicels; sporophyll broadly ovate, c. 1.5 × 1.3 mm, papery, margin membranous, erose, apex acute, with short acute tip.
   Distribution: Cosmopolitan.
   Ecology: Terresrial on wet ground on hill slopes, grasslands or roadsides, in fully exposed areas, above 1200 m elevation.
   Specimen examined: BO185868 (*JJ Smith* 386); BO1523996 (*Backer s.n.*); BO1524015 (*De Voogd s.n.*).

4. ***Phlegmariurus carinatus*** (Poir.) Ching, *Acta Bot. Yunnan.* 4 (1982) 120; Zhang & Iwatsuki, *Fl. China* vol. 2-3 (2013) 28
   Huperzia carinata (Desv. ex Poir.) Trevis., *Atti Soc. Ital. Sci. Nat.* 17 (1875) 247

Lycopodium carinatum Desv. ex Poir., *Encycl. suppl.* 3 (1813 [1814]) 555; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 278

Stems at least 50 cm long, slender, 1–3 times dichotomously forked. Leaves narrowly lanceolate, apex subulate, base attenuate, sessile, about 1.25 × 0.13 cm, chartaceous in texture, margin entire, appressed and revolute, midrib indistinct. Strobilus indistinct. Sporophylls about the same size as microphyll, oblong subdeltoid, to 5 mm long, 1.5 mm wide.

Distribution: South China, Ryukyu Isl., Taiwan, Indochina, Malesia and Polynesia.

Ecology: Epiphyte on mossy-tree trunk in shady places and humid areas.

Specimen examined: BO185203, BO185204 (*Backer* 1230).

5. ***Phlegmariurus phlegmaria*** (L.) Holub, *Preslia* 36(1) (1964) 21; Zhang & Iwatsuki, *Fl. China* vol. 2-3 (2013) 22

Huperzia phlegmaria (L.) Rothm., *Fedde, Repert*. 54 (1944) 62

Lycopodium phlegmaria L., *Sp. Pl.* 2 (1753) 1101; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 273 – 274

Stems at least 80 cm long, 1–3 times dichotomously forked. Leaves narrowly lanceolate to oblong-lanceolate, apex acuminate, base rounded to truncate, patent, about 0.5–1.6 cm × 0.15–0.6 cm, texture coriaceous, light green to yellowish green glossy, margin entire, midrib disticnt on both surface. Fertile branches repeated 2–4 times dichotomously forked, bearing 4-8 strobili at apex of a sterile branch. Strobilus distinct at apex of vegetative branches, 5.5–12.5 cm long.

Distribution: Widely distributed in the Old World tropics.

Ecology: Epiphyte on mossy-tree trunk in shady places and humid areas at medium and high altitudes.

Specimen examined: BO1515203 (*Dransfield* 4211); BO1517967 (*Dransfield* 4257); BO1528946 (*S. Yoshida* 1533); BO-186747 (*Raciborski s.n.*); BO1831616 (*Arief Hidayat* 522); BO186721 (*Backer* 1336); BO186724 (*Backer* 9365); BO186688 (*Backer* 10674); BO1515170 (*M.A. Donk s.n.*); BO1527685 (*M.A. Donk s.n.*); BO186637 (*Backer* 25866); BO1517692 (*Raciborski s.n.*); BO1456218 (*W.S. Hoover* 32803, *M.Hendra, & J. Hunter*); BO145622 (*W.S. Hoover* 32694, *M.Hendra, & J. Hunter*); BO31493 (*H.Wiriadinata* 31493, *W.S. Hoover, & J. Hunter*); BO1528955 (*Bakhuizen van den Brink* 3215); BO1528945 (*Afriastini* 19); BO186649 (*Backer* 23256); BO1515162 (*Schiffner s.n.*); BO186488 (*leg.ign s.n.*); BO1508739, BO1508738 (*MMJ van Balgooy* 2932 *& H. Wiriadinata*).

Notes: Uji (2002) and Hidayat & Wiriadinata (2003) found this species at GHSNP. In addition, we found this species at Cikaniki trail, GHSNP, in 2014 & 2017.

6. ***Phlegmariurus pinifolius*** (Trevis) Kiew, *Phytokeys* 96 (2018) 108

Huperzia pinifolia (Bl.) Trev., *Atti Soc. Ital. Sci. Nat.* 17 (1875) 247

Lycopodium pinifolium Bl., *Enum. Pl. Jav.* 2 (1824) 264

Stems at least 15–35 cm long, pendulous, 1–3 times dichotomously forked. Leaves narrowly ovate, apex acute, narrowing towards sessile base, patent, about 0.6–1.5 × 0.4–0.6 cm, chartaceous in texture, margin entire, midrib more or less distinct on lower surface. Strobilus indistinct to slightly distinct. Sporophylls usually patent, the same shape as microphylls, but much reduced in size.

Distribution: Sri Lanka, Thailand, Peninsular Malaysia, Borneo, Philippines, Sulawesi, Java, Moluccas (Seram), Sumatra, Solomon Isl., Santa Cruz Isl. (Vanikoro), and Vanuatu.

Ecology: Epiphyte on mossy-tree trunk in evergreen forest.

Specimen examined: BO1456221 (*W.S. Hoover* 32694, *M.Hendra, J. Hunter*); BO186859 (*Backer* 9288); BO186904 (*Bakhuizen van den Brink* 3649).

Notes: Uji (2002) recorded this species from GHSNP. This species was recently transferred to *Phlegmariurus* from *Huperzia* (Kiew & Kamin 2018).

7. ***Phlegmariurus proliferus*** (Bl.) A.R.Field & Bostock, *Phytokeys* 20 (2013) 46

Huperzia prolifera (Bl.) Trev., *Atti Soc. Ital. Sci. Nat.* 17 (1875) 248

Lycopodium proliferum Bl., *Enum. Pl. Jav.* 2 (1828) 265; Alderw., *Malayan Fern Allies* (1915) 36 – 37

Stems pendulous, once or twice dichotomously branched, 15–45 cm long. Leaves ascending, crowded, linear-lanceolate, 10–15 mm long, acute, entire, subrevolute at the edge, the costa

obscurely prominent above or beneath, grooved on the other side, the base decurrent, the higher leaves growing gradually shorter. Sporophyll about ½ as long as the microphylls, similar shape with microphylls or ovate-lanceolate.
Distribution: Peninsular Malaysia (Perak, Pahang), Java, Borneo (Sabah), Sulawesi, Lesser Sunda Islands (Lombok), Moluccas (Seram) and Solomon Isl.
Ecology: Epiphyte on mossy-tree trunk in primary or secondary forest.
Specimen examined: BO186883 (*Raciborski s.n.*); BO1831637 (*Arief Hidayat* 547); BO1522646 (*van Steenis* 12454); BO186837 (*Bakhuizen van den Brink* 3214); BO186886 (*Raciborski s.n.*).
Notes: This species recorded from GHSNP by Hidayat & Wiriadinata (2003).

8. ***Phlegmariurus squarrosus*** (G. Forst.) Löve & Löve, *Taxon* 26 (1977) 324; Zhang & Iwatsuki, *Fl. China* vol. 2–3 (2013) 26
   Huperzia squarrosa (G. Forst.) Trev., *Atti Soc. Ital. Sci. Nat*. 17 (1875) 247
   Lycopodium squarrosum G. Forst., *Fl. ins. austr. Prodr*. (1786) 479; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 278
   Stems at least 15–60 cm long, 1–3 times dichotomously forked. Leaves ± linear, apex acuminate, patent, 1.0–1.5 × 0.1–0.2 cm, coriaceous texture, margin entire, midrib more or less distinct on both surfaces. Strobilus indistinct. Sporophylls usually smaller than the microphylls, at the apex of vegetative branches, narrowly oblong to linear; sporangia reniform.
   Distribution: Madagascar and Indian Ocean Islands, South to East Asia, throughout Malesia, Australia (NE Queensland) and Pacific Islands.
   Ecology: Epiphyte on mossy-tree trunk or in rock crevices in shady or slightly exposed places above elevation of 1200 m
   Specimen examined: BO187227 (*Raciborski s.n.*); BO187334, BO187335, BO1518334 (*Backer* 25885); BO1522428, BO187223, BO187224 (*Backer* 9176); BO1522430, BO187225 (*Backer* 10879); BO187247 (*leg.ign. s.n.*); BO1831639 (*Arief Hidayat* 515).
   Notes: This species cited in GHSNP by Hidayat & Wiriadinata (2003). In addition, we found this species at Cikaniki trail, GHSNP, in 2017.

9. ***Phlegmariurus verticillatus*** (L.f.) A.R.Field & Testo, *Molec. Phylogen. Evol.* 94(B) (2015) 645
   Huperzia verticillata (L.f.) Trev., *Atti Soc. Critt. Ital.* 17 (1875) 248; Verdcourt, *Fl. of Tropical East Africa: Lycopodiaceae* (2005)
   Lycopodium verticillatum L.f., *Suppl. Pl.* (1782) 448; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 278
   Stems pendulous or occasionally on rocks; rooting stem 4–6 cm long, branching dichotomously several times, first branching at c. 4–8 cm from root; total length 25–50 cm long, 3–6(–10) mm wide near base, 2–3 mm wide at apex. Leaves ± adpressed to spreading, linear, 5–6(–10) mm long, 0.2–0.5 mm wide, with distinct midrib. Sporophylls similar with microphylls. Sporangia can occur all along the stems but mainly in the upper parts, not hidden, 2–2.5 mm wide.
   Distribution: Throughout the tropical regions of the world. In Malesia, it has been recorded from Peninsular Malaysia (Pahang and Selangor), Java and Borneo (Sabah and Sarawak).
   Ecology: Around 950–2100 m elevation.
   Specimen examined: BO1529047 (*M.A. Donk s.n.*); BO1831636 (*Arief Hidayat* 548); BO187463 (*Raciborski s.n.*); BO187464 (*Bakhuizen van den Brink* 6646); BO187462, BO187462 (*Bakhuizen van den Brink* 4008); BO187458, BO187459, BO187460 (*Backer* 10727).
   Notes: Hidayat & Wiriadinata (2003) found this species at GHSNP.

10. ***Pseudodiphasium volubile*** (G.Forst.) Holub, *Folia Geobot. Phytotax.* 18 (1983) 442
    Lycopodium volubile G.Forst., *Prodr.* (1786); Backer & Posthumus, *Varenfl. voor Jav.* (1939) 274
    Main stems scandent, branched, up to 5 m long, wiry, flexuose, with scattered minute appressed lvs. Leaves dimorphic. Lateral leaves flattened, subdistichous, spreading, 3–5 mm. long, lanceolate with broad decurrent base, ascending, falcate, acuminate, midrib evident; other leaves minute, linear, appressed. Strobili 2–10 cm long, terminal on paniculate branchlets. Sporophylls small, broad-ovate, abruptly narrowed to subulate tip.

Distribution: Peninsular Malaysia (Pahang), Borneo, Philippines, Java, Sulawesi, Lesser Sunda Isl. (Lombok), Moluccas (Seram), New Guinea, Solomon Isl., Vanuatu, Australia (NE-Queensland), New Caledonia, Society Isl. (Tahiti) and New Zealand (including the Kermadec and Chatham Isl.).
Ecology: Climbing on other plants, in light shade, at above 1500 m elevation.
Specimen examined: BO1540889 (*De Voogd s.n.*); BO1462041 (*Hovenkamp* 552); BO1538948 (*van Steenis* 8261); BO1534877, BO187546 (*Raciborski s.n.*); BO187517, BO187518 (*Bakhuizen van den Brink* 4157).

Ophioglossaceae

11. ***Ophioderma pendula*** (L.) Presl, *Suppl.* 56 (1845)
Ophioglossum pendulum L., *Sp. ed. II*, 2 (1763) 1518; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 269; Zhang *et al*., *Fl. China* vol. 2–3 (2013) 78
Leaves 1–3 or more. Sterile lamina sessile, pendulous and ribbonlike, usually lanceolate, sometimes dichotomously divided, up to 30–50(–100) × 1–3 cm; veins reticulate, without distinct midrib, forming diagonal and hexagonal areoles; veinlets ± visible. Sporophore arising from near base or middle part of sterile lamina and never longer than sterile lamina, pendent; stalk to 7 cm, spike 5–30 cm; sporangia 40–200 on each side.
Distribution: W. Africa, Sri Lanka, India, China, Taiwan, Korea, Japan, Malaysia, Philippines, Indonesia, Australia, Hawaii Isl.
Ecology: Epiphyte on tree trunks in tropical rain forests.
Specimen examined: BO1516153, BO1519771 (*Backer* 10633); BO1516108 (*MMJ van Balgooy* 2941 *& H. Wiriadinata*); BO1830141 (*Arief Hidayat* 542); BO1519797 (*van Steenis* 160); BO1519809 (*Backer* 25887).
Notes: Hidayat & Wiriadinata (2003) found this species at GHSNP.

12. ***Ophioglossum reticulatum*** L., *Sp. Pl. 2* (1753) 1063; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 268 – 269; Zhang *et al*., *Fl. China* vol. 2-3 (2013) 79
Plants 10–30 cm tall. Rhizomes erect, slender, bearing a few thick fleshy roots. Common stalk 4–8 cm, light green, gradually pale toward base. Sterile lamina with a short stalk or almost sessile, ovate or orbicular-ovate, 3–4 × 2.6–3.5 cm, herbaceous, base deeply cordate, margin ± wavy, apex rounded or subobtuse; veins distinct, reticulate. Sporophore arising from base of sterile lamina, slender, 10–15 cm long; spike 3–3.5 cm long, slender.
Distribution: Widely distributed in the tropics and subtropics regions of the world.
Ecology: Terrestrial in shaded forest or open places.
Specimen examined: BO1522234 (*Bakhuizen van den Brink* 2833); BO1522245 (*Bakhuizen van den Brink* 6306); BO1522162 (*Bakhuizen van den Brink* 5306); BO1462034 (*Hovenkamp* 562).

13. ***Sceptridium daucifolium*** (Wall. ex Hook. & Greville) Lyon, *Bot. Gaz.* 40
Botrychium daucifolium (Wall.) Hook. & Grev., *Ic. Fil.* 2(9) (1830) t. 161; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 271; Zhang *et al*., *Fl. China* vol. 2-3 (2013) 76
Stems or rhizomes erect, bearing many fleshy roots. Leaves 30–40 cm tall with stalk 10–12 cm. Lamina bipinnate to tripinnatifid, subpentagonal, 15–20 × 16–24 cm, herbaceous; pinnae 6 or 7 pairs, alternate; basal pinnae largest, triangular, 12–14 × 6–10 cm, bipinnatifid; pinnules 4 or 5 pairs, narrowly ovate to broadly lanceolate, lower basal pinnule largest, up to 8 × 3 cm, pinnatifid, ultimate segments sharply serrate, apex acute or acuminate; veins obvious, rachis and costae with sparse, white, long hairs. Sporophore arising from above middle of common stipe, 10–12 cm long, more or less as long as sterile lamina, with stalk 14–16 cm long, bi-tripinnate.
Distribution: India (NE and S), Sri Lanka, Himalayan region (Bhutan and Nepal), Myanmar, Vietnam, Sumatra, Peninsular Malaysia, Java, Borneo, Philippines, Sulawesi, Lesser Sunda Isl. (Bali, Flores), Moluccas (Seram).
Ecology: Terrestrial in shaded wet places, forest.
Specimen examined: BO-1409558 (*Raciborski s.n.*); BO-1830216 (*Arief Hidayat* 577).
Notes: Hidayat & Wiriadinata (2003) found this species at GHSNP.

Psilotaceae

14. ***Psilotum complanatum*** Sw., *Schrad., J. Bot.* 1800(2) (1801) 110; Backer & Posthumus, *Varenfl. voor Jav.* (1939) 286; Lindsay & Middleton, *Ferns of Thailand, Laos and Cambodia* (2012)

Rhizomes creeping. Aerial stems and branches flattened with a distinct midrib, usually 20–100 cm long, exceptionally to 2 m long, lower unbranched part 9–36 cm long and 2–4 mm wide, upper branches (13–)35(60) cm long, 2–4 cm wide, synangia inset laterally giving the stem a jagged appearance, appendages lateral and alternate, 1–2 mm long. Synangia 1(–3) mm diameter, yellowish green becoming yellow when mature.

Distribution: Widely distributed throughout the tropical regions of the world.

Ecology: Epiphyte on tree trunks in light shade.

Specimen examined: BO1876922 (*M.A. Donk* P321); BO1876923 (*Bakhuizen van den Brink* 405); BO1876935 (*J.G.B. Beumee* 53a); BO1548775 (*W.C. van Hennig s.n.*); BO1542774 (*Bakhuizen van den Brink* 6648); BO1548819 (*Bakhuizen van den Brink s.n.*); BO1540168 (*leg.ign.* s.n); BO1540167 (*van Slooten* 696).

Figure 1. Some Mycoheterotrophic plants in Gunung Halimun Salak National Park. A. *Gymnosiphon aphyllus*. B. *Cyrtosia javanica* (photo from Central Java specimen). C. *Didymoplexis* sp. D. & E. *Epipogium roseum*. F. *Gastrodia crispa*. Photo by: W.A. Mustaqim.

[ANGIOSPERMS – MONOCOTYLEDONS]

Burmanniaceae

15. ***Burmannia lutescens*** Becc., *Malesia I* (1877) 246; Jonker, *Fl. Malesiana* 4, 1 (1948) 19
Gonianthes candida Bl., *Cat. Gew. Buitenzorg* (1823) 20
Gonyanthes candida Bl., *Flora* 8 (1825) 123.
Achlorophyllous herbs, up to 23 cm. Stem thickly filiform to robust, simple or branched, usually bearing 1 flower or forked into inflorescences. Leaves reduced into small scale, 1–3.5 mm long. Flowers 3-winged, white, limb sometimes yellow or bluish, perianth tube trigonous, 2.5–5 mm long, inner perianth present although sometimes very small, perianth persistent in fruit. Stamens 3, connective without basal spur. Ovary 3-celled. Style thick, bearing 3-subsessile, funnel-shaped to bowl-shaped stigmas.
Distribution: Sumatra, Peninsular Malaysia, Java, Borneo and New Guinea (including New Ireland).
Ecology: Usually in the humus of shady moist forest, up to 1500 m.
Specimen examined: 400.15, BO1846407 (*K.B. Boedijn s.n.*); 400.19, BO1568339 (*Leeuwen-Reijnvaan* 8278); BO1568347 (*Bakhuizen van den Brink* 697); BO1845598 (*Raciborski s.n.*); BO1845599 (*R.H.C.C Schffer s.n.*).
Notes: This species known previously in GHSNP by Jonker (1948) and Mirmanto *et al*. (2008).

16. ***Gymnosiphon aphyllus*** Bl., *Enum. Pl. Jav.* 1 (1827) 29; Jonker, *Fl. Malesiana* 4, 1 (1948) 20.
Gymnosiphon borneense Becc., *Malesia I* (1877) 241
Gymnosiphon pedicellatum Schltr., *Bot. Jahrb.* 49 (1913) 105.
Achlorophyllous herbs, with stem to 17 cm, forked into a bifid cincinnus or bearing a simple cincinnus. Leaves reduced to scales, appressed, 1–1.5 mm long, acute. Inflorescences a loose, many flowered cincinni or bifid cincinni, many flowered. Flowers pedicellate, 1–5 mm long. Perianth white or lilac, tube cylindrical or trigonous, 4 mm long, limb c. 2.5 mm long, outer ones ovate, inner ones linear-lanceolate, minute, perianth deciduous in fruit. Stamens 3, anthers inserted above the middle of the perianth, sessile. Ovary 1-celled. Capsule about 3 mm long. **– Fig. 1A.**
Distribution: Southern Thailand and throughout Malesia.
Ecology: On humus, decaying wood or leaves in the shade moist forests, below 1500 m
Specimen examined: 401.24 (*Bakhuizen van den Brink* 4240).
Notes: This species still found in GHSNP at 2017.

17. ***Thismia javanica*** J.J.Sm., *Ann. Jar. Bot. Btzg.* 23 (1910) 32; Jonker, *Fl. Malesiana* 4, 1 (1948) 23.
Achlorophyllous herbs, stem simple or branched, up to 12 cm, 1–5-flowered. Leaves reduced to scales, 3 mm long, acute. Flower subtended by 3 involucral bracts. Flowers with perianth tube urceolate, longitudinal stripes 12, orange, inside with longitudinal bars connected by transverse bars, outer perianth lobes obtuse, ovate, inner perianth lobes free, triangular, terminated in up to 3 cm long, filiform tentacles. Stamens 6, anthers 3-toothed at the free apical margin, each terminating in a hair. On the outher side of the anther, inserted in the middle, a quadrangular appendages present, wider than stamen. Style orange, stigma sessile. Ovary obovoid. Capsule orange, c. 6 mm long. Seeds ellipsoid.
Distribution: Sumatra and Java (West).
Ecology: Humus, shaded forest, below 1000 m
Vernacular name: Angkrek rambut (Java).
Notes: This species still occurs on the Tanjakan Cikudapaeh trail, at 2017.

Orchidaceae

18. ***Aphyllorchis pallida*** Blume, *Tab. Pl. Jav. Orchid.* (1825) Set. 77.
All part glabrous, about 30 cm tall. Inflorescence erect, with 7–10 flowers. Flowers c. 4 mm across, not opening widely; dorsal sepal concave, oblong, c. 4.7 × 1.0 mm, acute; lateral sepals oblong ovate c. 4 × 1.3 mm, acute; petals elliptic oblong, slightly oblique, c. 4.5 × 2 mm, obtuse; lip c. 4 × 2 mm;

hypochile concave, 1.3 mm long; epichile 3-lobed, 2.6 mm long; column obovoid-cylindrical, slightly arcuate.
Distribution: Thailand, Vietnam and Malesia. In Malesia, it was recorded from Sumatra, Peninsular Malaysia, Java, Borneo and the Philippines.
Ecology: Humid, shaded habitat.
Specimen examined: BO0045487 (*Bakhuizen van den Brink* 5970).

19. ***Cyrtosia javanica*** Blume, *Bijdr. Fl. Ned. Ind.* (1825) 396, pl. 6
Plant small, c. 10 cm tall. Stem 1 or 2 arising from a rhizome, erect, 6–8 cm tall, with implicate scales at nodes. Inflorescence racemose, 5–7 flowers. Flowers not fully opening, yellowish brown, fleshy; dorsal sepal oblong c. 16 × 8 mm, slightly concave; lateral sepals broadly ovate c. 16 × 9 mm; petals oblong, c. 15 × 7 mm; lip adnate to base of column, suborbicular with thickened disk; column clavate c. 8 mm, winged at apex. **– Fig. 1B.**
Distribution: Widely distributed in Southeast Asia (including Taiwan) and throughout the regions of Malesia.
Ecology: Humid, shaded habitat.
Specimen examined: 716.1 (*A.J.P. Oort s.n.*); 716.2 (*van Leeuwen* 8270); BO0068841 (*Nangnong s.n.*).
Notes: Mahyar & Sadili (2003) recorded also this species in GHSNP area. We also found this species at Cikaniki area in 2012.

20. ***Cystorchis aphylla*** Ridl., *J. Linn. Soc. Bot.* 32 (1896) 400.
Plant erect, c. 15 cm tall. Inflorescence 2–17-flowered. Flowers brownish; sepals 6 mm long, lateral with swollen bases enclosing the spur of the lip forming a short 2 mm long mentum; lip as long as sepals, narrow, basal part concave, middle part narrower and densely papillose, tip small obtuse separated by a small constriction; column short.
Distribution: Thailand and Malesia as far as Moluccas.
Ecology: Humid, shaded habitat.
Specimen examined: 581.4, BO0112042 (*Bakhuizen van den Brink* 5982).
Notes: Mahyar & Sadili (2003) recorded also this species in GHSNP area.

21. ***Didymoplexis striata*** J.J.Sm., *Icon. Bogor.* (1903) t. 104 B
Plant small, erect. Inflorescence with 2–3 flowered. Flower 6–7 mm wide, brownish; lateral sepals triangular, falcate, concave, joined together at basal half, obtuse; petals and dorsal sepal joined together for most of its length, free parts triangular, obtuse; lip ovate to elliptic, rounded to obtuse; column straight.
Distribution: Thailand, Java, Borneo and Solomon Isl.
Ecology: Humid, shaded habitat.
Notes: This species metioned in GHSNP area by Backer & Bakh.f. (1968) and Comber (1990). In Java, only found in Mt. Salak without elevation record.

22. ***Eulophia zollingeri*** (Rchb.f.) J.J.Sm., *Orch. Java* (1905) 228
Plant 15–80 cm tall, tuberous. Inflorescence brownish red, erect, racemose, with several clasping sheaths, c. 8–20 flowered. Flowers dull purple-red to brownish yellow, 2.5–3 cm in diam; dorsal sepal elliptic oblong, 15–23 × 4–7 mm, acuminate; lateral sepals suboblong, slightly oblique, 16–25 × 6–9 mm, acuminate; petals oblanceolate, 11–18 × 5–7 mm, mucronate; lip adnate to column foot, subobovate to oblong-obovate, 14–15 × 15–18 mm, trilobed, very shortly saccate-spurred at base; column c. 5 mm.
Distribution: Distributed widely from tropical to subtropical Asia and also in Australia.
Ecology: Humid, shaded habitat.
Specimen examined: BO0066303 (*Backer* 22169).

23. ***Epipogium roseum*** (D.Don) Lindl., *J. Proc. Linn. Soc., Bot.* 1 (1857) 177
Plant 10–45 cm tall, tuberous. Inflorescence 2-16 flowered, floral bract ovate-lanceolate. Flowers resupinate, pendulous, opening widely or not, white with faint purple spots on lip; sepals linear-

lanceolate 8–11 × 2–3 mm, subacute; petals similar to sepals, slightly oblique, 7–10 × 2–3 mm, acute to acuminate; lip elliptic-ovate, concave, 8–12 × 6–7 mm, spurred at base; column 2.5–4.5 mm long. **– Fig. 1D–E.**
Distribution: Widespread from Tropical Africa to Tropical Asia as far as New Guinea, also in Australia and Pacific Islands (Vanuatu).
Ecology: Open or shaded habitat.
Specimen examined: BO0064586 (*A. Rant s.n.*); BO0064578 (*Middeloer s.n.*); BO0064584 (*Backer* 10793).
Notes: We also found this species two times, in 2014 and 2017.

24. ***Gastrodia crispa*** J.J.Sm., *Bull. Jard. Bot. Buitenzorg* III, 3 (1921) 248
Plant erect, c. to 30 cm tall, 2–6 flowered. Flower 1.75 cm long, pale greenish-brown, with some warty outside; sepals and petals united into an oblong campanulate tube; sepals petals all joined together with free part of sepals oblong obtuse and petals ovate obtuse; lip c. 7–8 mm long with two callus; column 6–7 mm long, truncate. **– Fig. 1F.**
Distribution: Endemic to West Java.
Ecology: Humid, shaded habitat.
Specimen examined: BO1371668 (*Asep Sadili* 239 *& Nurdin*); BO1371669 (*Asep Sadili* 270 *& Nurdin*); BO1416068 (*Asep Sadili* 242 *& Nurdin*).
Notes: This species found in GHSNP area by Comber (1990), Mahyar & Sadili (2003), and our personal observation (2014, 2017).

25. ***Gastrodia javanica*** (Blume) Lindl., *Gen. Sp. Orchid. Pl.* (1840) 384
Plant erect, c. to 70 cm tall, 10–12 flowered. Flower 1.5 cm long, pale dull purplish brown-inside with yellow lines on the lateral sepals; sepals petals all joined together except for free tips about 3 mm long, the lateral sepals separated at base; lip narrowly diamond-shaped, the sides raised; column straight, as long as the lip.
Distribution: Thailand, Sumatra, Peninsular Malaysia, Java, Borneo, Philippines and Lesser Sunda Islands.
Ecology: Humid, shaded habitat.
Specimen examined: BO0066494 (*Bakhuizen van den Brink* 5272).

26. ***Gastrodia spathulata*** (Carr) J.J.Wood, *Orchids Mount Kinabalu* 2 (2011) 355
Neoclemensia spathulata Carr, *Gardens' Bull.* 8 (1935) 180, fig. 45; Wood *et al., The plants of Mount Kinabalu: 2. Orchids* (1993) 265
Plant erect, tuberous. Rhizome cylindrical, villose. Inflorescence erect, terminal, laxly 2–3 flowered; floral bracts appressed to pedicel. Flower medium-sized, white with orange petals and greenish-brown lip; sepals adnate, forming a tube, recurved and papillose at apex; petals adnate at base to lateral sepals, much shorter than sepals, linear-spathulate, fimbriate; lip adnate to base of column, entire, clawed, claw oblong or subquadrate and bearing 2 subglobose; column stout, with acute stelidia (description based Wood *et al*. 1993).
Distribution: Java (West, only from Leuwi Jamang, GHSNP) and Borneo (Sabah, only from Mount Kinabalu).
Ecology: Humid, hill forest, elevation 1100 m
Specimen examined: BO1405196 (*Asep Sadili* 623 *& Dian Komara*).
Notes: This species has been reported recently in Java (Suetsugu *et al*. 2018).

27. ***Gastrodia verrucosa*** Blume., *Mus. Bot.* 2 (1856) 175
Plant erect, tuberous, c. 10 cm long. Inflorescence 2–4 flowered, fleshy, with 2–3 tubular sheaths. Flower pale pinkish-brown, warty outside; sepals and petals united into a short campanulate tube; sepals with the free parts orbicular-ovate; petals orbicular ovate, lip attached below sinus between lateral sepals, ovate-lanceolate, 6–7 × 4–5 mm, obtuse to acute, margin irregular dentate; column 7–8 mm long with triangular-acute wing on each side.
Distribution: Thailand, Sumatra, Peninsular Malaysia and Java.
Ecology: Humid, shaded habitat.

Specimen examined: BO0066385 (*leg.ign. s.n.*).

28. ***Lecanorchis javanica*** Blume, *Mus. Bot.* 2 (1856) 188
Plant erect, thin, angular, 8.5–40 cm long. Flowers opening widely, 1.6 cm wide; dorsal sepal reflexed, lanceolate, concave, 8 × 2 mm, obtuse; lateral sepals obliquely lanceolate, concave, 8–9 × 2–3 mm, obtuse; petals recurved, lanceolate to spathulate, concave, 8 × 2–3 mm, obtuse; lip 9 mm long, midlobe obovate-triangular, crenulate-finely erose; column slender, clavate, 5–6 mm long.
Distribution: Thailand, Vietnam, Peninsular Malaysia and Java (West).
Ecology: Humid, shaded habitat.
Specimen examined: BO1416072 (*Uway W. Mahyar & A. Sadili* 277); BO0069689 (*Dransfield* 4239).
Notes: This species mentioned found in GHSNP area by Mahyar & Sadili (2003) and Mirmanto *et al*. (2008).

29. ***Lecanorchis multiflora*** J.J.Sm., *Bull. Jard. Bot. Buitenzorg* II, 26 (1918) 8
Plant c. 60 cm long, sometimes branched. Inflorescence many flowered. Sepals and petals opened widely, pale green or white, 9–10 × 3 mm. Lip trilobed, 8–10 mm long, sidelobes rounded, midlobe rounded covered with white hairs and two low keels, column 6 mm long.
Distribution: China, Thailand, Sumatra, Peninsular Malaysia, Java and Borneo.
Ecology: Humid, shaded habitat.
Specimen examined: BO1407626 (*A. Sadili* 407 *& Nurdin*); BO1416073 (*Uway W. Mahyar & A. Sadili* 368); BO1407890 (*A. Sadili* 461 *& Dian Komara*); BO1407353 (*A. Sadili* 510 *& Dian Komara*).
Notes: This species mentioned found in GHSNP area by Mahyar & Sadili (2003) and Mirmanto *et al*. (2008).

30. ***Stereosandra javanica*** Blume, *Mus. Bot.* 2 (1856) 176
Plants c. 30 cm tall. Rhizome 1.5–4 × 1–2.5 cm. Inflorescence 5- to 10-flowered, floral bracts erect, 6–7 mm. Flowers pendulous; sepals and petals similar, lanceolate, c. 7–9 × 2.5 mm, acute; lip ovate-lanceolate, c. 8 × 3.5 mm, concave, containing 2 low, wartlike calli at base. Column short, c. 2 mm excluding anther.
Distribution: Southeast Asia, throughout Malesia and also in the Solomon Islands.
Ecology: Humid, shaded habitat.
Specimen examined: BO1407870 (*A. Sadili* 599 *& Dian Komara*).
Notes: This species mentioned found in GHSNP area by Mahyar & Sadili (2003).

[ANGIOSPERMS – DICOTYLEDONS]
Gentianaceae
31. ***Exacum tenue*** (Bl.) Klack., *Bot. Jahrb. Syst.* 126 (2006) 478
Cotylanthera tenuis Bl., *Bijdr. Fl. Ned. Ind.* 13 (1826) 708; Backer & Bakh.f. *Fl. Java* 2 (1965) 438
Erect, small achlorophyllous herbs, branched or simple. Leaves reduced into opposite scales, appressed, ovate, 1–5 mm long. Flowers borne solitary at the apex of stem, perianth 4-merous. Calyx fleshy, white or yellowish white, divided halfway down or slightly deeper, lobes erecto-patent, triangular. Corolla contorted in bud, deeply divided, contorted at bud, tube 1–3 mm long, lobes finally widely patent, oblong or subobovate, white or pale lilac, 4–9 × 1.25–3.5 mm. Stamens with filaments shorter than corolla lobes, anthers erect, exceeding the filament, bright yellow, 2-celled, style filiform, stigma capitate.
Distribution: East India (Odisha), Sumatra, Java, Borneo, Sulawesi and New Guinea (Averyanov *et al*. 2014).
Ecology: Lowland closed rain forest, among leaf litter.
Specimen examined: BO1355566 (*Th Valeton s.n.*).

Polygalaceae

32. ***Epirixanthes cylindrica*** Bl., *Cat. Gew. Buitenzorg (Blume)* 82 (1823); Backer & Bakh.f. *Fl. Java* 1 (1963) 200
Erect, delicate mycoheterotrophic herbs, stem yellowish white or violet, mostly with rigid, fastigiate branchlets, 7–25 cm high. Leaves simple, spirally arranged, minute, scale like, ovate or ovate-oblong, appressed. Inflorescence a very dense spike, with a rounded top, fertile portion 8–20 mm long, 4–6 mm thick, bracts obovate or subspathulate, c. 2 mm long. Flowers with sepals free, 1.5–1.75 mm long. Petals 3, yellowish white. Anthers 5. Style shorter than 1/3 mm. Fruit somewhat exceeding the calyx, obovoid-urceolate, 1.25–1.75 mm long, contracted at the top.
Distribution: Sumatra, Java (West), Borneo and New Guinea.
Ecology: Humus soil, in shaded rain forest, among leaf litter.
Specimen examined: BO1791075 (*Schiffner* 3123); BO1791076 (*van Steenis* 139); BO1791095 (*van Steenis* 12429); BO1478684 (*Bakhuizen van den Brink s.n.*); BO1478685 (*Bakhuizen van den Brink s.n.*).

33. ***Epirixanthes elongata*** Bl., *Cat. Gew. Buitenzorg (Blume)* 82 (1823); Backer & Bakh.f. *Fl. Java* 1 (1963) 200
Erect, delicate mycoheterotrophic herb, stem violet or yellow brown, mostly with rigidly erect branches, 6–25 cm high. Leaves simple, spirally arranged, minute, scale like, ovate or ovate-oblong, appressed. Inflorescence a dense spike, bracts ovate-lanceolate, very acute, 1.25–1.75 mm long, violet or yellow, spikes with fertile portion 1–3.5 cm with fruiting part 1.5–3 mm thick. Flowers with sepals free, acute, yellowish white, whether or not with a violet median streak or blotch. Petals 3, 1.5–2 mm long, yellowish white. Stamens 5, with tube adnate to corolla-tube. Ovary 2-celled, style 0.5–1.25 mm long, equaling ovary or somewhat longer. Fruit indehiscent, enclosed by the calyx, transversely oval, 1–1.25 mm wide.
Distribution: East India, southern Myanmar, southern China, northern Vietnam, Sumatra, Peninsular Malaysia, Java (West), Borneo and Moluccas (Ambon and Seram).
Ecology: Humus soil, in shaded rain forest, among leaf litter.
Specimen examined: BO1791065 (*Schiffner* 2124); BO1791082 (*van Steenis* 3000); BO1478696 (*Backer* 10849); BO1478692 (*Bakhuizen van den Brink* 4132); BO1478694 (*Bakhuizen van den Brink* 4142); BO1794912 (*Bakhuizen van den Brink s.n.*).

CONCLUSION

Based on this study, there are 33 species (7 families, 22 genera) of mycoheterotrophic plants in the Gunung Halimun Salak National Park. This represents about half of the total species in Java, with 33 out of 67. *Gastrodia crispa*, a species endemic to Java, can be found in this area. We concluded that this National Park provides important habitats for mycoheterotrophic plants species on Java. Continuous protection of the Gunung Halimun Salak National Park is essential to ensure the future existence of mycoheterotrophic plants.

ACKNOWLEDGEMENT

The authors thank Deedee Alfarishy and Reza Saputra for assistance during the field studies and to Wita Wardani for assisting us to obtain permission to study specimens held at Herbarium Bogoriense (BO).

REFERENCES

Alderwerelt, C.R.W.K. 1915. *Malayan fern allies. Handbook to the determination of the fern allies of the malayan islands (incl. those of the Malay Peninsula, the Philippines, and New Guinea).* The Department of Agriculture, Industry and Commerce Netherlands India, Batavia: 288 pp.

Angiosperm Phylogeny Group (APG). 2016. An update of the Angisoperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181(1): 1-20.

Averyanov, L.V., K.S. Nguyen, & H.T. Nguyen. 2014. Checklist of mycoheterotrophic species of the genus *Exacum* (Gentianaceae) and new species, *Exacum zigomorpha*, from northern Vietnam. *Phytotaxa* 183(2): 108-113.

Backer, C.A. & O. Posthumus. 1939. *Varenflora voor Java. Overzicht der op Java voorkomende varens en varenachtigen, hare verspreiding, oekologie en toepassingen*. Uitgave van's lands plantentuin, Buitenzorg: XLVII + 370 pp.

Backer, C.A. & R.C. Bakhuizen v.d. Brink Jr. 1963. *Flora of Java (Spermatophytes only) vol. I*. Wolters-Noordhoff N.V., Groningen: viii + 761 pp.

Backer, C.A. & R.C. Bakhuizen v.d. Brink Jr. 1965. *Flora of Java (Spermatophytes only) vol. II*. Wolters-Noordhoff N.V., Groningen: viii + 761 pp.

Backer, C.A. & R.C. Bakhuizen v.d. Brink Jr. 1968. *Flora of Java (Spermatophytes only) vol. III*. Wolters-Noordhoff N.V., Groningen: viii + 761 pp.

Boonkerd, T., R. Pollawatn, & P. Darumas. (2005). Taxonomic revision of the Lycopodiaceae in Southeast Asia: final report. Chulalongkorn University: viii + 75 pp.

Cameron, D.D. & J.R. Leake. 2007. A different kind of parasitic plant: A brief history of myco-heterotrophy and epiparasitism. *Haustoria* 51: 4-5.

Campbell, F. 2014. A summary of holomycotrophic orchids. *The MIOS Journal* 15(4): 6-17

Comber, J.B. 1990. *Orchids of Java*. The Bentham-Moxon Trust, Bangkok: 407 pp.

Hidayat, A. & H. Wiriadinata. 2003. Kekayaan tumbuhan paku di kawasan Cikaniki, tanjakan Kudapaeh, Cikeris, Gunung Botol, dan Gunung Bentanggading, Taman Nasional Gunung Halimun, Jawa Barat. Laporan teknik LIPI tahun 2003. Pusat Penelitian Biologi – LIPI, Bogor: 1-7 pp. (MS)

Hsu, T.-C., T.-Y.A. Yang, F. Pitisopa, M. Fanerii, & C.-W. Li. 2016. New records and name changes for the orchids in the Solomon Islands. *Taiwania* 61(1): 21-26.

Imhof, S. 2010. Are monocots particularly suited to develop mycoheterotrophy? *In:* Seberg, O., G. Petersen, A. Barfod, J.I. Davis. Eds. *Diversity, phylogeny, and evolution in the monocotyledons*. Aarhus University Press, Kopenhagen: 11-23.

Jonker, F.P. 1948. Burmanniaceae. *In:* C.G.G.J. van Steenis. Ed. *Flora Malesiana Ser. I vol. IV.* Nordhoff-Kolff N.V., Jakarta: 13-26.

Kiew, R. & I. Kamin. 2018. Two new *Phlegmariurus* species (Lycopodiaceae) and one new combination from Peninsular Malaysia. *Phytokeys* 96: 99-110.

Leake, J.R. 1994. The biology of myco-heterotrophic ('saprophytic') plants. *New Phytol.* 127: 171-216.

Leake, J.R. 2005. Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the 'saprophytic' plant myth. *Mycologist* 19(3): 113-122.

Lindsay, S. & D.J. Middleton. (2012 onwards). Ferns of Thailand, Laos and Cambodia. Retrieved June 3, 2017, from http://rbg-web2.rbge.org.uk/thaiferns/

Mahyar, U.W. & A. Sadili. 2003. *Jenis-jenis anggrek Taman Nasional Gunung Halimun*. Biodiversity Conservation Project LIPI-JICA-PHKA, Bogor: ii + 208 pp.

Merckx, V.S.F.T. 2013. Mycoheterotrophy: An introduction. *In:* Merckx, V.S.F.T. ed. *Mycoheterotrophy: The biology of plants living on fungi*. Springer Science+Business Media, New York: 1-17 pp.

Merckx, V.S.F.T., J.V. Freudenstein, J. Kissling, M.J.M. Christenhusz, R.E. Stotler, B.C. Stotler, N. Wickett, P.J. Rudall, H.M. v.d. Kamer, & P.J.M. Maas. 2013a. Taxonomy and classification. *In:* Merckx, V.S.F.T. ed. *Mycoheterotrophy: The biology of plants living on fungi*. Springer Science+Business Media, New York: 19-101 pp.

Merckx, V.S.F.T., E.F.Smets, & C.D. Specht. 2013b. Biogeography and conservation. *In:* Merckx, V.S.F.T. ed. *Mycoheterotrophy: The biology of plants living on fungi*. Springer Science+Business Media, New York: 103-156 pp.

Metusala, D. & J. Supriatna. 2017. *Gastrodia bambu* (Orchidaceae: Epidendroideae), A new species from Java, Indonesia. *Phytokeys* 317(3): 211-218.

Mirmanto, E., H. Wiriadinata, M.F. Royyani, S. Ichikawa, & Ismirza. 2008. *Merajut pesona flora hutan pegunungan tropis di Gunung Salak*. Puslit Biologi-TNGHS-GHSNPMP, Bogor: 82 pp.

Pteridophyte Phylogeny Group (PPG). 2016. A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54(6): 563-603.

Suetsugu, K., A. Hidayat, & H. Tsukaya. 2018. First record of the Mycoheterotrophic plant *Gastrodia spathulata* (Orchidaceae) from West Java, Indonesia. *Acta Phytotax. Geobot.* 69(2): 135-137.

Tsukaya, H. & A. Hidayat. 2016. A new species of *Gastrodia* (Orchidaceae: Gastrodieae, Epidendroideae) from Java. *Phytokeys* 273(1): 77-80.

Uji, T. 2002. Keanekaragaman dan potensi flora di Gunung Halimun dan sekitarnya di Taman Nasional Gunung Halimun. *Berita Biologi* 6(1): 1-12.

Verdcourt, B. 2005. *Flora of Tropical East Africa: Lycopodiaceae*. Kew Publishing, Kew: 17 pp.

Wood, J.J., R.S. Beaman & J.H. Beaman. 1993. *The plants of Mount Kinabalu: 2. Orchids*. Royal Botanic Gardens, Kew: xi + 411 pp.

Zhang, L.B. & K. Iwatsuki. 2013. Lycopodiaceae. *In* Z.Y. Wu, P. H. Raven & D. Y.Hong. Eds. *Flora of China, Vol. 2–3 (Pteridophytes)*. Science Press, Beijing; Missouri Botanical Garden Press, St. Louis: 13-24.

Zhang, X.C., Q.R. Liu & N. Sahashi. 2013. Ophioglossaceae. *In* Z.Y. Wu, P. H. Raven & D. Y.Hong. Eds. *Flora of China, Vol. 2–3 (Pteridophytes)*. Science Press, Beijing; Missouri Botanical Garden Press, St. Louis: 73-80.

Appendix. List of MHPs species from Java

This list collected from various sources, such as Jonker (1948), Backer & Bakh.f. (1963, 1965, 1968), Comber (1990), Uji (2002), Hidayat & Wiriadinata (2003), Mahyar & Sadili (2003), Mirmanto *et al.* (2008), Tsukaya & Hidayat (2016), Metusala & Supriatna (2018), Suetsugu *et al.* (2018). Notes: W = Western Java (Banten, West Java), C = Central Java (Jogjakarta, Central Java), E = Eastern Java (East Java).

[FERNS & LYCOPHYTES]

Lycopodiaceae: *Diphasiastrum complanatum* (W,C), *Diphasiastrum wightianum* (W,C,E), *Huperzia miniata* (W,C), *Huperzia serrata* (W,C,E), *Huperzia zollingeri* (W,C), *Lycopodium clavatum* (W,C,E), *Phlegmariurus carinatus* (W,C,E), *Phlegmariurus nummulariifolius* (W,C,E), *Phlegmariurus phlegmaria* (W,C,E), *Phlegmariurus pinifolius* (W,C,E), *Phlegmariurus proliferus* (W), *Phlegmariurus squarrosus* (W,C,E), *Phlegmariurus tetrastichus* (E), *Phlegmariurus verticillatus* (W), *Pseudodiphasium volubile* (W,C,E). **Psilotaceae:** *Psilotum complanatum* (W,C), *Psilotum nudum* (W,C,E). **Ophiglossaceae:** *Helminthostachys zeylanica* (W,C,E), *Japanobotrychium arisanense* (W,C), *Ophioderma pendulum* (W,C,E), *Ophioderma intermedium* (W), *Ophioglossum reticulatum* (W,C,E), *Ophioglossum parvifolium* (W,C,E), *Sceptridium daucifolium* (W,C), *Sceptridium ternatum* (W,C,E).

[ANGIOSPERMS – MONOCOTYLEDONS]

Burmanniaceae: *Burmannia bifaria* (W), *Burmannia championii* (W), *Burmannia coelestis* (W,E), *Burmannia lutescens* (W,C), *Burmannia steenisii* (E), *Gymnosiphon aphyllus* (W), *Gymnosiphon neglectus* (W,C), *Thismia clandestina* (W), *Thismia javanica* (W). **Orchidaceae:** *Aphyllorchis pallida* (W), *Cyrtosia javanica* (W,C,E), *Cyrtorchis aphylla* (W,C,E), *Didymoplexis cornuta* (W), *Didymoplexis flexipes* (W), *Didymoplexis micradenia* (W), *Didymoplexis obreniformis* (W), *Didymoplexis pallens* (W,C,E), *Didymoplexis striata* (W), *Epipogium roseum* (W,C,E), *Erythrorchis altissima* (W), *Eulophia zollingeri* (W,C,E), *Galeola nudiflora* (W,C,E), *Gastrodia abscondita* (W), *Gastrodia bambu* (W,C), *Gastrodia callosa* (W), *Gastrodia crispa* (W), *Gastrodia javanica* (W), *Gastrodia selabintanensis* (W), *Gastrodia spathulata* (W), *Gastrodia verrucosa* (W), *Lecanorchis javanica* (W), *Lecanorchis multiflora* (W,C,E), *Silvorchis colorata* (W), *Stereosandra javanica* (W,C,E).

[ANGIOSPERMS – DICOTYLEDONS]

Triuridaceae: *Sciaphila arfakiana* (W), *Sciaphila maculata* (W), *Sciaphila nana* (W), *Sciaphila tenella* (W). **Gentianaceae:** *Exacum tenue* (W). **Polygalaceae:** *Epirixanthes cylindrica* (W), *Epirixanthes elongata* (W), *Epirixanthes papuana* (W).

Pollen Viability of 19 Indonesian Bananas (*Musa* L.) Collection of Purwodadi Botanic Gardens: Premilinary Study For Breeding

[1]Janis Damaiyani and Lia Hapsari
Purwodadi Botanic Garden, Indonesian Institute of Sciences
Jl. Surabaya – Malang Km 65, Purwodadi, Pasuruan, East Java, 67613
*Corresponding author email: j.damaiyani@gmail.com

ABSTRACT

Pollen viability on bananas (*Musa* L.) both wild species and cultivated varieties/cultivars is a basic information for further banana breeding program. The objective of this study was to evaluate the viability of pollen grains of 2 wild species (*M. acuminata* ssp. *rutilifes* and *M. balbisiana*) and 17 banana cultivars collection of Purwodadi Botanic Garden– Indonesian Institute of Sciences. Pollen viability analysis was conducted by staining test using 1% solution of 2,3,5-*Triphenyltetrazolium chloride* (TTC), then observed with light microscope Olympus CX31 at 40 magnification. Red-stained pollen grains were considered viable/fertile. Percentage of pollen stainability was calculated to determine the level of pollen viability of each banana sample. Clustering analysis was also conducted using PAST software to determine the pollen viability pattern among bananas. Results showed that pollen viability of bananas were varies, differed between genomic groups but clustered within genomic groups, so that pollen viability may utilised in the banana taxonomy. Among wild species, *M. acuminata* ssp. *rutilifes* (AAw) has higher pollen viability than *M. balbisiana* (BBw) *i.e.* 99.75% and 46.36% respectively. Banana cultivars with genomic group AA, AAA, and AAB has pollen viability of more than 60%, *i.e.* Mas, Raja Molo, Ambon Hong, Seribu, Nangka, Morosebo, Raja Kenanga and Cebol. Pisang Mas (AA) and Raja Molo (AAA) have high pollen viability *i.e.* 94.83% and 94.33%, respectively. Pisang Jambe (AA) was an outlier with low pollen viability (19.51%), and need further observation. Whilst, banana cultivars with genomic group ABB has pollen viability less than 30%; with only 2 banana cultivars *i.e.* Kepok Putih and Sri that has viable pollen (>30%). This preliminary result suggests that in addition to wild banana species, banana cultivars with two or more A genomes also might be useful as male parents with high viability of pollen grains.

Key words: banana, *Musa*, pollen viability, Purwodadi Botanic Garden

INTRODUCTION

Edible bananas (*Mus*a spp.) are important food crops worldwide (Ortiz *et al*., 1998). It plays a considerable socio-economic role in many countries, in view of the importance as foodstuff and source of foreign currency on the local and international market (Soares *et al*., 2008). It is also valuable in cultural life, especially in the rural communities, as one of essential component for offerings and decorations in the ritual ceremonial activities, such as thanksgiving of pregnancy, birth, circumcision, marriage, house warming, until the funeral (Hapsari *et al*., 2017). Nowadays, global banana farmings are facing problems due to biotic and abiotic stresses such as pests and diseases outbreaks *viz.* nematodes, wilts (*Fusarium*, blood disease, moko, *Xanthomonas*), viruses (bunchy top, streak virus), sigatoka, etc. (Molina *et al*., 2007; Hapsari & Masrum, 2012) and changing environmental and climatic conditions *e.g*. drought, salinity, extreme temperature, strong winds, etc. (Ravi & Vaganan, 2016). Therefore, mitigation through breeding programs are urgently to be conducted in order to improve resistancies of existing banana varieties.

The generally parthenocarpic and sterility characteristic of most cultivated banana varieties make banana breeding difficult and expensive (Mukasa & Rubaihayo, 1993; Molina *et al*., 2005; Turyagyenda, 2006). Banana breeding programs spend considerable time in either developing or identifying suitable male parents (due to low male fertility) with characteristics of interest for breeding. Knowledge on pollen viability is one of essential factor in the selection of male parents for breeding program (Sathiamoorthy & Rao, 1987; Soares *et al*., 2008; Mukasa and Rubaihayo, 1993; Ssebuliba *et al*., 2008).

Being part of the primary center of origin and diversity of bananas, Indonesia has a large number diversity of bananas both wild seeded species and edible seedless cultivars. There are about 12 wild

banana species (genus *Musa*) and not less than 200 edible cultivars available in Indonesia (Nasution, 1991; Nasution & Yamada, 2001; Hapsari, 2014), of which about 90 edible cultivars reported in East Java (Hapsari *et al*., 2015a; Hapsari *et al*., 2015b, Hapsari *et al*., 2017; Hapsari *et al*., 2018). All those banana germplasm materials are valuable sources for further banana improvements so that needs to be collected, characterized and evaluated. In banana breeding, wild diploid clones have largely been used as male parents. In recent years several cultivated varieties diploids and triploids have also been utilised such as Dwarf Cavendish (AAA), Gros Michel (AAA), and Rasthali (AAB) (Sathiamoorthy & Rao, 1987).

Studies on pollen of Indonesian bananas are still very limited. Damayanti (2002) reported the pollen viability and germination on 4 cultivars (Mas, Raja Uli, Kepok) and one wild *Musa ornata*. Result showed that the wild *M. ornata* had the highest pollen viability followed by cultivars Uli, Mas Kepok and Raja. Furthermore, diploid pollen were more viable than triploid. In addition, Rachman *et al*. (2012) reported the pollen storage and germination on 5 wild *M. acuminata* sub-species (*breviformis*, *zebrina*, *malaccensis*, *bantamensis* and *banksii*). Results showed that *M. acuminata* ssp. *banksii* had highest pollen germination (21.78%) and storage was declined the pollen germination significantly to all sub-species. However, silica gel was recommended to be used until 5 weeks of storage and temperature of -5ºC and 5ºC was recommended for pollen storage.

Purwodadi Botanic Garden– Indonesian Institute of Sciences is an ex-situ conservation institution of dry lowland plants, including bananas (Musaceae). The first banana collection was recorded in 1976. Last inspection on 2015, the total collection was recorded 134 numbers, comprised 6 wild species and 128 cultivars. The banana collections were obtained from explorations to several areas in Indonesia, especially in eastern Indonesia (Hapsari, 2011; Hapsari *et al*., 2015a). Those banana collections are valuable genetic resource as a material for future breeding program. Hence, the objective of this study was to evaluate the viability of pollen grains of 19 Indonesian bananas. It comprised of two wild banana species, which represents the ancestral genomes (AAw and BBw). Seventeen banana cultivars were selected based on the male flower availability during the observation period, which represents three different genomic groups i.e. AAA, AAB and ABB. The result of this study may become valuable basis information for further banana breeding program.

MATERIAL AND METHODS

Study area

Purwodadi Botanic Garden– Indonesian Institute of Sciences is located in Purwodadi, Pasuruan, East Java. Further, the banana field collection is located at Region II, Environment VI, Vak XXIV A-B-D-E (Figure 1).

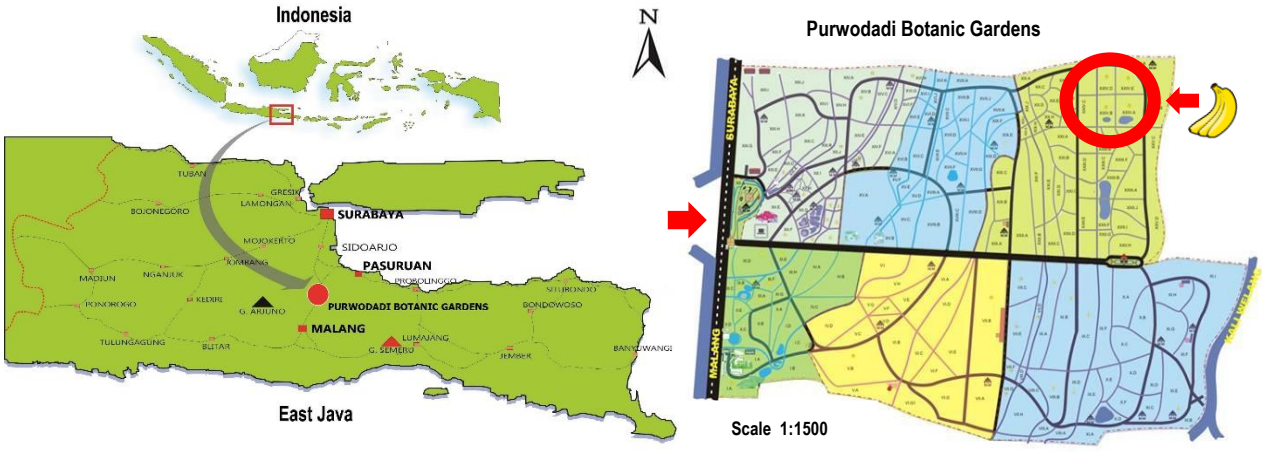


Figure 1. Location map of Purwodadi Botanic Garden and the banana field collection (Source: Documentation and Information Unit, and Registration Unit of Purwodadi Botanic Garden)

Plant materials

Nineteen banana accessions from the field collection of Purwodadi Botanic Garden– Indonesian Institute of Sciences collected from East Java, Central Java, Yogyakarta and West Java were examined in this study. The selection of banana accessions were based on the fresh male flower availability at field collection during the observation periode on January to February 2017. It comprised two wild banana species which represents the ancestral genomes (AAw and BBw), whilst seventeen banana cultivars represents three different genomic groups i.e. AAA, AAB and ABB (Table 1).

Table 1. Identity data of Indonesian banana accessions examined in this study

Species Name/cultivars	Genomic Group	Location and Collection Number	Registration Number	Locality
M. acuminata ssp. *rutilifes*/ Cici	AAw	XXIV.D. 12-a	P19810983	Tuban, East Java
M. balbisiana/ Klutuk Hijau	BBw	XXIV.D. 1-abc	P1980041	Purwosari, Pasuruan, East Java
Mas	AA	XXIV.E. 93-ab	P2013050155	Banyuwangi, East Java
Jambe	AA	XXIV.E. 19-abc	P19770482	Tulungagung, East Java
Ambon Hong	AAA	XXIV.D. 25-a	N/A	East Java
Raja Molo	AAA	XXIV.E. 31-a	P1973019	Lawang, Malang, East Java
Morosebo	AAA	XXIV.D. 7-bc	P197906175	Sarangan, Madiun, East Java
Raja Kenanga	AAA	XXIV.E. 24-a	P19810568	Purwodadi, Pasuruan, East Java
Cebol	AAA	XXIV.E. 70	P2012030021	Sukokerto, Pajarakan, Probolinggo
Nangka	AAA	XXIV.E. 7-bc	P19810567	Purwodadi, Pasuruan, East Java
Seribu	AAB	XXIV.D. 6-a	P1978073	Bogor, West Java
Kepok Putih	ABB	XXIV.D. 70	P19940531	Yogyakarta
Sri	ABB	XXIV.B. 2-a	P19720219	Pasuruan, East Java
Ebung	ABB	XXIV.D. 17-abc	P19760731	Siman, Ponorogo, East Java
Tlekung	ABB	XXIV.B. 22-a	N/A	Tlekung, Batu, East Java
Kates	ABB	XXIV.D. 97-ab	P2010091	Tutur, Pasuruan, East Java
Raja Bandung	ABB	XXIV.D. 69-ab	P19940525	Yogyakarta
Raja Prentel	ABB	XXIV.E. 2-bc	P1972027	Nongkojajar, Pasuruan, East Java
Bandung	ABB	XXIV.E. 11-abc	N/A	Central Java

Notes:
Passport data source: Registration Unit of Purwodadi Botanic Garden
Genomic group reference: Hapsari *et al*., 2015a; Hapsari *et al*., 2015b; Hapsari *et al*., 2018

Pollen staining and viability test

Male flowers were excised from the male buds at anthesis time between 7.00 to 9.00 am (Figure 2.a-b) and brought immediately to the laboratory to be observed its pollen viability. Fresh pollen grains were manually dislodged from each anther (Figure 2.c-d) with the aid of a sterile needle and gently spread on a glass slide.

Pollen viability analysis was conducted by staining test using 1% solution of 2,3,5-*Triphenyltetrazolium chloride* (TTC) (Beyhan & Serdar, 2008). Enough amount of TTC were dropped on the pollen grains, then a cover-glass was placed over the pollen grains and the slides were stored at room temperature (25°C) for one hour. After an hour, the slides were then observed with light microscope Olympus CX31 at 40 magnification. Pollen grains that took up the stain were considered viable/fertile while those that did not were sterile. Viable pollen grains were physically counted in ten randomly selected microscope fields per sample (only completely rounded and deeply stained grains were recorded).

Figure 2. The inflorescence of banana: a. Male bud, b. Male flowers, c. Anthers, d. Filament and e. Pollen grains

Data analysis

Percentage of pollen stainability was calculated to determine the level of pollen viability of each sample following formulae by Ssebuliba *et al*. (2008). Clustering analysis was also subjected on percentage of pollen viability using Paleontological statistics/PAST software (paired group/UPGMA algorithm and Euclidean similarity index) to determine the pollen viability pattern among 19 bananas examined (Hammer *et al*., 2001).

$$Pollen\ viability\ (\%) = \frac{number\ of\ stained\ pollen\ grains}{total\ number\ of\ pollen\ grains}\ \text{x}\ 100$$

RESULTS AND DISCUSSIONS

Pollen stainability

Viable pollen of bananas using TTC staining method showed by red color grains, while the sterile pollen grains were transparent/un-stained (Figure 3). TTC staining was based on the reduction of a colorless soluble *Tetrazolium* salt to a reddish insoluble substance called *Formazan* (Shivanna & Rangaswamy, 1992). It is indicated the presence of active dehydrogenase enzymes in the viable pollen grains (Beyhan & Serdar, 2008). TTC is considered as the most efficient dye for staining to reveal the pollen viability (Soares *et al*., 2013), with faster and easier results to observe the actual viability of pollen (Sulusognu & Cavusoglu, 2014). Another staining method used in studies of banana pollen *e.g.* by *Iodium-Kalium iodida*/I_2KI (Damayanti, 2002), *Aceto-carmine* glycerol jelly/ACGJ (Ssebuliba *et al*., 2008; Oselebe *et al*., 2014), and Alexander's stain colors (Fortescue & Turner, 2004; Goigux *et al*., 2013).

The result showed that pollen viability was varying among 19 banana accessions examined in this study. Different species and cultivars of banana possess different levels of competency in the production of microspores, which correlated positively with levels of pollen fertility (Fortescue & Turner, 2004). Pollen viability was ranged from a mean of 7.69% to 99.76%, with high value of variance 1085.36 (standard deviation 32.95). Pisang Bandung (ABB) has the lowest pollen viability, whereas wild *M. acuminata* ssp. *rutilifes* (AAw) has the highest pollen viability (Table 2). The high variability of pollen viability was presummable due to the high genetic diversity of bananas in Indonesia. This result is similar to some previous studies which reported large variability in pollen viability among East African Highland bananas (Ssebuliba *et al*., 2008), Ugandan bananas (Mukasa & Rubahaiyo (1993), Indian bananas and Autralian bananas (Fortescue & Turner, 2004), also Nigerian bananas (Oselebe *et al*., 2014).

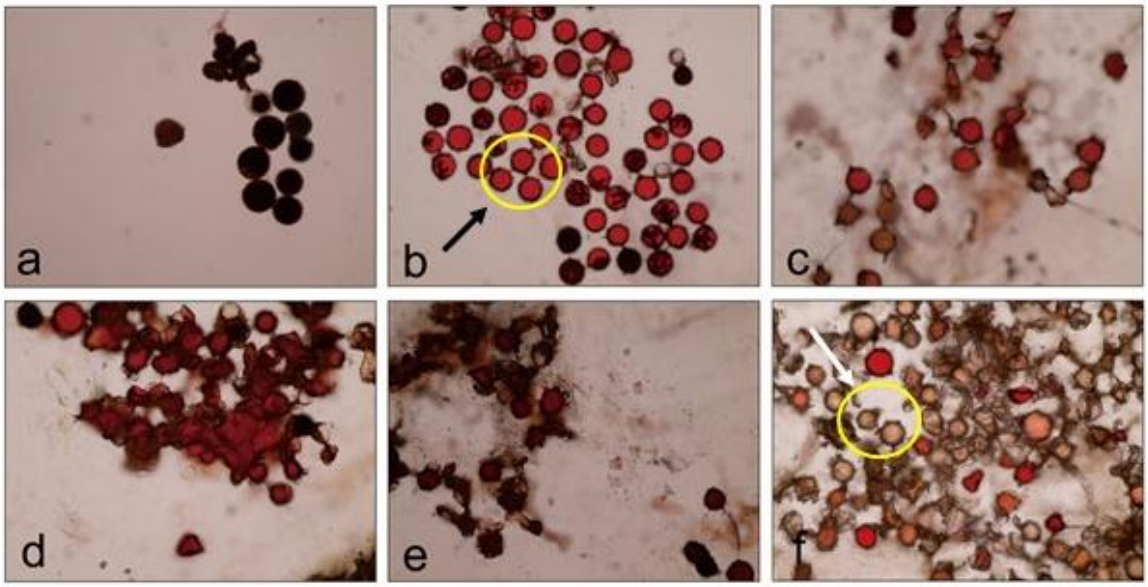


Figure 3. TTC stained pollen grains of bananas: a. *M. acuminata* ssp. *rutilifes* (AAw), b. *M. balbisiana* (BBw), c. Pisang Mas (AA), d. Pisang Nangka (AAA), e. Pisang Seribu (AAB), and f. Pisang Ebung (ABB). Notes: deep red-stained pollen grains = fertile (black arrow), un-stained pollen grains = sterile (white arrow)

Pollen viability of wild banana species

Among wild banana species, *M. acuminata* ssp. *rutilifes* (AAw) has higher pollen viability than *M. balbisiana* (BBw) i.e. 99.75% and 46.36%, respectively (Table 2). In banana breeding, the wild diploid species have largely been used as parents due to resistances to most of pests and diseases. Further, *M. acuminata* (A genome donor) and *M. balbisiana* (B genome donor) is assumed to have contributed most to the origin of cultivated bananas (Simmonds & Shepherd, 1955), therefore they become essential materials for banana improvement program. Wild diploid bananas are generally both male and female fertile (Adeleke *et al*., 2004). Wild *M. acuminata* sub-species which has high pollen fertility are mostly use as male parents (Sathiamoorthy and Rao, 1987; Mukasa and Rubaihayo, 1993; Dumpe and Ortiz, 1996; Turyagyenda, 2006; Ssebuliba *et al*., 2008), whereas wild *M. balbisiana* which has less pollen fertility as female parents (Davey *et al*., 2013).

Previous pollen study by Fortescue and Turner (2004) reported that seeded diploid species of Southern Australian bananas *M. balbisiana* had significantly more viable pollen than *M. acuminata* were 98% and 71%, respectively. *M. balbisiana* from The Phillipines (Butohan 2, Los Banos and Tani) also showed high pollen viability of 97-99% (Dumpe & Ortiz, 1996). It was on the contrary to result of this study in which *M. acuminata* has much higher pollen viability than *M. balbisiana*. It was indicated that *M. balbisiana* from Java examined in this study was less fertile than *M. balbisiana* from Southern Australia and The Phillipines. The distribution of wild *M. balbisiana* is somewhat disjunct from South China to New Guinea (Perrier *et al*., 2011). It was presummable that *M. balbisiana* from Java was experienced speciation and genetically variable to *M. balbisiana* from Australia and The Phillipines. Molecular analysis to reveal the genetic variability among those *M. balbisiana* is required. In addition, *M. ornata* also reported had significantly more viable pollen than *M. acuminata* (Fortescue and Turner, 2004). According to Damayanti (2002), indeed *M. ornata* high in pollen viability (98.6%) but still less than *M. acuminata* ssp. *rutilifes* in this study. *M. ornata* is wild banana species mostly utilized for ornamentals (Hapsari, 2014; Hapsari *et al*., 2017).

Table 2. Pollen viability result of 19 Indonesian banana accessions examined in this study

Species name/cultivars	Genomic Group	Mean Number of Pollen evaluated	Mean Viable Pollen	% Viable Pollen
M. acuminata ssp. *rutilifes*/ Cici	AAw	34.00 ± 19.21	33.89 ± 19.21	99.76 ± 0.72
Mas	AA	154.40 ± 56.83	147.20 ± 56.35	94.83 ± 3.09
Jambe	AA	62.00 ± 19.46	12.60 ± 5.44	19.51 ± 7.20
Raja Molo	AAA	132.70 ± 43.05	126.60 ± 44.35	94.33 ± 5.10
Ambon Hong	AAA	86.10 ± 40.36	76.60 ± 38.98	87.8 ± 5.20
Seribu	AAB	41.50 ± 19.43	31.10 ± 13.03	78.16 ± 15.32
Nangka	AAA	74.30 ± 19.18	56.20 ± 14.81	75.8 ± 5.70
Morosebo	AAA	32.40 ± 9.25	23.90 ± 9.27	72.67 ± 9.52
Raja Kenanga	AAA	54.50 ± 25.64	42.40 ± 23.69	71.68 ± 21.96
Cebol	AAA	35.44 ± 16.22	24.33 ± 10.51	69.35 ± 8.17
M. balbisiana/ Klutuk Hijau	BBw	55.60 ± 52.24	30.80 ± 33.69	46.36 ± 12.30
Kepok Putih	ABB	101.80 ± 44.67	42.40 ± 24.83	41.98 ± 12.23
Sri	ABB	42.30 ± 21.80	13.30 ± 8.19	31.54 ± 14.38
Ebung	ABB	77.60 ± 28.21	18.60 ± 11.11	23.45 ± 7.40
Tlekung	ABB	48.90 ± 24.61	12.60 ± 12.62	22.71 ± 11.36
Kates	ABB	65.10 ± 32.14	11.20 ± 8.66	14.55 ± 7.19
Raja Bandung	ABB	18.90 ± 7.40	2.60 ± 1.96	14.33 ± 11.29
Raja Prentel	ABB	48.70 ± 17.24	4.30 ± 3.06	8.11 ± 6.19
Bandung	ABB	54.60 ± 21.53	4.60 ± 3.20	7.69 ± 4.03

About fifteen sub-species of wild *M. acuminata* were recognized in Indonesia comprised sub-species of *alasensis, halabanensis, acuminata, nakaii, cerifera, longepetiola, bantamensis, rutilifes, breviformis, zebrina, malaccensis, sumatrana, tomentosa, microcarpa*, and *flava* (Nasution, 1991). Due to its potential characteristics, each sub-species is valuable as parents for further breeding. Further, *M. acuminata* ssp. showed high pollen viability; includes ssp. *banksii* had 94-98% viable pollen, ssp. *malaccensis* had 98-99% viable pollen (Dumpe & Ortiz, 1996), and ssp. *rutilifes* had 99.76% viable pollen (this study). However, its germination percentage were considered low. Study on fresh pollen germination *in vitro* of wild *M. acuminata* sub-species by Rachman *et al*. (2012) showed that sub-species of *bantamensis* has the highest germination percentage (15.21%), followed by *breviformis* (10.61%), *malaccensis* (7.91%) and *zebrina* (6.97%).

Pollen viability among banana cultivars

Among banana cultivars, the pollen viability of Pisang Mas (AA) was the highest (94.83) followed by AAA group (78.61%), AAB group (78.16%) and ABB group (20.43%). Edible diploid cultivars were considered has fertile pollens, whilst the triploid bananas are mostly sterile (Heslop-Harrison and Schwarzacher, 2007). Pisang Jambe was considered as an outlier. It was included as AA group (Hapsari *et al*., 2015; Hapsari *et al.*, 2018) and supposed to had high viable pollen, but according to this study it was considered low in pollen viability (19.51% ± 7.20%). Further observation of a complete pollen characteristic subjected to Pisang Jambe is required.

Pisang Mas as diploid cultivar (AA) showed the highest percentage of viable pollen (94.83%). Diploid individual plant performs normal meiotic chromosome behavior with the chromosome pairs segregate normally so that produce fertile pollens (Adeleke, 2004; Fuchs *et al*., 2011). This result in accordance to some previous pollen studies on bananas that AA diploids had on average the highest pollen viability as compared to the triploids and tetraploids. Dumpe & Ortiz (1996) suggest that crosses using diploid accessions as male parents were more likely to be successful than crosses with polyploid male parents. Furthermore, diploid cultivars (AA) are used in banana breeding for crosses with triploids (cultivars AAB) to generate AAAB tetraploids with satisfactory levels of fruit quality, yield and disease

resistance (Silva *et al.*, 2001). Another Indonesian diploid banana cultivar reported by Damayanti (2002) also showed high pollen viability is Pisang Uli (71.9%). Pollen study on more Indonesian diploid banana cultivars is recommended *e.g.* Berlin, Berlian, Orlin, Jaran, Rayap, Rejang, Trimulin, Gadis, Lidi, Jarum, Lilin, Masam, Kole, Pinang, and other possible synonims cultivar. (Valmayor *et al*., 2000; Jumari & Pudjoarinto, 2000; Hapsari & Masrum, 2011; Hapsari *et al*., 2015; Hapsari *et al*., 2017)

The triploid banana cultivars from genomic groups of AAA and AAB examined (Raja Molo, Ambon Hong, Seribu, Nangka, Morosebo, Raja Kenanga and Cebol) have pollen viability of more than 60%. Pisang Raja Molo (AAA) showed the highest pollen viability among triploid cultivar (94.33%). This study showed that pollen viability of Indonesian triploid bananas were considered higher than Australian and Indian triploid bananas. Australian triploid cultivars in average had 6–10% viable pollen, with Gros Michel (AAA) had the highest percentage of viable pollen at 13% and Green/Red (AAA) had 3%, whereas Indian triploid cultivars had 21–29% viable pollen (Fortescue & Turner, 2004).

Meanwhile, there was a tendency of low pollen viability has been observed in the ABB cultivars. In majority, the percentage of pollen viability of ABB cultivars (Ebung, Tlekung, Jambe, Kates, Raja Bandung, Raja Prentel and Bandung) less than 30%, only 2 banana cultivars *i.e.* Kepok Putih and Sri that have viable pollen of >30%. Raja Prentel and Bandung were the lowest pollen viability (<10%) (Table 2). In triploid individual plant showed meiotic irregularities includes the chromosome pairs segregate abnormally, lagging chromosomes, and univalent formation so that causing pollen infertility (Adeleke, 2004; Fuchs *et al*., 2011). However, some fertile pollen occured is probably the result of a few gametes having complete genomes through balanced genome segregation (Heslop-Harrison & Schwarzacher, 2007).

This preliminary result suggests that in addition to wild banana species, banana cultivars with two or more A genomes also might be useful as male parents with high viability of pollen grains. Further and more detail studies of anther and pollen both wild banana species and cultivars particularly on diploids are recommended including pollen output, maturity development stages, characteristics, germination, physiological and biochemical process related to pollen germination and tube growths, compatibility, storage, and culture.

Pollen viability clustering pattern

Interestingly, the pollen viability percentage of bananas examined were differed between genomic group but clustered within genomic groups, unless a cultivar of Pisang Jambe (Table 2, Figure 4). Clustering analysis based on percentage of pollen viability, the nineteen Indonesian banana accessions were separated into two groups. Group I consists of banana accessions of genomic groups AAw, AAA and AAB with pollen viability >60%, whereas Group II consists of banana accessions of genomic groups BBw, ABB and Pisang Jambe (AA) as an outlier with pollen viability <50% (Figure 4). The dendogram is in accordance with its taxonomic classification and phylogenetic relationships (Hapsari *et al.*, 2018), except the outlier Pisang Jambe (Figure 4).

In Group I, wild *M. acuminata* ssp. *rutilifes* (AAw) was clustered with pisang Mas (AA), Raja Molo and Ambon Hong (pollen viability of >85%). Pisang Morosebo (AAA), Raja Kenanga (AAA) and Cebol (AA) are included in Cavendish sub-group (Hapsari *et al*., 2017), and therefore based on its pollen viability also clustered together in the same sub-group (Pollen viability 69-73%). Pisang Nangka was morphologically identified as AAB (Jumari & Pudjoarinto, 2000), but molecular study revealed that it was identified as AAA (Hapsari *et al*., 2015b, Hapsari *et al*., 2018). However, this study showed that Pisang Nangka was clustered with Pisang Seribu which has AAB genome with pollen viability 75-78% (Figure 3). Whilst, in Group II, wild *M. balbisiana* (BBw) was clustered in a sub-group with Pisang Kepok Putih (ABB), and act as an outgroup of ABB cultivars (pollen viability of >40%). The other ABB cultivars were clustered in two sister sub-groups, with Pisang Jambe (AA) as an outlier (pollen viability <40%). This clustering pattern based on pollen viability of ABB cultivars was similar to its morphological characteristics pattern as reported by Gusmiati *et al*., 2018. Thus, this study also proves that knowledge on pollen viability may utilised in the taxonomy of bananas as suggested by Sathiamoorthy & Rao (1987).

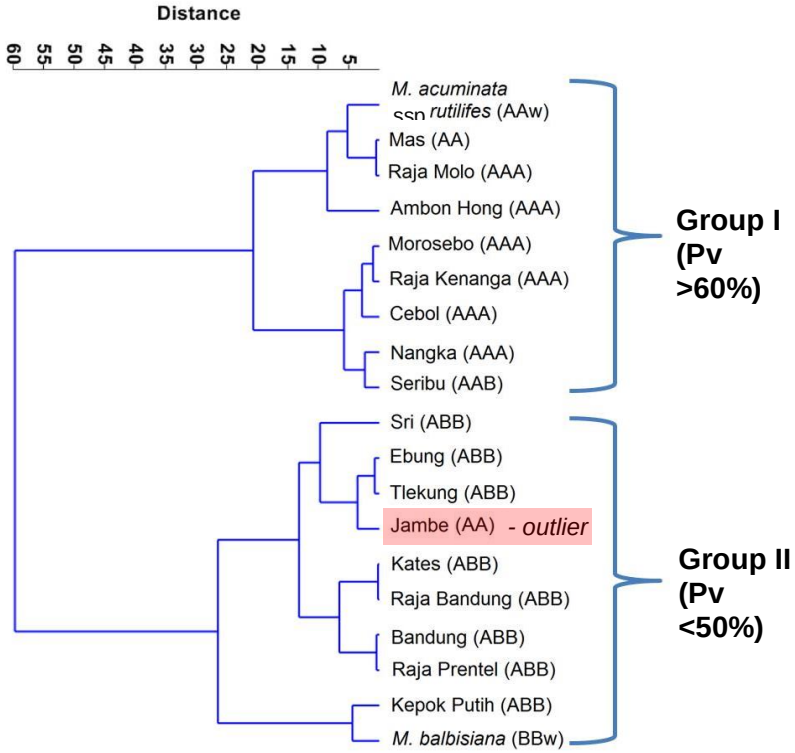


Figure 4. Dendogram clustering of 19 Indonesian bananas based on percentage of pollen viability. Note: Pv = pollen viability.

CONCLUSION

This study concluded: (i) pollen viability was varying among all the Indonesian banana accessions, (ii) pollen viability differed between genomic groups but clustered within genomic groups, so that pollen viability may utilised in the banana taxonomy (iii) wild species *M. acuminata* ssp. *rutilifes* has higher pollen viability than *M. balbisiana*, (iv) banana cultivars with genomic groups AA, AAA, and AAB has high pollen viability of more than 60%, (v) Pisang Jambe (AA) was an outlier and had low pollen viability, further observation is required, (vi) there was a tendency of low pollen viability in the wild BB and ABB cultivars (<50%). Thus, this preliminary results suggest that in addition to wild banana species, banana cultivars with two or more A genomes might be useful as male parents with high viability of pollen grains. Further and more detail studies of anther and pollen both wild banana species and cultivars particularly on diploids are recommended

ACKNOWLEDGEMENTS

The authors would like to acknowledge Registration Unit of Purwodadi Botanic Gardenfor providing the passport data of banana collection examined in this study, also to Documentation and Information Unit for providing the garden map. Sincere thanks are dedicated to the staff of Laboratory In vitro of Purwodadi Botanic Gardenfor the assistancy during pollen observation.

REFERENCES

Adeleke, M.T.V., M. Pillay, and B.E. Okoli. 2004. Relationships between meiotic irregularities and fertility in diploid and triploid *Musa* L. *Cytologia* 69(4): 387–393.

Beyhan, N., and U. Serdar. 2008. Assessment of pollen viability and germinability in some European chestnut genotypes (*Castanea sativa* L.). *Horticultural Science, Hoboken*. 35:171-178.

Damayanti, N.S. 2002. Pollen viability and anther culture in several banana cultivars (*Musa* spp.). Undergraduate Thesis. Biology Department. Faculty of Mathematic and Natural Sciences. Bogor Agricultural University. Bogor. [Indonesian]

Davey, M.W., R. Gudimella, J.A. Harikrishna, L.W. Sin, N. Khalid, and J. Keulemans. 2013. A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter-and intra-specific *Musa* hybrids. *BMC Genomics* 14(1):683.

Dumpe, B.B., and R. Ortiz. 1996. Apparent male fertility in *Musa* germplasm. *Horticulture Science* 31(6):1019-1022.

Fortescue, J.A., and D.W. Turner. 2004. Pollen fertility in Musa: Viability in cultivars grown in Southern Australia. *Australian Journal of Agricultural Research* 55(10): 1085-1091.

Fuchs, M.C.P., S.M. de Sousa, I. Sanmartin-Gajardo, and L.F. Viccini. 2011. Chromosome number in meiotic stage cells and pollen viability of *Vanhouttea hilariana* Chautems, *Vanhouttea brueggeri* Chautems and an interespecific hybrid (Gesneriaceae). *Anales de Biología* 33: 35-40.

Goigux, S., F. Salmon, and F. Bakry. 2013. Evaluation of pollen fertility of diploid and doubled-diploid clones of Mlali and their potential use for banana Breeding. *Acta Horticulturae* 986: 195-204.

Gusmiati, L.H., L. Hapsari, and D. Wahyudi. 2018. Morphological Diversity and Clustering of 10 Cooking Bananas (Musa cv. Group ABB) Collection of Purwodadi Botanic Garden - LIPI. *Floribunda* 5 (8): 299–314.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. PAST: Paleontological statistics software for education and data analysis. *Palaeontologia Electronica* 4(1): 1–9.

Hapsari, L. 2011. Two decades of Musaceae collection of Purwodadi Botanic Garden (1990-2010). *Jurnal Berkala Penelitian Hayati Edisi Khusus* 5A: 147-151. [Indonesian]

Hapsari, L., and A. Masrum. 2012. Preliminary screening resistance of Musa germplasms for Banana Bunchy Top Disease in Purwodadi Botanic Garden, Pasuruan, East Java. *Buletin Kebun Raya* 15(2): 58-70.

Hapsari, L. 2014. Wild *Musa* species collection of Purwodadi Botanic Garden: Inventory and its morpho-taxonomic review. *The Journal of Tropical Life Science* 4(1): 70-80.

Hapsari, L., D.A. Lestari, and A. Masrum. 2015a. *Album of the Banana Collection of Purwodadi Botanic Garden Seri 1: 2010-2015*. Purwodadi Botanic Garden – Indonesian Institute of Sciences. [Indonesian]

Hapsari, L., D. Wahyudi, R. Azrianingsih, and E.L. Arumingtyas. 2015b. Genomic identification of bananas (*Musa* spp.) from East Java assessed by PCR-RFLP of the Internal Transcribed Spacer ribosomal DNA. *International Journal of Biosciences* 7(3): 42-52.

Hapsari, L., J. Kennedy, D.A. Lestari, A. Masrum, and W. Lestarini. 2017. Ethnobotanical survey of bananas (Musaceae) in six districts of East Java, Indonesia. *Biodiversitas* 18(1): 160-174.

Hapsari, L., A. Rodiyati, and E.L. Arumingtyas. 2018. Genetic Variability and Relationship of Banana Cultivars (*Musa* L.) From East Java, Indonesia based on the Internal Transcribed Spacer Region nrDNA Sequences. *Journal of Tropical Biology and Conservation* 15:101–120.

Heslop-Harrison, J.S., and T. Schwarzacher. 2007. Domestication, genomics and the future for banana. *Annals of Botany* 100(5):1073–1084.

Jumari, and A. Pudjoarinto. 2000. Phenetic relationship of banana cultivars in Java. *Biologi* 2 (9): 531-542. [Indonesian]

Molina, A.B., J-V. Escalant, and I. Van den Bergh. 2005. In. Advancing banana and plantain R and D in Asia and the Pacific – Vol 13. Proceedings of the 3rd BAPNET Steering Commitee meting held in Guangzhou, China, 23-26 November 2004. Molina AB, Xu LB, Roa VN, Van den Bergh I, Borromeo KH (Eds.). International Network for the Improvemnet of Banana And Plantain – Asia Pasific, Los Banos, Laguna, Phillipines.

Molina, A.B. 2007. Challenges facing the banana industry and their impact on trade. Paper presented in Intergovernmental Group on Bananas and Tropical Fruits and the Common Fund for Commodities Workshop on Opportunities and Challenges in the World Markets for Fruits and Tropical Products in cooperation with the Trade Promotion Centre of the Chinese Ministry of Agriculture Guangzhou, China, 29 November – 1 December 2007. Food and Agriculture Organizatin of the United Nations.

Mukasa, S.B., and P.R. Rubaihayo. 1993. Male fertility in Uganda Banana Germplasm. *African Crop Science Journal* 1(2):87-93.

Nasution, R.E., and I. Yamada. 2001. *Wild bananas in Indonesia*. Puslitbang Biologi-LIPI. Bogor. [Indonesian]

Nasution, R.E. 1991. A taxonomic study of the species Musa acuminata Colla with its intraspecific taxa in Indonesia. *Memoirs of Tokyo University of Agriculture* 32: 1-122.

Ortiz, R., F. Ulburghs, and J.U. Okoro. 1998. Seasonal variation of apparent male fertility and 2n pollen production in plantain and banana. *Horticulture Science* 33(1):146-148.

Oselebe, H.O., C.V. Nnamani, and E. Ikeh. 2014. Pollen diversity, viability and floral structure of some *Musa* genotypes. *Nigeria Journal of Biotech*nology 27:21-27.

Perrier, X., E. De Langhe, M. Donohue, C. Lentfer, L. Vrydaghs, F. Bakry, F. Carreel, I. Hippolyte, J-P. Horry, C. Jenny, V. Lebot, A-M. Risterucci, K. Tomekpe, H. Doutrelepont, T. Ball, J. Manwaring, P. de Maret, and T. Denham. 2011. Multidisciplinary perspectives on banana (*Musa* spp.) domestication. Proceeding of National Academy of Sciences USA108(28): 11311–11318.

Rachman, E., Y.S. Poerba, and F. Ahmad. 2012. Pollen storage of wild varieties of *Musa acuminata* Colla ensuring pollen source for cultivated banana breeding program. *Berita Biologi* 11(2): 167-175. [Indonesian]

Ravi, I., and M. Vaganan. 2016. Abiotic stress tolerance in banana. In: Abiotic Stress Phisiology of Horticultural Crops. Rao NKS, Shivashankara KS, Laxman RH (Eds.). Springer India. Pp. 207-222.

Sathiamoorthy, S., and V.N.M. Rao. 1987. Pollen production in relation to genome and ploidy in banana clones. In: National Seminar on Banana Production Technology. Muthukrishnan CR, Abdul Khader JBMM (Eds.). Tamil Nadu Agricultural University, Coimbatore, India. Pp 46-49.

Shivanna, K.R., and N.S. Rangaswamy. 1992. *Pollen biology: A laboratory manual*. Springer-Verlag Berlin Heidelberg. Germany.

Silva, S.O., M.T. Souza Jr, E.J. Alves, J.R.S. Silveira, and M.B. Lima. 2001. Banana breeding program at Embrapa. *Crop Breeding and Applied Biotechnology* 1: 399-436.

Simmonds, N.W., and K. Shepherd. 1955. The taxonomy and origins of the cultivated banana. *Botanical Journal of the Linnean Society* 55: 302-312

Soares, T.L., D. de Oliveira e Silva, M.A.P. de Carvalho Costa, J.A. dos Santos-Serejo, A. da Silva Souza, L.S.M. Lino, E.H. de Souza, and O.N. de Jesus. 2008. *In vitro* germination and viability of pollen grains of banana diploids. *Crop Breeding and Applied Biotechnology* 8: 111-118.

Soares, T.E., O.N. De Jesus, J.A. Dos Santos-Serejo, and E.J. De Oliveira. 2013. *In vitro* pollen germination and pollen viability in passion fruit (*Passiflora* spp.). *Revista Brasileira de Fruticultura* 35(4):1116-1126.

Ssebuliba, R.N., A. Tenkouano, and M. Pillay. 2008. Male fertility and occurrence of 2n gametes in East African Highland bananas (*Mus*a spp.). *Euphytica* 164:53–62. DOI 10.1007/s10681-007-9637-6.

Sulusoglu, M., and A. Cavusoglu. 2014. In vitro pollen viability and pollen germination in cherry laurel (*Prunus laurocerasus* L.). *The Scientific World Journal* ID657123:1-7. DOI: 10.1155/2014/657123.

Turyagyenda, F.L. 2006. Characterization of a segregating population of *Musa* for parthenocarpy and male fertility. *Info Musa* 15(1-2): 44-45.

Valmayor, R.V., S.H. Jamaluddin, B. Silayoi, S. Kusumo, L.D. Danh, O.C. Pascua, and R.R.C. Espino. 2000. Banana cultivar names and synonyms in Southeast Asia. International Network for the Improvement of Banana and Plantain – Asia and the Pacific Office. Los Banos, Laguna, Philippines.

Predicting suitable habitat of the threatened and endangered tree *Dipterocarpus littoralis* in West Nusakambangan Nature Reserve, Indonesia using maximum entropy modelling

Mahendra Primajati[1], Arief Hamidi[1], Iyan Robiansyah[2], Fazlurrahman Shomat[1], Setija Budiawan[1]
[1]Fauna & Flora International – Indonesia Programme, Komplek Margasatwa Baru, Jl. Margasatwa Baru No. 7A, Pondok Labu, Jakarta 12450, Indonesia; [2]Pusat Konservasi Tumbuhan Kebun Raya – LIPI, Jl. Ir. H. Juanda No.13, Bogor, Jawa Barat.
Email: mahendra.primajati@fauna-flora.org; mahendra.primajati@gmail.com
Tel: +62-21-2276-4295

ABSTRACT

Plahlar (*Dipterocarpus littoralis*), endemic to the Nusakambangan Island, is listed as Critically Endangered by the IUCN mainly due to restricted distribution, slow regeneration, and high level of illegal harvesting. Nevertheless, studies on Plahlar conservation status is still lacking and limited to the West Nusakambangan Nature Reserve (WNR). In many cases, baseline data on potential suitable habitat is an essential step toward effective species management planning. Using Maximum Entropy (MaxEnt) analysis, 83 Plahlar occurrence and seven predictors were used to predict Plahlar suitable habitat in the WNR, including geophysical and spectral variables from Landsat-8 satellite imagery. The MaxEnt model performed well with the mean Area Under Curve of 0.85 (stdev: 0.035). Elevation, distance from the coastline, and slope had the highest percent contribution, explaining 34%, 21.7%, and 16.5% variations of the model respectively. The model predicted only 16% of the WNR is suitable for Plahlar, indicating a narrow habitat preference. This study highlights the urgent need of effective conservation actions to save the species from the brink of extinction.

Keywords: habitat preferences, MaxEnt, species distribution modeling, small island.

INTRODUCTION

Plahlar (*Dipterocarpus littoralis*) is an endemic species growing naturally only on Nusakambangan Island, Central Java. Plahlar has been classified as Critically Endangered by the IUCN mainly due to restricted distribution, slow regeneration, and rampant illegal logging (Ashton, 1998). As Dipterocarp, the wood of this tree has a high economic value because of its strong quality, straight and durable which is suitable for the manufacture of building materials and boats (Robiansyah & Davy, 2015; Whitten, Soeriaatmadja & Afif, 1996). This economic value makes Plahlar wood favored by local illegal loggers.

No previous detailed habitat suitability mapping has been conducted, although population status and habitat preferences for this endangered species in West Nusakambangan Nature Resevere was already carried out by Robiansyah & Davy in 2015. Habitat suitability modeling is essential for the conservation and management of critically endangered species, especially those are understudied such as Plahlar. As such the geographical range of the Plahlar in West Nusakambangan Nature Reserve remains unclear and an updated range map for this species is urgently needed to aid conservation planning. Therefore, the collection of information related to Plahlar habitat is indispensable for the conservation efforts of the West Nusakambangan Nature Reserve area. Species distribution modeling (SDM) are becoming increasingly popular in ecology and are being widely used in many application, especially in ecology of species. One of the most widely used SDMs is Maximum Entropy modeling (MaxEnt). MaxEnt estimates the species potential geographic distribution by finding the distribution of maximum entropy (closest to uniform) subject to the constraint (van Proosdij *et al*., 2015). For predicting threatened species, MaxEnt appears to be robust even if only few occurrence records are available (Wilting, Cord, Hearn, Hesse, & Mohamed, 2010).

The objective of this study was to generate a prediction of suitable Plahlar habitat from ecological niches modeled using presence only data. Secondly was to identify any geophysical and spectral signature factors from remote sensing data that indicate the growing space and habitat suitability of Plahlar. Plahlar habitat mapping is performed to show which sites are suitable areas for this tree. In addition, this mapping activity can also be used as input in managing the nature reserve area and provide information for Plahlar national conservation strategic and action plan.

MATERIAL AND METHODS

Study Area

The study area for the predictions of the Plahlar habitat suitability was specifically carried out in West Nusakambangan Nature Reserve, Nusakambangan Island, Central Java, Indonesia (Figure 1). The status has been reinforced by Ministry of Forestry Decree Letter No. SK.359 / Menhut-II / 2004 dated October 1, 2004 to became Cagar Alam Nusakambangan Barat (CA-NKB) or west Nusakambangan Nature Reserve (WNR) after arranged the border limit in 2009 to 656.06 Ha. This area is within the administrative area of Tambakreja Village, South Cilacap Subdistrict, Cilacap Regency. The WNR area has the following limits: North & East borders with forest area owned by Ministry of Law and Human Rights; South and West part bordered by the Indonesian Ocean. Regional climate for the whole Nusakambangan Island is tropical, lowland, and evergreen rain forest (Robiansyah & Davy, 2015), with mean annual temperature of 26.5ºC and annual precipitation 3,304 mm (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). Forest range from coastal to elevation 112 m asl (Farr et al., 2007), with the domination of geological formations of karstic rocks from the shoreline to the highest location.

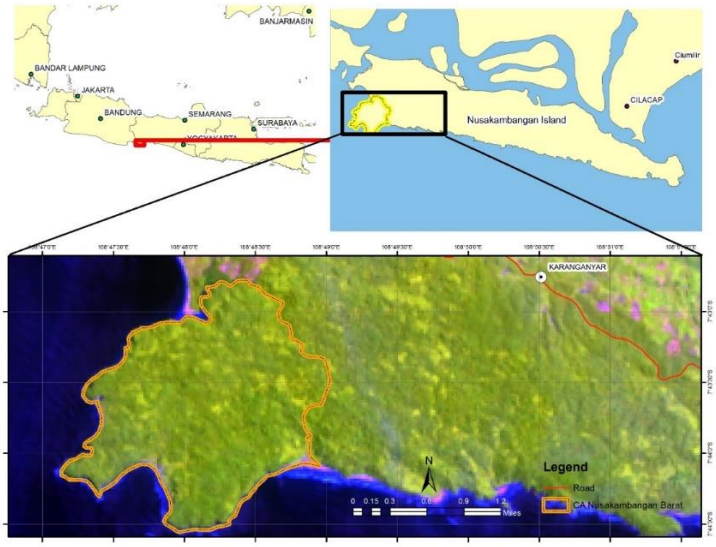


Figure 1. Location of West Nusakambangan Nature Reserve in Nusakambangan Island. Inset show study area covered with forested land cover and 30 presence location was used to model the habitat suitability of Plahlar.

Species Occurrence Data

Plahlar is a medium to big tree with its diameter at breast height potentially exceeds 1 m and reach 35 m in height. The bole is straight and bark rather smooth than flaky. Leave simple and clustered around the twig apices, the lamina is broadly ovate sized 16-25 (up to 52 in young trees) by 10-18 (up to 28 in young) cm, thinly coriaceous and having 19-24 pairs of nerves. The fruit with a pair of wing which is actually a 5-persistent corollas that 2 of them are longer and resemble to a pair of wing, and one wing can sized 24 by 4 cm. The nut is fairly big with diameter up to 3.5 cm (P. S. Ashton, 1982). The species may resemble to *D. hasseltii* and *D. retusus* with regards to its wide leaves and big fruit than other Dipterocarps. A total of 83 Plahlar localities were recorded by FFI and BKSDA between 2014 and 2015 in WNR. Only individual trees with a diameter at breast height (DBH) of 20 cm and larger were recorded and georeferenced (Figure 1).

Environmental Variables

We used 10 environment predictors based on their relevance to the biological factors of Plahlar, including geophysical and spectral variables from Landsat-8 satellite imagery (Kumar & Stohlgren, 2009; Young, Carter, & Evangelista, 2011). Spectral reflectance and vegetation indices derived from satellite data have been widely used to assess variations in the physiological state and biophysical properties of vegetation (Gill et al., 2017; Huete, Didan, Leeuwen, Miura, & Glenn, 2011; Lewis, Phinn, & Arroyo, 2013). Landsat-8 satellite image data has been used to see Plahlar's spectral reflectance potential. Using a spectrophotometer, a preliminary study on the reflectance spectrum of Plahlar leave surfaces was done to investigate the infrared wave absorbance values, resulting a mean reflection of 1.053 ± 0.16µm (Kristio Budiasmoro, personal communication). Using this wavelength, we identified three out of eleven Landsat-8 bands as suitable identifiers to detect Plahlar occurrence, including red bands (l8-b4, 0.64 - 0.67µm), near infrared (l8-b5, 0.85 - 0.88µm), and short wave infrared (l8-b6, 1.57-1.65µm) (Appendices 1). In addition, we used a normalized different vegetation index (l8_ndvi) to detect changes in canopy composition (P. Li, Jiang, & Feng, 2013). We classified the land cover classification (l8_class) of the Landsat-8 satellite image into forest cover (1), medium forest (2), open forest (3), non-forest (4), non-vegetation (5), and water body cover (6) (SNI, 2010; USGS, https://earthexplorer.usgs.gov/).

Five geophysical data were used as predictors of Plahlar habitat distribution (Robiansyah & Davy, 2015), including altitude (nkb_aster_dem), slope (nkb_aster_slope), direction of the slope (nkb_aster_aspect), relative distance from river (nkb_riv_dist), and the relative distance from the coastline (nkb_coast_dist). The altitude, slope, aspect, and river geophysical data are derived from the Advanced Spaceborne Thermal Emission and Reflection Radiometer Global Digital Elevation Model (ASTER GDEM) also obtained from USGS (http://gdex.cr.usgs.gov/gdex/). All variables were resampled to 15 meters / pixels, and use the WGS84 - UTM 49S projection system. In this analysis we did not use bioclimatic data from Worldclime – Global Climate Data, because of the spatial resolution of this data is 1 km^2, which is too coarse to use in the current modeling (Hijmans et al., 2005).

The relationship between variables are examined using spearman's rank correlation, variance inflation factor (VIF), and scatterplot matrix. Removing highly correlated variable is recommended by several studies given the complex feature created by MaxEnt (Merow, Smith, & Silander, 2013). Examining all paired variables was performed in R software using CAR and ModelMap package to investigate auto-correlation between variables. The overall patterns from scatterplot matrix (Figure 2) suggest possible linear relationship. All spectral reflectance variables are positively correlated, whereas only slope and coast distance are negatively correlated. To be more specific to select appropriate variable, we used VIF to indicates how much the variance of the variables estimate is being inflated by multicollinearity (Oyana & Margai, 2016). Variables with VIF greater than 5 indicates multicollinearity, thus were omitted from the model (Lesage, Banerjee, Fischer, & Congdon, 2009; Oyana & Margai, 2016). We then retained seven variables in the model, including slope, aspect,

altitude (DEM), distance from the coast, distance from river, land-cover classification, and band 6 of Landsat-8 satellite image in the model (Table 1).

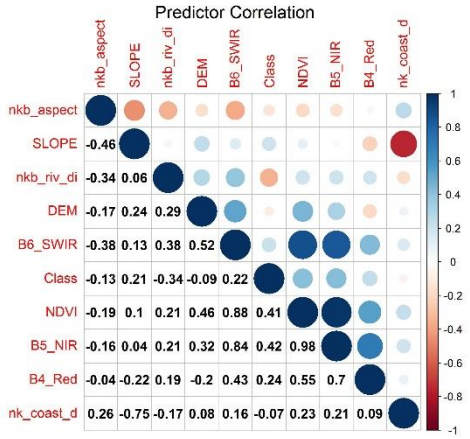


Figure 2. Correlation matrix of all paired variables

Table 1. Preliminary assessment for colliniearity statistics result. (*chosen variable for MaxEnt)

Model Variable	Collinearity Statistics VIF
l8_b4	62.17
l8_b5	1064.08
l8_b6*	4.93
NDVI	839.37
nkb_aspect*	1.55
nkb_riv_dist*	1.93
nkb_coast_dist*	4.76
nkb_aster_dem*	3.28
nkb_aster_slope*	4.35
l8_class*	1.93

Modeling Procedure

We used maximum entropy (MaxEnt), a machine learning program that estimates the probability distribution of species occurrence based on environmental constraints. MaxEnt has been widely used for modeling species distribution and become consistently competitive among many suitable habitat modeling methods (Kumar & Stohlgren, 2009; Phillips, Anderson, & Schapire, 2006; Elith et al., 2011). It requires presence only data and environment variables for the study area. We performed 50 replications, sub-sample replication, 25% random test percentage, and 700 maximum iteration. Regularization multiplier parameter of 1 and 2.5 were used to avoids fitting locally, reducing the effect of spatial autocorrelation and model over-fitting (Norris, 2014). Response Curve and Jackknife analysis were performed in the modeling process. The logistics probability output of MaxEnt generates values ranging from 0 to 1. We maximized the sum of training data sensitivity and specificity (Max TSS) for maximum probability species presence and minimum training presence logistics (Min TP) threshold rule for lowest probability species presence in the direction of define the final most, medium and not suitable predictive map. Max TSS known to be had a promising result for threshold use when presence only data are used in the model (Liu, White, & Newell, 2013).

RESULT AND DISCUSSION

Area Under Characteristics (AUC) = 0,851

Figure 3 below shows the Area Under the Receiver Operating Characteristics (ROC) or it can be referred to AUC. AUC value allows us to compare the performance of one model with anothers, and it is very useful to evaluate the MaxEnt model that we make. AUC value = 0.5 indicates that the model's performance is not better than random, while a value close to 1.0 gives an indication of better model performance. After performing 50 runs of replication for the approximate conformity of this Plahlar habitat, the AUC chart is obtained as shown below:

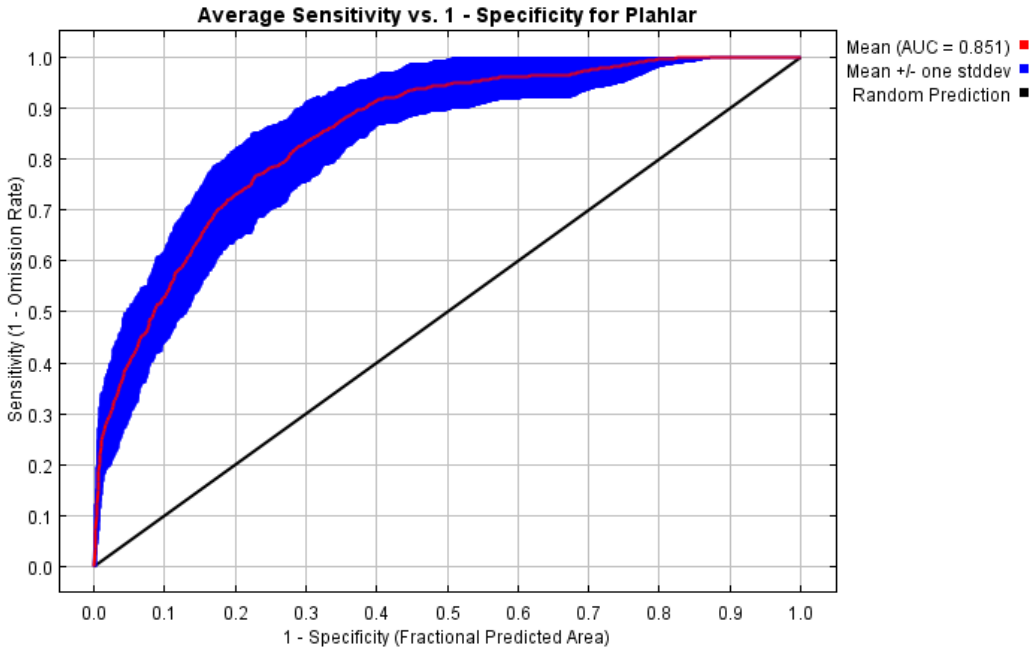


Figure 3. The receiver operating characteristic (ROC) curve final MaxEnt model for Plahlar

Variable Contribution Analysis

Table 2 shows environmental variables and contribution percentages of each variable used in the habitat distribution model of Plahlar. Although the percent contribution should be interpreted with caution and should be accompanied with permutation importance, the higher the percentage value of the contribution, the higher the influence or impact variable in predicting the existence of the species (S. Phillips, 2008). According to this analysis, elevation (nkb_aster_dem) and relative coast distance (nkb_coast_dist) is the highest predictive contributor with contribution percentage and permutation importance value reaches 34%, 43.5 and 21.7%, 19.4 respectively. Slope and aspect are in a place close together, with slope (nkb_aster_slope) variable give more contribution to the model rather than aspect (nkb_aspect).This finding is in accordance with the previous study which states that the presence of Plahlar was significantly associated with low elevation and south-west facing sites (Robiansyah & Davy, 2015).

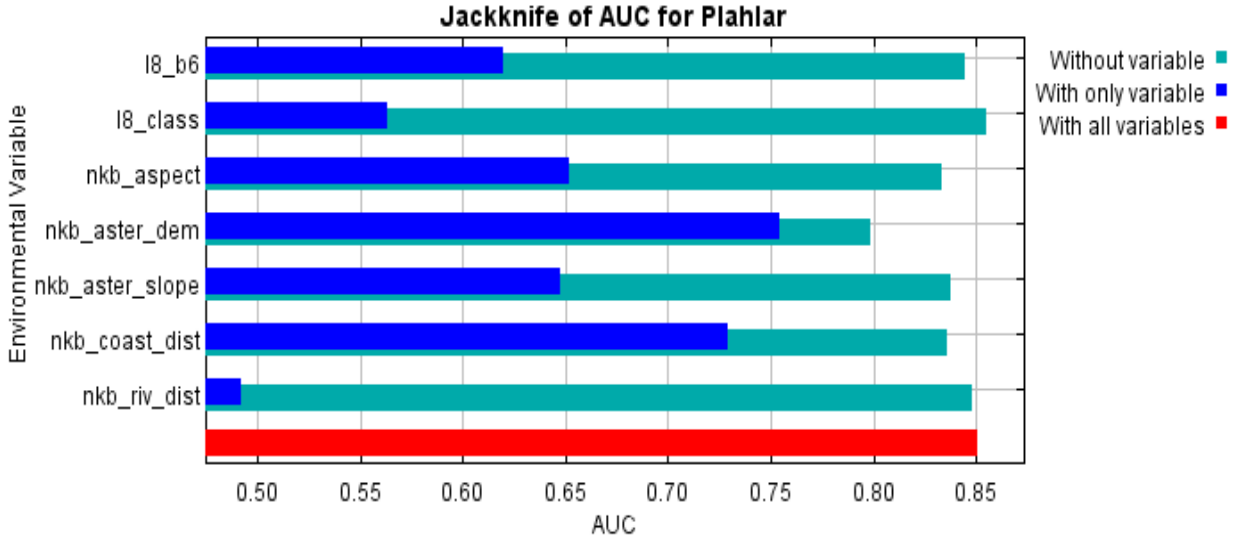
Table 2. Percent contribution and permutation of importance Plahlar MaxEnt model.

Variable	Percent contribution	Permutation importance
nkb_aster_dem	34	43.5
nkb_coast_dist	21.7	19.4
nkb_aster_slope	16.5	9.4
nkb_aspect	15	13.3
l8_b6	10.8	8.7
nkb_riv_dist	1.6	4.5
l8_class	0.5	1.2

From Table 2, the geophysical factor dominates the top four that can influence this prediction model. These factors include elevation, slope of the land, and distance from the coastline. All the three variables have percentage contribution over 15%. While the two lowest geophysical variables

are the relative distance from river (nkb_riv_dist) and land-cover classification is not dominant, with percent contribution less than 2%. One satellite image spectral reflectance variable has been found to give a contribution percentage value above 10%, this variable is Landsat-8 band 6 (l8_b6). Band 6 is short wave infra-red (SWIR) with reflectance range 1.57-1.65 µm which have been known to discriminates moisture content of soil and vegetation, also signature for minerals (B. Li, Ti, Zhao, & Yan, 2016; Mars & Rowan, 2010). This could happen in WNR when vegetation density in Plahlar location is very low, the reflected radiation is dominated by soil or ground exposure, and the effect of multiple reflections within the plant canopy is greatly reduced (Price, 1992). The karstic dominated area could be the possible effect of this variable became a medium contributor when some of the Plahlar occurrence located in sparse forest area, low density of vegetation, and expose of forest floor. In this analysis, satellite image reflectance variables l8_b4, l8_b5, and vegetation index (NDVI) were excluded from the model not only to avoid the multicollinearity between other variable, but also because in preliminary MaxEnt test result are found that this variable not significantly contribute in predicting the Plahlar habitat.

Below is a jackknife graph that shows the performance of each variable if the model is run separately using the variable itself or not using that variable at all. The variable with the highest gain when used separately is the elevation (nkb_aster_dem), as it has the most influential information in the prediction model. The variable that most decreases the gain when omitted is the slope of the land (nkb_aster_slope), because it has the most information that is not present in other variables at the time the model is operated.



The determination of thresholds logistics probability should not be arbitrary and should consider the relative importance of omission and commission error (Liu et al., 2013; Norris, 2014). We classify the logistics probability result using Max TSS and Min TP threshold rule to minimize differences between data values in the same class and maximize differences between classes (Brewer & Pickle, 2002; Jenks & Caspall, 1971). If we assign suitable habitats for Plahlar with Max TSS logistics threshold more than 35%, the occupancy areas with a high habitat suitability rate is only 16% of the total area of WNR area (

Table *3*).

The central and northern sides of the darker zone (Figure 4) are the best predictable habitat areas for Plahlar to growth based on geophysical and spectral satellite imagery. From the results of the prediction of habitat distribution, data matching was performed with the Plahlar occurence from the

survey results. It was found that 72 out of 83 occurrence or 87% of Plahlar were in locations that were inside the predicted area of high probability habitat. This ensures that the prediction of the habitat can adequately describe the area of growing Plahlar that is suitable in the WNR area. From the habitat distribution map (Figure 4), it can be estimated that Plahlar gathered more in the middle of the island which has a tendency to have a height between 40-60 meters above sea level, slope between 5-15 degrees, and a relative distance of 500-800 meters from the coast line (Appendices 2). These three most decisive biophysical factors are not found on the east side of the WNR which makes the area the lowest probability area as a Plahlar habitat as indicated as light gray on the map.

Table 3. Classification of threshold probability from MaxEnt result

Probability	Class Probabilities	Area (Ha)	%	Number of Plahlar Occurence	% Plahlar Occurence
High	0.35 – 0.95	106.17	16.18	72	87
Medium	0.05 - 0.35	269.21	41.03	10	12
Low	0 – 0.05	280.67	42.78	1	1
Total		656.05	100	83	100

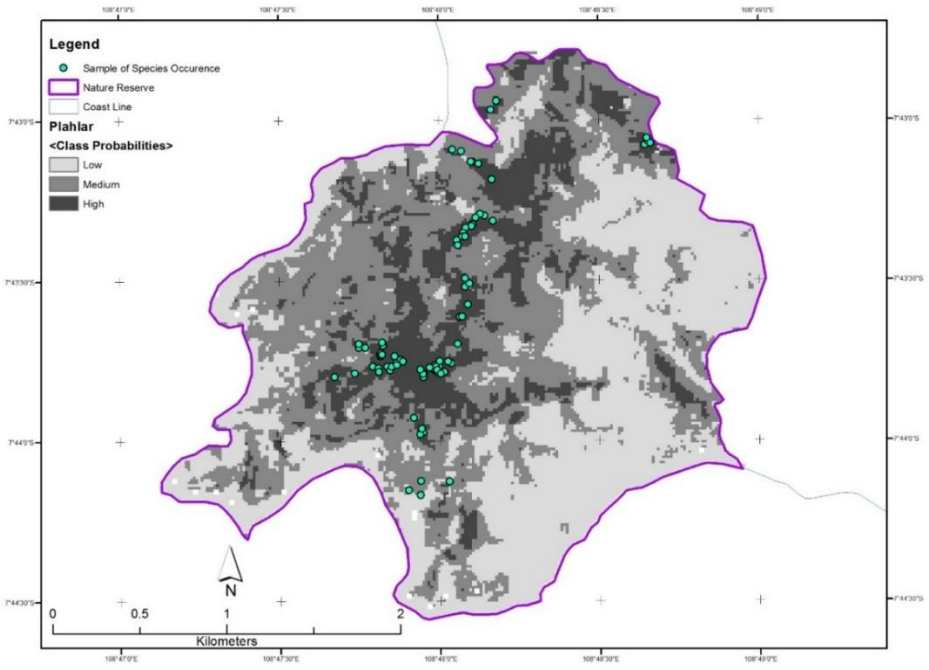


Figure 4. Predicted suitable habitat distribution of Plahlar. Maps show the spatial distribution of suitable habitat within the prediction area obtained from 83 point of Plahlar occurrence.

Then the consequence of this small area of suitable habitat from the result map (Figure 4) is to reduce the disturbance inside the WNR for enhancing the survival rate of this species. Because if we look outside the boundary, there are still logging activities that can be found around the area, and this is further exacerbated by the lack of a good understanding of the surrounding community about the conservation status of Plahlar. There are several methods that fit to do so to prevent the loss of this spesies. Assisted/Accelerated Natural Regeneration (ANR) could be one best solution to be performed on this situation. Another way is to alter the growth of *Arenga obtusifolia* (Langkap) as an invasive

species and clean up the creepers of *Meremia peltata*, to keep the rise of termites undermining the growth of its seedlings (Partomihardjo et al, 2014; Robiansyah & Davy, 2015). Reintriduction outside of WNR are another possible effort which is more easily for innovative propagation compared in within the WNR area where is impossible due to its protection status. Further investigation to search the population of Plahlar outside the WNR boundary is vital to support this effort.

CONCLUSION

In this analysis, we can generate a model map of the habitat suitability of Plahlar (*Dipterocarpus littoralis*) which is one of the threatened plants in its original habitat in WNR. The resulting model is based on the prediction of some geophysical and spectral variables, especially the most influential are geophysical factors such as elevation, distance from shoreline, and land slope respectively. These three variables provide the greatest contribution in estimating the suitability of Plahlar.

It is possible to model species occurrence with MaxEnt, but it should be done when we can interpret the logistic output as corresponding to a temporal and spatial scale of sampling that result in a 50% chance of the species being present in high probability of suitable areas (Elith et al., 2011; S. J. Phillips et al., 2006). This assumption can be accepted for Plahlar, although the allegedly most suitable habitat location of Plahlar is not always found in the real growing Plahlar area, but it is the best model for determining growing habitat with the same environmental variables as the area found by this model result. The existence of this habitat probability map will make it easier for regional managers and other researchers to develop population inventory based on the best location of the Plahlar habitat from MaxEnt so that it can help save the costs and time of the survey in the future.

For theoretical and statistical advances in species distribution modeling must be accompanied by the collection of more detailed field data. This model can be improved with including microclimate and soil type variables. Moreover, this analysis has not yet involved island wide data collected because some of the data is in the on-going process. Therefore, for future analysis is expected to add other environmental variables, especially microclimate variables. The use of this method is very useful if data extrapolation is also carried out outside the WNR area, so that information on predicting habitat distribution across the island of Nusakambangan can be obtained. To make it easier to estimate the location, as well as the size of the population and the possibility of suitable habitats in the context of the conservation of the Plahar, in this case it gives the best possible point for the effort to plant cultivated seeds.

ACKNOWLEDGMENTS

This work was undertaken with full support of funding from the Global Tree Campaign (GTC) programme and Fauna & Flora International. We would also thank to Balai Konservasi Sumber Daya Alam (BKSDA) of Central Java province, for the research permit and field support especially for Tri Satyatama, Dedy Rusyanto, and Teguh Arifiyanto. Tim Relawan Konservasi Selok (Rekonsel), for the field support and friendship during the survey. David Gill (GTC Project Manager). Kristio Budiasmoro from Environmental Studies Center of Sanata Dharma University.

REFERENCES

Ashton, P. 1998. *Dipterocarpus littoralis* (Vol. 2307–8235).

Ashton, P. S. 1982. Dipterocarpaceae. *Flora Malesiana I*, *9*, 237–552.

Brewer, C. a., & Pickle, L. 2002. Evaluation of Methods for Classifying Epidemiological Data on Choropleth Maps in Series. *Annals of the Association of American Geographers*, *92*(4), 662–681. http://doi.org/10.1111/1467-8306.00310

Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E., & Yates, C. J. 2011. A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, *17*(1), 43–57. http://doi.org/10.1111/j.1472-4642.2010.00725.x

Farr, G, T., Rosen, P. a, Caro, E., Crippen, R., Duren, R., … Burbank, D. 2007. The Shuttle Radar Topography Mission. *American Geophysical Union*, 1–43. http://doi.org/10.1029/2005RG000183

Gill, M. (Vice-C. G. B. / P. K. C., Jongman, R. (JongmanEcology /Wageningen U. R., Luque, S., More, B., Paganini, M., & Szantoi, Z. 2017. *A Sourcebook of Methods and Procedures for Monitoring Essential Biodiversity Variables in Tropical Forests With Remote Sensing*. Global Observation of Forest Cover and Land Dynamics (GOFC-GOLD).

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978. http://doi.org/10.1002/joc.1276

Huete, A., Didan, K., Leeuwen, W. Van, Miura, T., & Glenn, E. 2011. *Land Remote Sensing and Global Environmental Change* (Vol. 11). http://doi.org/10.1007/978-1-4419-6749-7

Jenks, F., & Caspall, C. 1971. Error on Choroplethic Maps: Definition, Measurement, Reduction. *Annals of the Association of American Geographers*, *61*(2), 217–244.

Kumar, S., & Stohlgren, T. J. 2009. MaxEnt modeling for predicting suitable habitat for threatened and endangered tree Canacomyrica monticola in New Caledonia, *1*(4), 94–98.

Lesage, J., Banerjee, S., Fischer, M. M., & Congdon, P. 2009. Spatial statistics : Methods , models & computation. *Computational Statistics and Data Analysis*, *53*(8), 2781–2785. http://doi.org/10.1016/j.csda.2008.11.001

Lewis, D., Phinn, S., & Arroyo, L. 2013. Cost-Effectiveness of Seven Approaches to Map Vegetation Communities — A Case Study from Northern Australia's Tropical Savannas. *Remote Sensing*, *5*(January 2013), 377–414. http://doi.org/10.3390/rs5010377

Li, B., Ti, C., Zhao, Y., & Yan, X. 2016. Estimating soil moisture with Landsat data and its application in extracting the spatial distribution of winter flooded paddies. *Remote Sensing*, *8*(1). http://doi.org/10.3390/rs8010038

Li, P., Jiang, L., & Feng, Z. 2013. Cross-Comparison of Vegetation Indices Derived from Landsat-7 Enhanced Thematic Mapper Plus (ETM+) and Landsat-8 Operational Land Imager (OLI) Sensors. *Remote Sensing*, *6*(1), 310–329. http://doi.org/10.3390/rs6010310

Liu, C., White, M., & Newell, G. 2013. Selecting thresholds for the prediction of species occurrence with presence-only data. *Journal of Biogeography*, *40*(4), 778–789. http://doi.org/10.1111/jbi.12058

Mars, J. C., & Rowan, L. C. 2010. Spectral assessment of new ASTER SWIR surface reflectance data products for spectroscopic mapping of rocks and minerals. *Remote Sensing of Environment*, *114*(9), 2011–2025. http://doi.org/10.1016/j.rse.2010.04.008

Merow, C., Smith, M. J., & Silander, J. A. 2013. A practical guide to MaxEnt for modeling species' distributions: What it does, and why inputs and settings matter. *Ecography*, *36*(10), 1058–1069. http://doi.org/10.1111/j.1600-0587.2013.07872.x

Norris, D. 2014. Model thresholds are more important than presence location type : Understanding the distribution of lowland tapir (Tapirus terrestris) in a continuous Atlantic forest of southeast Brazil Predicting the geographic distributions of species is a growing fi. *Tropical Conservation Science*, *7*(3), 529–547.

Oyana, T. J., & Margai, F. M. 2016. *Spatial Analysis, Statistics, Visualization, and Computational Methods*. Boca Raton: Taylor & Francis Group.

Phillips, S. 2008. A Brief Tutorial on MaxEnt. *AT&T Research*, 1–38. http://doi.org/10.4016/33172.01

Phillips, S. J., Anderson, R. P., & Schapire, R. E. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3–4), 231–259.

Price, J. C. 1992. Estimating vegetation amount from visible and near infrared reflectances. *Remote Sensing of Environment*, *41*(1), 29–34. http://doi.org/10.1016/0034-4257(92)90058-R

Robiansyah, I., & Davy, A. J. 2015. Population Status and Habitat Preferences of Critically Endangered Dipterocarpus littoralis in West Nusakambangan , Indonesia. *Makara Journal of Science*, *19*(4), 150–160. http://doi.org/10.7454/mss.v19i4.5169

SNI. 2010. *Klasifikasi Penutup Lahan SNI 7645*.

van Proosdij, A. S. J., Sosef, M. S. M., Wieringa, J. J., & Raes, N. 2015. Minimum required number of specimen records to develop accurate species distribution models. *Ecography*, (MAY), n/a-n/a. http://doi.org/10.1111/ecog.01509

Whitten, T., Soeriaatmadja, R. E., & Afif, S. A. 1996. *The Ecology of Java and Bali*. (S. Johannesen, Ed.) (Vol. 2). Singapore: Eric Oey.

Wilting, A., Cord, A., Hearn, A. J., Hesse, D., & Mohamed, A. 2010. Modelling the Species Distribution of Flat-Headed Cats (Prionailurus planiceps), an Endangered South-East Asian Small Felid, *5*(3). http://doi.org/10.1371/journal.pone.0009612

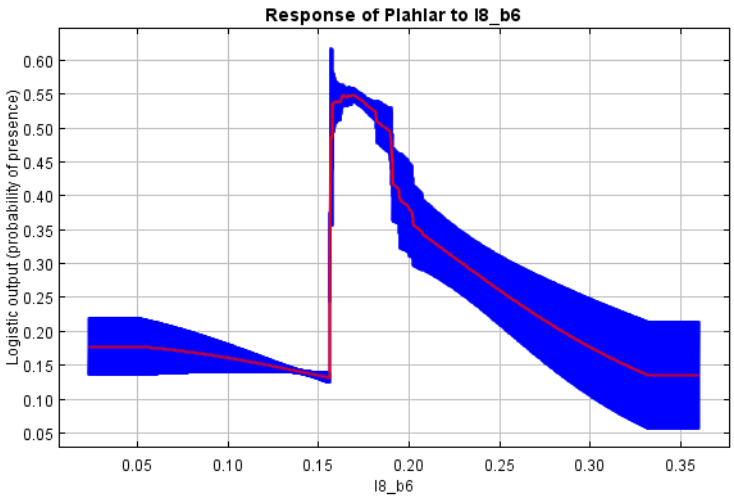
Young, N., Carter, L., & Evangelista, P. 2011. A MaxEnt Model v3.3.3e Tutorial (ArcGIS v10), 1–30.

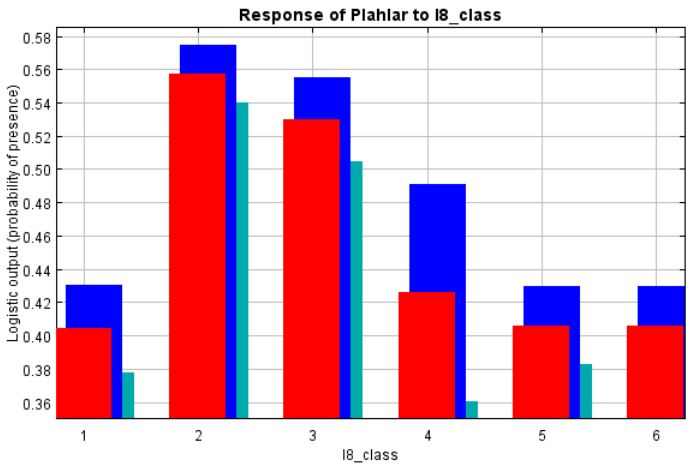
Appendices 1. Spectral and geophysical variable used in this model

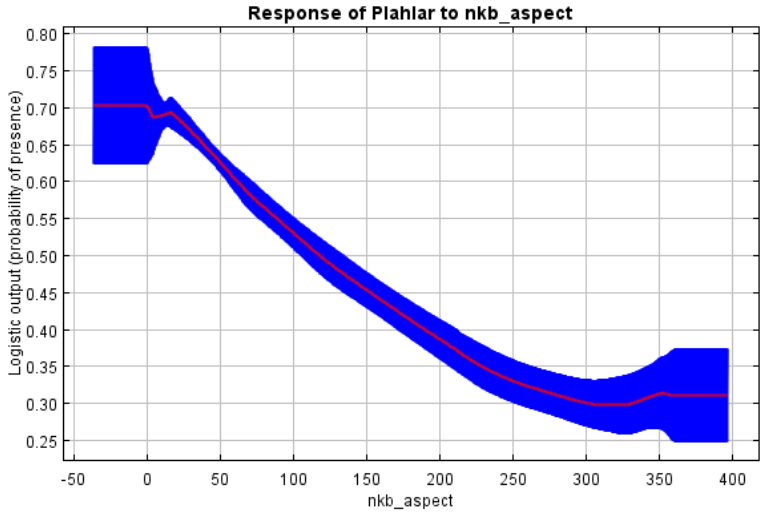
Variable	Data Source	Data Type	Native Resolution	Variable Description
Top of Atmosphere Reflectance Band 4 (l8_b4)	Landsat-8 Satellite Imagery	Continuous	30 m	Discriminates vegetation slopes.
Top of Atmosphere Reflectance Band 5 (l8_b5)	Landsat-8 Satellite Imagery	Continuous	30 m	Emphasizes biomass content and shorelines.
Top of Atmosphere Reflectance Band 6 (l8_b6)	Landsat-8 Satellite Imagery	Continuous	30 m	Discriminates moisture content of soil and vegetation.
Top of Atmosphere Reflectance Normalized Difference Vegetation Index (NDVI)	Landsat-8 Satellite Imagery	Continuous	30 m	Vegetation indices based on remotely-sensed spectral reflectance in the near-infrared and visible bands, have been widely used for monitoring vegetation cover and health condition, plant phenology, and ecosystem changes.
Landcover Classification (l8_Class)	Landsat-8 Satellite Imagery	Categorical	30 m	The land cover classes' definitions following the internationally accepted definitions (UNFCCC, FAO, IPCC) and are tailored to comply with the Indonesia National Standard for Land Cover Classification, SNI 7645:2010.
River Distance (nkb_riv_dist)	ASTER GDEM; RBI	Continuous	30 m	Relative distance from detected rivers. Generated from digital elevation model data.

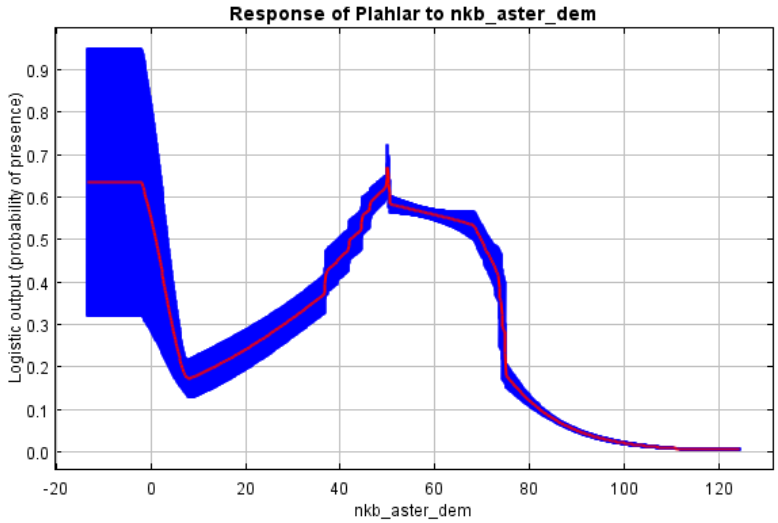
Variable	Data Source	Data Type	Native Resolution	Variable Description
Coast Distance (nkb_coast_dist)	RBI, FFI Survey 2015	Continuous	30 m	Relative distance from detected coast linel. Generated from satellite imagery data, RBI Maps and gps tracking data.
Digital Elevation Model (nkb_aster_dem)	Aster GDEM	Continuous	30 m	Elevation data generated from Advanced Spaceborne Thermal Emission and Reflection Radiometer.
Slope (nkb_aster_slope)	Aster GDEM	Continuous	30 m	Relative slope of the land generated from Aster GDEM data.
Aspect (nkb_aspect)	Aster GDEM	Continuous	30 m	Identifies the downslope direction of the maximum rate of change in value from each cell to its neighbors. It can be thought of as the slope direction. Generated from Aster GDEM data.

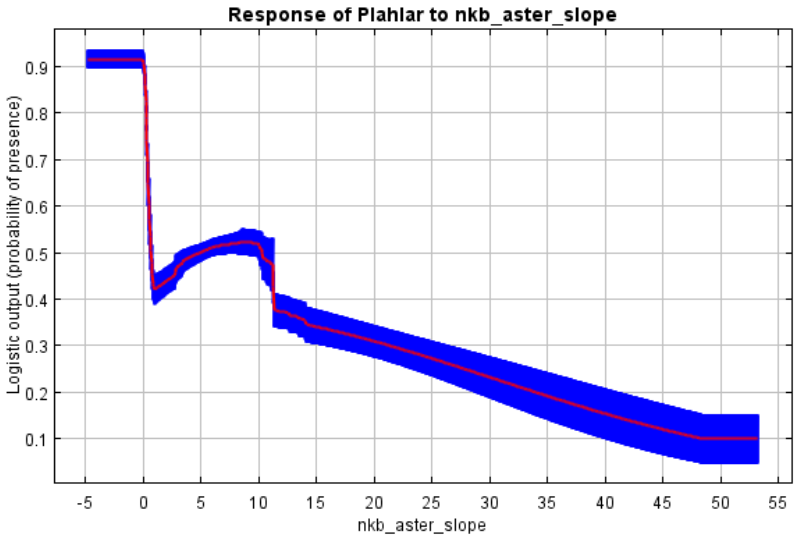
Appendices 2. Response curve each environmental variable

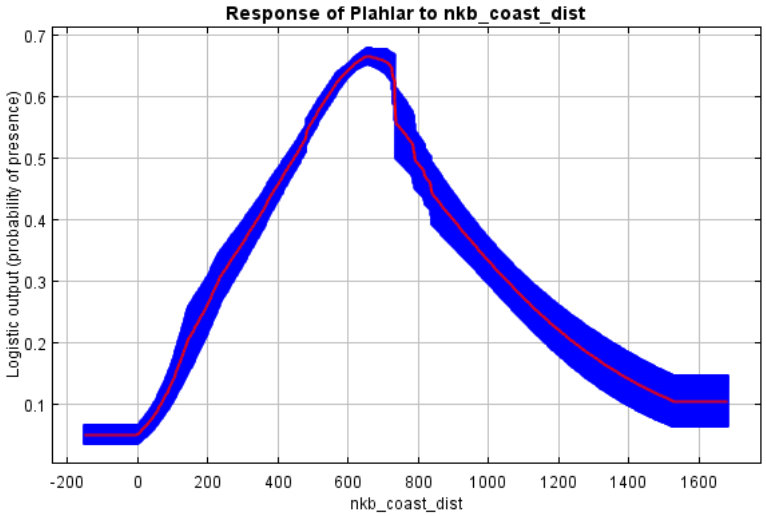


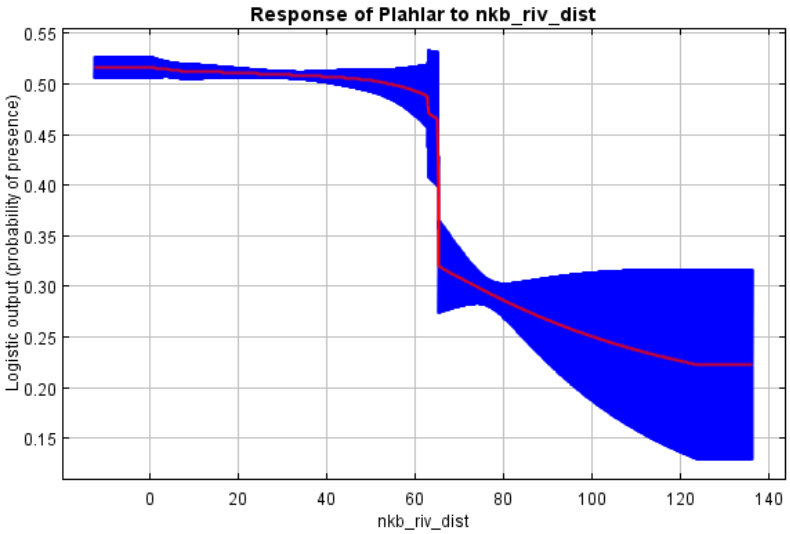



Response of Plahlar to nkb_aspect
Logistic output (probability of presence)
nkb_aspect


Response of Plahlar to nkb_aster_dem
Logistic output (probability of presence)
nkb_aster_dem


Response of Plahlar to nkb_aster_slope
Logistic output (probability of presence)
nkb_aster_slope


Response of Plahlar to nkb_coast_dist
Logistic output (probability of presence)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
-200
0
200
400
600
800
1000
1200
1400
1600
nkb_coast_dist


Response of Plahlar to nkb_riv_dist
Logistic output (probability of presence)
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0
20
40
60
80
100
120
140
nkb_riv_dist

An Analysis of the Characteristics of Peatlands in Sriwijaya Botanical Gardens in South Sumatera

Sri Maryani[1], Bastoni[2], M. Agust Nurwahyudi[3],
[1,3]Board of Regional Research and Development, South Sumatera Province
[2]Forestry Research Center Palembang, Indonesia
Jalan Demang Lebar Daun No. 4864 Palembang, Indonesia Ph./Fax +62711374456/+62711350077
Email: smaryani2014@gmail.com

ABSTRACT

South Sumatra province has 1.4 million ha peat area or 16.3% of the total area. It is one of the potential natural resources to be managed and utilized for the benefit and welfare of the entire community. One form of the conservation and utilization of peat area in the Province of South Sumatra is the development of Botanical Garden. Botanical Garden is part of the wealth of the nation that has many functions; in addition for the knowledge center of botany, conservation, education, and research area, it can serve as outdoor recreation facilities. The experiment was conducted from February 2015 to April 2016 in the site of Sriwijaya Botanical Gardens in South Sumatera. Peat soil characteristics in Sriwijaya Botanical Gardens were analyzed by data distribution and variations in elevation, thickness of the peat and groundwater, physical and chemical properties of groundwater, and also vegetation observations. Based on the elevation, Sriwijaya Botanical Gardens are included in lowland category, while the type of peat is very deep, with relatively shallow water table. This research data can be implemented in all developmental activities in Sriwijaya Botanical Gardens area without ignoring the principles of land conservation.

Keywords: conservation, land characteristics, peat, Sriwijaya Botanical Gardens.

INTRODUCTION

Peatlands are one of the ecosystems formed in anaerobic conditions (with poor drainage) in the tidal marsh or swampy area high with organic matter (> 50%) as a result of the accumulation of crop residues. Peatlands provide some ecological services and economic and social potential for development as a life-support system (Galbraith et al., 2005; Egoh et al. 2007). Peat soil is composed of soil organic matter, either with a thickness of more than 45 cm or layered with mineral soil at a thickness of 80 cm and has a thick layer of organic material of more than 50 cm. Peat soil or organic soil is also known as Organosol or Histosol (Agus F. & Subiksa IGM, 2008). Peatland area in Indonesia is estimated to be 11 million ha, spread across the islands of Sumatra, Kalimantan, and Irian Jaya (Papua), covering nearly 1/3 of swamp lands in Indonesia. South Sumatra province has 1.4 million ha peatlands or 16.3% of the total area, and the condition is one of the natural resource potentials to be managed and utilized for the benefit and welfare of the whole society (Forestry Service, 2015) (Figure 1).

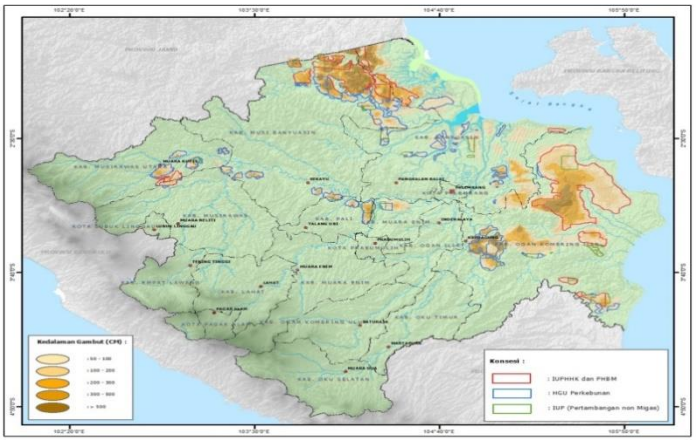


Figure 1. Map of Distribution of Peatlands in South Sumatra (Forestry Service of South Sumatera Province, 2015)

One form of the conservation and utilization of peatlands in the province of South Sumatra is the development of Sriwijaya Botanical Gardens. The Botanical Gardens is part of the wealth of the nation that has many functions, which in addition to serving as a knowledge center for botany, conservation, education and research area, it functions as outdoor recreation facilities, especially for species of medicinal plants in Indonesia (Figure 2).

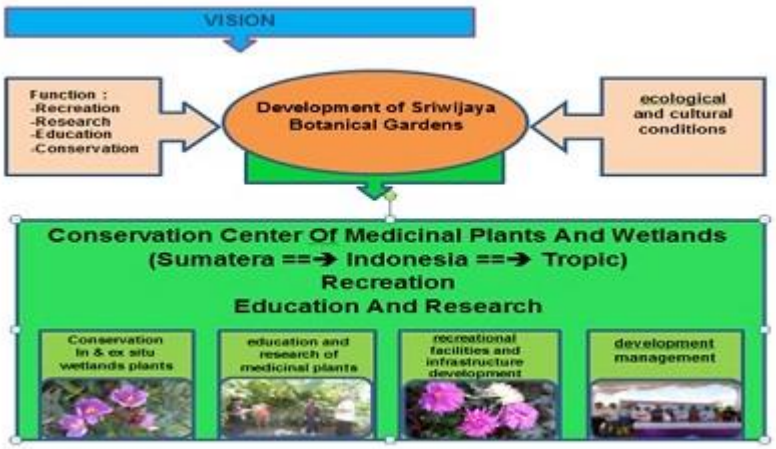


Figure 2. Chart of Vision and Development of Sriwijaya Botanical Gardens

Basically, the main area of the Botanical Gardens is designed for the collection of plants, thus requiring the physical building in the botanical garden to be planned carefully in order to add to the beauty of the gardens (in addition to its functionality) and not to interfere with the presence of plant collections or thematic zones.

Thematic zones are plant collections grouped by their utility or special properties and laid out as a park. The fact that the state of the soil, climate, vegetation composition, and plant diversity of a region different from that of other regions means that botanical gardens in Indonesia also have different characteristics and natural potentials, each with its own charm. Sriwijaya Botanical Gardens has a strategic function to collect and conserve diverse species of medicinal plants and wetland plants. The land area allocated to Sriwijaya Botanical Gardens is about 100 hectares with land typology dominated by peat swamp land.

Some characteristics of wetlands that determine the success of planting include the thickness of the peat, peat maturity, depth, duration of the puddle on land that has not been drained, and seasonal

fluctuations in groundwater levels on land that has been drained. The data is expected to be available on an operational basis for planting in wetlands (Bastoni, 2009). The data on layer depth, pyrite (FeS_2), chemical properties of soil and water is needed if the management of the Botanical Gardens is to introduce exotic plant species (not the typical plants of local wetlands).

The existing Botanical Gardens is located on peatlands that have been planted with oil palms. The transformation of peatlands into palm oil plantations on peat swamp forest is the dominant factor causing the degradation of peatlands (Riwandi, 2003). Land clearing activities ignoring the biophysical characteristics of the environment will degrade peatlands and turn them into wastelands (Noorginayuwati et al., 1997 and Sutikno et al. 1998 as cited in Noor, 2001).

Palm oil plantations on peatlands are expected to apply the principles of ecological areas based on the optimization and preservation of resources; otherwise, the resulting changes will make all the difference and lead to loss of ecological functions in the peatlands. Land clearing and canalization lead to changes in hydrology. These changes trigger changes in the level of soil fertility, land subsidence, and dryness (Las et al., 2008). Some characteristics of wetlands will affect cultivation methods and types of plants that will be planted in Sriwijaya Botanical Gardens, Ogan Ilir.

Land productivity can mean the ability of a land to support sustainable use. This means if the land is for example used for agriculture, it will be able to produce the crop according to the input given. Meanwhile, crop productivity is influenced by genetic factors and environmental factors. So, the level of crop production is strongly influenced by the presence of a suitable combination of genetic and environmental plants (phenotype). Crops that are genetically productive will not be able to produce optimally when they are not supported by suitable growing environment. Construction of botanical gardens on areas with wetland typology will require the type of selection, adaptation and modification of cultivation techniques different from conventional practices on dry land (Bastoni, 2009). This is what underlies the need for research on the characteristics of peatlands in Sriwijaya Botanical Gardens which is supported by data on distribution and variations in elevation, thickness of the peat and groundwater levels, physical and chemical properties of groundwater, and also observation of the vegetation.

METHODOLOGY

Time and place

The research was conducted from February to April 2015 and the analysis continued from January – April 2016 at the construction site of Sriwijaya Botanical Gardens in the Bakung Village Sub-District, North Inderalaya, Ogan Ilir Regency, in South Sumatera Province.

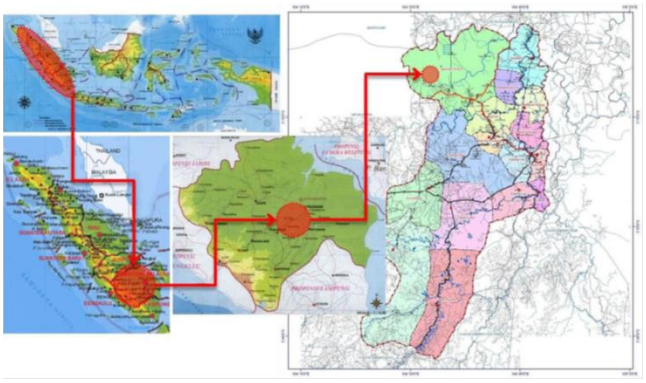


Figure 3. Location of Sriwijaya Botanical Garden

Material and Equipment

The equipment used in this research included GPS, peat soil drills, pH meter, location maps, digital camera, plastic for samples, rubber, paper label, permanent markers, plastic bags, plastic bottles,

cloth for cleaning drills, stationery, and tools for analysis in the laboratory.

Research Stages

The research was carried out in stages. First was orientation. At this stage, secondary data was collected, including data on the conditions of the location, climate, and base map; followed by designing survey maps; plotting the observation point; taking care of administration and licensing; and preparing equipment, materials, and supplies for survey.

Data analysis

Research on peat soil characteristics in Sriwijaya Botanical Gardens focused on analyzing the following data:

- Distribution of and variations in elevation, thickness of peat, and groundwater levels.
  Field observations at 20 sample points at research site.
- Physical and chemical properties of groundwater
  Collecting soil samples in 20 observation points. Then the samples were composited into 7 groups based on the types of soil layer, namely the charcoal layer with a depth of 0-4 cm, sapric (3.4-24 cm), hemic-fibric I (<50 cm), hemic-fibric II (50-100 cm), hemic-fibric III (100-150 cm), peat-mineral transition, and mineral soil, and they were subsequently analyzed in the laboratory.
- Observation of vegetation
  Field observations at 20 sample points at research site.
- Effects of peat characteristics in Sriwijaya Botanical Gardens
  Comparing the results of previous studies with several real events at the location of Sriwijaya Botanical Gardens.
  Sample points were determined for 20 points per 100 hectares (Arsyad, 2010). The area of Sriwijaya Botanical Garden is 1,090 meters long x 865 meters wide.

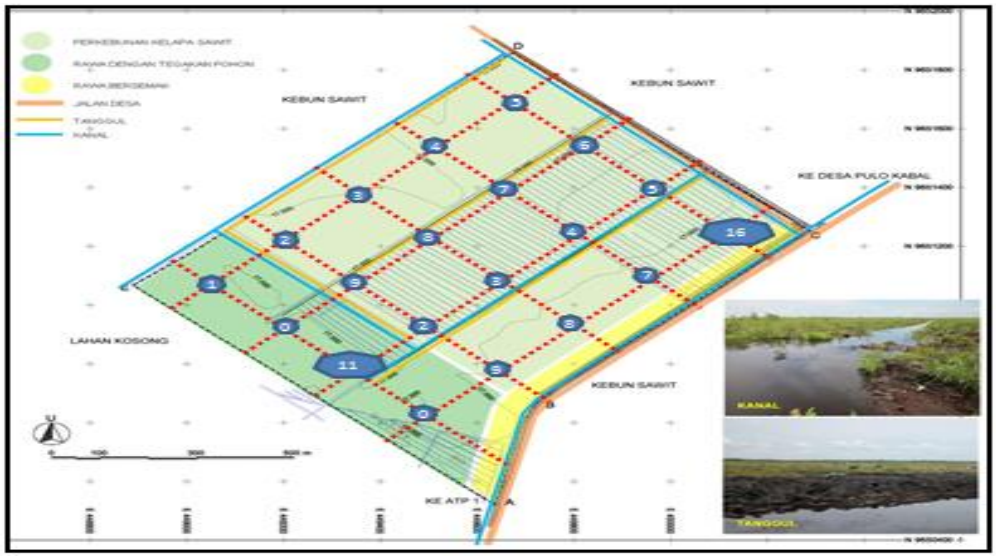


Figure 4. Sampling Location

RESULTS AND DISCUSSION

1. Elevation characteristics of the research location

The location in North Inderalaya, Ogan Ilir, on which Sriwijaya Botanical Gardens will be built is a peatland, which has been converted into oil palm plantations, with the growing age of 4 years. Elevation measurements at the 20 observation points found observation points with higher elevations, namely

numbers 5, 10, and 19 (21 m above sea level), while the lowest elevation was found at the observation point numbers 8 (16 m above sea level) and 13 (15 m above sea level).

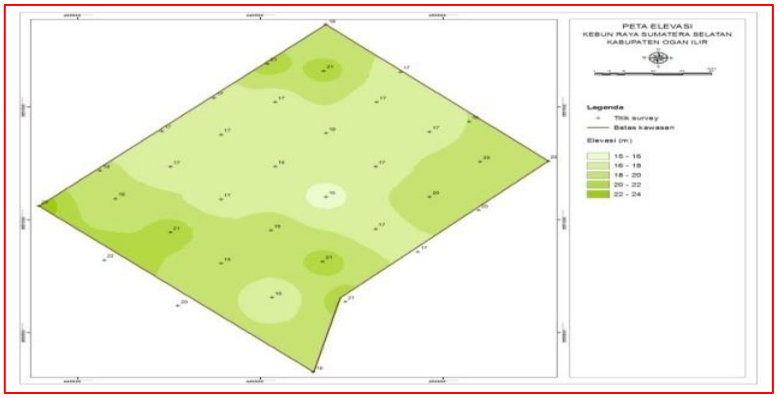
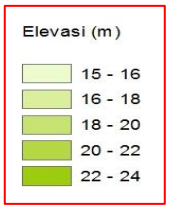


Figure 5. Elevation characteristics in the research location



The elevations of research site ranged from 15-23 m above sea level, with an average elevation of 18 m above sea level, and the mode is 17 m.

2. Characteristics of peat thickness in the research location

Peat was especially dominant in the west part of the Botanical Gardens (Figure 5).

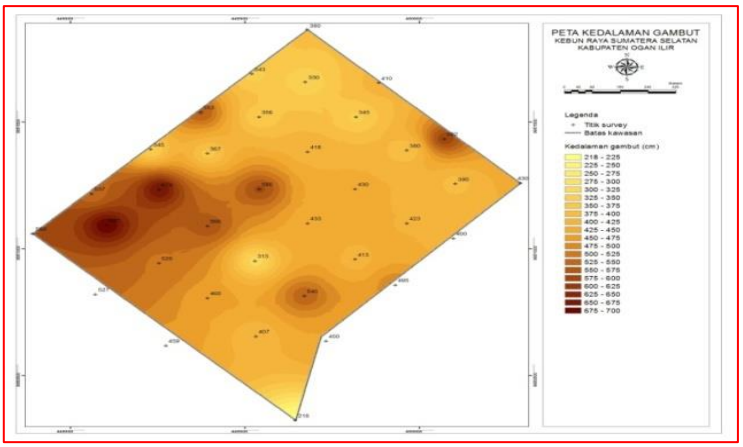


Figure 6. Characteristics of peat thickness in research locations

The range of peat thickness in the research site was 218-697 cm, with an average thickness of 452 cm, so the typology is included in the deep-peat class (figure 7):

Figure 7: Peat Profile in research locations

Figure 7 shows some examples of peat profiles found in the research area. The peat or organosol land often refers to a pile of organic matter derived from the remains of plants that are decaying and have been going on in a long term with the condition of always getting flooded. The organic materials are decomposed and carried by aerobic bacteria and influenced by the original vegetation, climate, topography, and chemical properties of water.

Image (a) is a sapric peat material, in which mostly the organic materials have undergone decomposition. This layer begins with a layer of charcoal + peat ± 5 cm. This soil has a relatively high density and low water holding capacity. It is generally gray, grayish green or bluish-containing sulfidic material/pyrite (FeS_2). Figure (b) is a fibric peat layer, which is newly decomposed, with the characteristics of tissue (fiber) plants still being evident (easily recognized). Meanwhile, Figure (c) is the coating type that begins with mineral soil. Possible origins of mineral soil are soil embankment for planting media with a depth of ± 24 cm, followed by fibric and hemic peat layers.

Figure (d) is a layer that is dominated by hemic peat followed by a layer of sapric peat, while Figure (e) is a layer of mineral soil which begins with a layer of ex-fire charcoal, a transition layer between charcoal and peat, and then a layer of mineral soil interspersed with peat layers. Most of the environment is waterlogged or watery peat soil, very shallow soil with halophilic vegetation, such Gelam (Melaleuca spp.).

3. Characteristics of groundwater in the research location

The front elevations of groundwater in the research locations ranged from 4-30 cm, with an average height 15 cm, and are included in deep and shallow groundwater. On the measurement of Advance Air Land (MAT) in the rainy season (February-April 2015), the maximum height MAT was - 19 cm; the water in the trenches around the research location was stagnant. (Figure 8)

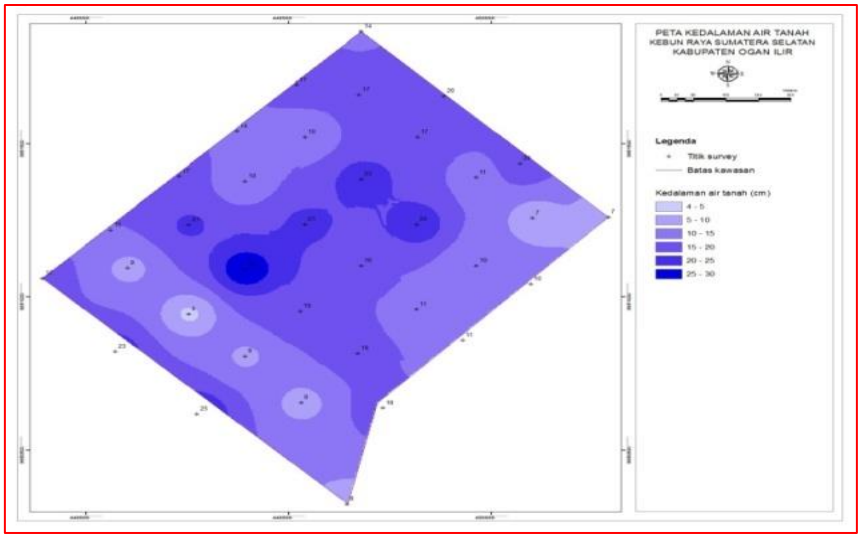


Figure 8. Characteristics of the Front Elevation of Groundwater at the Research Location

4. Physical-Chemical Characteristics of Groundwater

The characteristics of the peat water were: pH levels were low (3-4) or highly acidic, high organic content, high levels of iron and manganese, and yellow or brownish (concentrated). Brownish red color is the natural color of peat water containing colloidal particles. Positively charged organic matter cannot be deposited by gravity, so the forces should be added in order to deposit the particles. It is largely thought to be the result of humification process (*humic acid and fulvic acid*), in addition to minerals (Fe and Mn). Both compounds are heterogeneous in molecular weight, carboxyl content, total acidity, and solubility in acid-base. PH levels of groundwater samples in the location under research ranged from 4.77 to 5.75, so the water was acidic; TSS content of > 1000 mg/L, Fe 7.21 to 11.01 mg/L, Mn content ranging from 0.11 to 0.18 mg/L, BOD5 ranging from 52-163 mg/L, COD 1165-530 mg/L, H2S of 0.01 mg/L, and PO4 2.85 to 2.89 mg/L. These parameters exceeded the quality standards set by South Sumatra Governor's Regulation No. 16 year 2005.

5. Characteristics of Vegetation: Types of Trees (Tree Species) predominantly found in the areas under research were: Gelam (*Melaleuca leucadendron*), swamp Pulai (*Alstonia peneumatophora*), land Pulai (*Alstonia scholaris*), and Perepat (*Combretocarpus rotundatus*). As for the types of understorey plants or species found: fern shrimp (*Stenochlaena palustris*), ground fern (*Nephrolepis exaltata*), Belidang (*Fimbristylus annua*), Seduduk (*Melastoma malabathricum*), Resam (*ferns*), and Rayutan (*liana woody).*

Effects of peat characteristics at Sriwijaya Botanical Gardens in South Sumatera on its development

Different land cover types will have different absorption rates of carbon gas; for instance, for land cover of trees the absorption rate of carbon gas is 129.92 kg/ha/h, but land cover of bush and shrubs will absorb carbon gas for about 12.56 kg/ha/h (Prasetyo et al., 2002 in Adiastari, 2010). Sriwijaya Botanical Gardens with mostly bush and shrub land covers has less carbon absorption rate; thus, the planting in that area must be specifically based on plant canopies. Data on elevation, peat thickness, groundwater, and also physical-chemical characteristics of groundwater, can be used in all development activities of Sriwijaya Botanical Gardens. For instance, in the area with higher elevation and very deep peat, it is suggested not to erect a building, but the infrastructure can be replaced by planting endemic plants such as Gelam (*Melaleuca leucadendron*), swamp Pulai (*Alstonia peneumatophora*), land Pulai (*Alstonia scholaris*), and Perepat (*Combretocarpus rotundatus*). Besides, we can make a preparation to face land fire, especially in dry season, in the area with deep peat and lower groundwater by making artesian well. Currently, Sriwijaya Botanical Gardens has 2 artesian wells with 100 meter depth, and 10 artesian wells with 50 meter depth which are located separately. If the peat experiences excessive

dryness, it will be damaged because of the colloidal particles. Particles of the outer layer of peat are rich in resin that inhibits the re-absorption of water after drying and finally the peat can no longer absorb nutrients and hold water. As a result, the peat will experience drought and become flammable. Figure (9) shows us the land fire in October 2015 in Sriwijaya Botanical Gardens. Likewise, the condition of high level groundwater in Sriwijaya Botanical Gardens also affects all development activities there (figure 10).

Figure 9. Land fire in Sriwijaya Botanical Gardens in October 2015.

Figure 10. Condition of high level groundwater in Sriwijaya Botanical Gardens in March 2016

CONCLUSION

Based on the parameter of elevation, the lands in Sriwijaya Botanical Gardens in Ogan Ilir Regency, are included in the category of lowlands, while the peat is classified as very deep, with the lowest type of groundwater level. Plant cultivation activity in peatlands should implement water management technology, tailored to the characteristics of the peat and groundwater levels; for example, by making drainage channels to maintain a stable water level. Besides, in the construction of the infrastructure at the site of Sriwijaya Botanical Gardens, the related parties should take into account the characteristics of porous peat, so that the principles of conservation are maintained.

REFERENCES

Agus F., and Subiksa IGM 2008, Peatland: Potential for Agricultural and Environmental Aspects, Research Land and the World Agroforestry Center (ICRAF), Bogor.

Balai soil Research Institute, Department of Agriculture, 2003, Evaluation of Land for Agricultural Commodities, Puslitbangtanak, Bogor.

Balitbangnovda South Sumatra Province, in 2013, the Environmental Management efforts (UKL) and Environmental Monitoring (UPL) Botanical Garden Development Selatan.

Barus Sumatra FY 2004, Abiotic Environmental factors and Biodiversity Plankton As Water Quality Indicators of Lake Toba, Journal of Human and Environment, Volume XI 2, 64-72.

Budianta Ristiani D. and D. , 2013, the Soil Fertility Management, UNSRI Press, Palembang.

Fahrunsyah, 2012, Study of Chemical Characteristics of Soil and Soil Fertility Status In Region Food Crop Production Center Tana Tidung, Ziraa'ah Journal, Volume 33 No. 1, p. 1-9.

Ferad Puturuhu 2010, Micro Effect Groundwater Relief And Conditions Of Morphology Land On Land Sagu Tawiri Desa Teluk Ambon Ambon, Journal of Agriculture, vol. 6. No 2, p 78-83.

Hadi A., 2007, Principles of Sampling Environmental Management, PT. Gramedia Pustaka Utama, Jakarta.

Hardjowigeno, S. 2010. Soil Science.Prints All 7. Academica Pressindo. Jakarta.

Indriani D.P., Marisa H., and Zakaria, 2009, the Plant Species Diversity In Mangrove Region Nipah (Nypa fruticans Wurmb) in District Pulau Rimau Kab. Banyuasin South Sumatra, Science Research Journal, Vol. 12 no. 3 (D), p. 1-4.

Indriyanto 2010, Forest Ecology, Earth Literacy Publisher, Mould-3, Bandar Lampung.

Mardiyanti DE, Abrams KR, and Baskara M., 2013, the Plant Species Diversity Dynamics of Post-Planting Rice, Journal of Crop Production Volume I no , 1, p.

A New Hybrid from Interspecific Hybridization *Begonia masoniana* Irmsch. Ex Ziesenhx *Begonia kui* C.-I Peng

Hartutiningsih-M.Siregar & Sri Wahyuni
Centre for Plant Conservation - Botanic Gardens, Indonesian Institute of Sciences
Jl. Ir. H. Juanda No. 13, Bogor 16003, West Java, Indonesia.Ph./Fax.: +62-251-8322187
*email: hartutiningsih@yahoo.co.id

ABSTRACT

Leaf colour of Begonias can be inherited through artificial hybridization between two different species. This study aims to generate a new variety and also to increase its genetic diversity. The study was conducted at Bogor Botanic Gardens. An artificial cross between *B. masoniana* as the female parent and *B. kui* as male parent was carried out, generating the F1 (*B.* 'Masokui'). The resulting F1 is characterized by several morphological characters of a new variety. Out of the 37 characters observed, nine different characters were found in the new variety. The difference in *B.* 'Masokui' was observed in its leaf properties, in which the surface of the upper leaves is spotted with large specks (pustulate). The upper surface leaves have three shades of colour, the leaves have a petiole and an inflorescence peduncle and the inherited pubescent tepals show characters dominantly derived from the female parent, so that morphologically the new variety is similar to the female parent. The inherited properties that can be observed on the leaves are dominated by phenotype as if they are only inherited from one of its parents, namely the female parent. Distribution of colours on the leaves is a combination of both parents, while the properties of male and female flowers are inherited from the male parent. After completing the stages of F1 crossing, PPVTPP registration process commenced. The result is a new variety named *B.* 'Masokui' registered by No. 512/PVHP/2017. Vegetative propagation was done through leaf cuttings to multiply the superior clones. This new variety is a beautiful, leafy begonia with an exotic look that has economic values. It is expected that in the future the flower can be cultivated and propagated by breeders, ornamental plant community, or the people in general.

Keywords: *B. masoniana, B.kui, B.* 'Masokui', interspecific hybridization

INTRODUCTION

Indonesia has many species of wild Begonias that can be developed into new ornamental plants. This is part of the justification of research aimed at preserving the potential of Indonesian plant diversity. In the past, Begonias did not receive much attention from the floriculture industry. However, Begoniaceae is a family of flowering plants with significant diversity within Indonesia and has become more popular in recent years because of its spectacular ornamental characteristics. Thus, there has been an increasing interest in developing the potential of the genus for ornamental purposes. This has necessitated new initiatives in biodiversity exploration, sustainable collection activity, and careful ex-*situ* conservation in order to conserve and develop species of ornamental Begonias (Kiew, 2005).

Begoniaceae is separated into two ornamental types: flowering Begonias and beautiful leafy Begonias. The uniqueness and beauty of its leaves (i.e. their shape and colour), the compactness of the plant and its vigour and its resistance to diseases are basic characters that have to be given priority when developing a new variety of ornamental leaf Begonias.

The leaf surface of begonias is of various types: flat surface, for example *B.decora*, wrinkled or rugose (*B. rajah*), bullate (*B. acida*), with bumps resembling warts (*B. paulensis*), inflated (muricate) surface (*B.*'Pebble Lane') and with evenly small spots like acne (*B. imperialis*). The hairiness can be classified into downy leaf surface (*B. acetosa*), shaggy-like surface (*B. masoniana*) and long-haired (*B. sizemorae*). In terms of leaf motif, some are plain, patches, striped, or striated with a similar motif, as in group *B.rex*, or spotted like *B. maculate*. Leaf colours: brightly coloured Begonias such as green, silver; or a combination of several colours such as pink, red, purple, grey, black, or other mixed colours. Leaf texture: the leaves are thick and have a lot of succulents that can save water and carbohydrates such as the leaves group of *B.rex* (Krempin, 1993; Kiew, 2005).

Meanwhile, hybridization is a classical method to obtain new plants with desirable properties and new genetic combination resulting from the crossing of two different genotyping individuals (variety

or species).This method is frequently applied on cultivated plants to transfer genes for resistance to pest and diseases or tolerance to drought in wheat, tomato, sugarcane and other cultivated varieties; for example, chili. It also used to increase the genetic diversity of plants (Syukur *et al*., 2012).

There are six varieties of Begonias at Bogor Botanic Gardens generated from artificial hybridization, namely *B.*'Lovely Jo' (*B. puspitae x B. pasamanensis*); *B.*'TutiSiregar' (*B. listada x B. acetosa*); *B.*'Blirik', *B.* 'Fiandani', *B.*'GreenPeltate' and *B.*'Natunapangean' (*B. natunaensis x B. puspitae*) (Salisbury, 2008; Hartutiningsih, 2016). Meanwhile, the other two varieties from the crossing between *B. longifolia* x *B.* 'Silver Queen'were obtained from EkaKarya Bali Botanic Gardens (*B.*'Longisilver' and *B.* 'Longigreen').

The future hybridization programs will keep focusing on the interspecific hybridization among the native wild species as well as the exotic begonias that have been previously collected. The results of the research on plant breeding will be given legal protection on intellectual property for plant varieties by getting included in a list of new breeding varietiesby the Centre for Plant Variety Protection and Agriculture Licensing (PPVTPP). The Centre is the government agency tasked to protect plant varieties by issuing copyright protection of plant varieties (Hindarwati, 2006).

MATERIAL AND METHODS

The study was conducted at the green house nursery of Bogor Botanic Gardens. The nursery is located at an elevation of 250 m asl, with a daily temperature range of 28°C–33°C and humidity between 60% and 90%. The genetic materials used were the collections of Bogor Botanic Gardens: *B. Masoniana* ex Ziesenh as the pollen recipient (female parent) and *B. kui* C.-I Peng as the male parent and F1 plant *B.* 'Masokui' (*B. masoniana*× *B. kui*). Breeding was done by crossing the two species with genetic disassortative mating (Syukur *et al*., 2012).

The artificial crossing between *B. masoniana* × *B. kui* was carried out on February 7, 2013. Five artificial crosses were made and only one cross was successful. The fruit was harvested on March 25, 2013. The seeds were sown on March 28, 2013 and germinated on April 10, 2013. After 5 months, one of the eight surviving seedlings was selected and transplanted into individual pots with a mix of medium husk charcoal and compost (2:1). Once adapted, it was propagated vegetatively by leaf cutting. The resulting F1 plants were characterized based on the Guidelines published by the Ministry of Agriculture of the Republic of Indonesia, Centre for Plant Variety Protection (2014) (Hindarwati, 2006; Maff 2010 & 2011).

RESULTS AND DISCUSSION

The female parent used, *B. masoniana*, is a species which is well adapted to various altitudes (Krempin, 1993; Kiew, 2005). It is characterized by rhizomatous habits. The leaves are attractive and over grown with rough hairs, forming a popular shield with iron cross Begonias. The leaves' upper surface has a texture of large, mottled leaves (*pustulate*), while the bottom surface of the leaves is sparsely hairy with coarse green colour, petiole length of 12–20 cm, an inflorescence, asymmetrical branching, peduncle length of 12−20 cm, and a single type flower (Table 1). This species has been cultivated for a long time. Its beautiful leaves are very potential to be made a parent for breeding program. Some successfully cultivated cultivars are *B.* 'Goshe' (*B. dregei* and *B. masoniana*), *B.* 'Orphan Annie' (*B. goegoensis* and *B. masoniana*) and *B.* 'Wanda' (*B. versicolor* and *B. masoniana*) (Tebbit, 2005). The iron cross Begonias are native of southern China and northern Vietnam (Kiew *et al*., 2015).

The male parent is *B. kui*, with mottled upper leaves' texture, short petioles, an inflorescence, symmetrical branching, and a short peduncle (4−5 cm). *B. kui* is a Vietnam endemic species, as described by Peng *et al.* (2007). *B.* 'Masokui' is a primary hybrid crossed between the *B. masoniana* (collection plant) as a female parent and *B. kui* as a male parent. The fruit produced was harvested and the seed was sowed directly with seedling germination. The mass selection of F1 was based on plant vigour. The F1 was selected from the best plant that has new, unique characters, unlike the two parents, namely a new variety named *B.* 'Masokui'. The name is derived from the combined names of both parents *B. Masoniana* (maso) with the male elder *B. kui* (kui). The plant first flowered in 2014 (Fig. 1).

This new hybrid is a new variety of beautiful, leafy Begonias with an exotic look. The morphology of the plant includes strong and robust varieties suitable as beautiful ornamental potted plants (Fig. 2).

The resulting F1 hybrids are characterized based on the Guidebook issued by the Ministry of Agriculture of the Republic of Indonesia, Centre for Plant Variety Protection, (2014), (Hindarwati, 2006; Maff 2010 & 2011), as follows: spotted upper surface of the leaves (pustulate); three shades of colours on the upper surface of the leaves; the primary colour of the upper surface of the leaves is green (GG 189 A), the secondary colour green (GG 188 A), the tertiary colour green (GPN 187A); the secondary colour is distributed on the middle; the upper surface is green (GG 138 B) and the surface densely hairy. The lower surface of the leaves is green (YG 146 B), and the secondary colour is green (GP 183 C). The petioles are 21−30 cm long, reddish green (GR 178 B) and hairy. The inflorescence has symmetrical branching, peduncle (25–28 cm long), and green colour (GO 176 B, GG 195 A).The flower is unisexual; male flowers 2 (2), red (R 56 D), female flowers 3 red (R 56 B), and adaxially hairy.

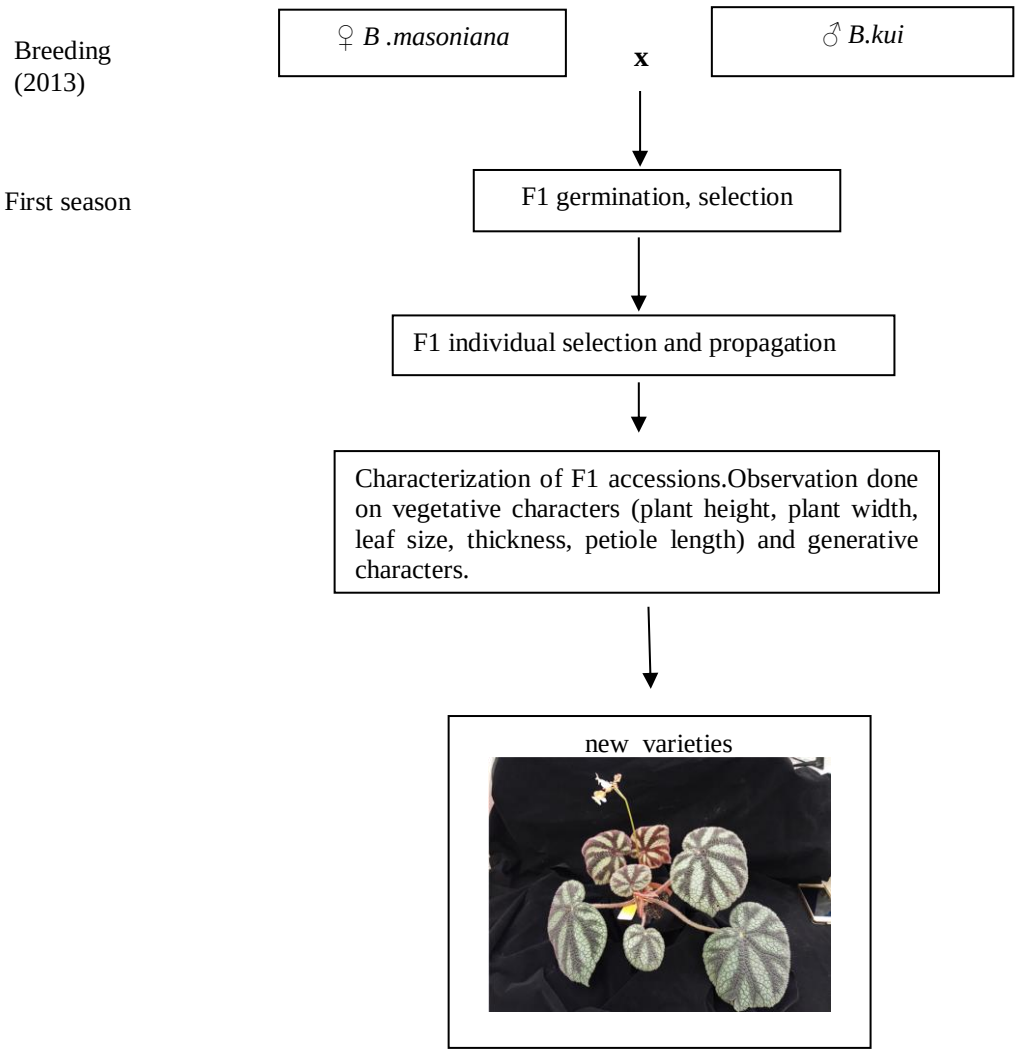


Figure 1. New *Begonia* hybrid varieties and the breeding scheme that produced them

The results show that the qualitative characters are fixed, similar for the two stem cells, leaf type, leaf shape, leaf tip, leaf margin, leaf base, stipule, petiole, and flower type (Table 1). The following is the characterization differences between the male, female, and F1 derivatives as displayed in Table 1.

Table 1. Morphological characters of ♀ *B. masoniana*, ♂ *B. kui*and*B.* 'Masokui'

No	Morphological Characters	♀ *B. masoniana*	♂ *B. kui*	F *B.* 'Masokui'
1.	Plant: stem	Rhizomatous	Rhizomatous	Rhizomatous
2.	Plant: height (cm)	Short (12–20)	Short (10–12)	Short (30–35)
3.	Plant: width (cm)	Medium (35–40)	Medium (20–22)	Medium (40–50)
4.	Leaf blade: type	Single	Single	Single
5.	Leaf blade: Leaf blade size (cm)	Medium (13–17x10–15)	Medium (6–9.5x7–10)	Medium(10–13x15–17)
6.	Leaf: thickness (cm)	Thin (0.3–0.4)	Thin (0.3–0.4)	Thin (0.3–0.4)
7.	Varieties with single leaf only: Position of petiolar attachment	Basifixed	Basifixed	Basifixed
8.	Leaf blade: shape	Orbicular	Orbicular	Orbicular
9.	Leaf blade concavity	Great pustulate	Small pustulate	Great pustulate
10.	Leaf blade: base	Cordate	Cordate	Cordate
11.	Leaf blade: apex	Acuminate	Acuminate	Acuminate
12.	Leaf blade: lobation	Absent	Absent	Absent
13.	Leaf blade: margin	Serrate	Serrate	Serrate
14.	Stipule	Triangular	Triangular	Triangular
15.	Leaf: number of colours on upper side	3	2	3
16.	Leaf blade: main colour of upper side surface	Green (GG N137 A)	Green (GG 139 A)	Green (GG 189A)
17.	Leaf blade: secondary colour on upper side	Green (GG 138 C)	Green (GG 138 C)	Green (GG 188A)
18.	Leaf blade: tertiary colour on upper side	Green (GP 187A)	Green (GG N 186 A)	Green (GPN 187 A)
19.	Leaf blade: distribution of secondary colour on upper side	Middle	whole	whole
20.	Leaf: Variegation on upper side	Present	Present	Present
21.	Leaf blade: width of coloration along veins on upper side	Wide	Wide	Wide
22.	Leaf blade: distribution of coloration along veins on upper side	Entire	Irregular	Entire
23.	Leaf blade: colour of veins on upper side	Yellow (YG 147 C)	Yellow (YG 147 A)	Green (GG 138 B)
24.	Leaf blade: intensity of hair on upper side	Thick coarse	Thick smooth	Thick smooth
25.	Leaf blade: main colour of abaxial surface	Green (GG 139 C)	Green (GG 197 C)	Yellow (YG 146 B)
26.	Leaf blade: secondary colour of abaxial surface	Green (GP 187C)	Green (GP 187 C)	Green (GP 183C)
27.	Petiole: length (cm)	12–20	4–5	21–30
28.	Petiole: colour	Green (GR 178 A)	Green (GO 177 A)	Green (GR 178 B)
29.	Petiole: hair	Thick	Thick	Thick

No	Morphological Characters	♀ B. masoniana	♂ B. kui	F B. 'Masokui'
30.	Inflorescence: pedicel/peduncle length (cm)	(12–20)	(4–5)	(25–28)
31.	Inflorescence: pedicel/peduncle colour	Top Green (GG 181 A) Down Green (GR 181 C)	Top Green (GO 177 C) Down Green (GG 195 A)	Top Green (GO 176 B) Down Green (GG 195 A)
32.	Flower: type	Single	Single	Single
33.	Male flower: colour	Green (GG 195 A)	Red (R 55 B)	Red (R 56 D)
34.	Male flower: number of tepal	2(2)	2(2)	2(2)
35.	Female flower: colour	3	3	3
36.	Female flower: number of tepal	Green (GG 195 B)	Red (R 56 A)	Red (R 56 B)
37.	Female flower: hair of tepal	Present	Absent	Present

Out of the 37 characters observed, there are nine different characters as presented in Table 2. The different leaf properties in *B.* 'Masokui' include the surface of the upper leaves that is spotted with large specks (pustulate); 3 (three) colour shades on the upper surface of the leaves; a petiole and inflorescence peduncle; the appearance of the inherited flower tepals hair of this character dominantly derived from the female parent, so that the morphological characters are similar to the female parent. The inherited properties that can be observed on the leaves are dominated by phenotype as if they are only inherited from one of its parents, namely the female parent. Distribution of colours on the leaves is a combination of both parents, while the properties of male and female flowers are inherited from the male parent.

Figure 2. 1. Morphological features of *Begonia* 'Masokui',
2. Upper leaf surface,
3. Abaxial leaf surface,
4. Inflorescence,
5. Male flowers and female flowers.

In the US Department of Agriculture, hybridization experiments were conducted on Begonias species belonging to the *B. semperflorens* group, *Begonia*, which flowered continuously. This group is the most widely cultivated. Chen *et al.* (2012) reported a hybrid between *B. semperflorens* species and *B.* 'Orange Rubra' with in vitro cultures. This hybrid shows different levels of mixing properties between the two parents according to the genome composition and some have the desired character of both parents.

The next stage after F1 crossing was completed was to meet the requirements to start the PPVTPP registration process. The result is a new variety named *B.* 'Masokui' registered by No 512/PVHP/2017. The vegetative propagation was done through leaf cuttings to multiply these superior clones. This new variety is a beautiful, leafy Begonia with an exotic look that has economic value. In the future, it is expected that the flower can be cultivated and propagated by its breeder, ornamental plant community or the public. All the stages of hybridization have been completed and the hybrid meets the DUS test (Distinct, Uniform and Stable) in accordance with the Guidelines for the Conduct of Test for Distinctness, Uniformity and Stability, which continued with the committee examination and certification. The final output is the certificate of plant variety protection from the Ministry of Agriculture.

CONCLUSION

The hybridization between *B. masoniana x B. kui* species resulted in new a variety that has more interesting, unique and better morphological leaves than its parent, namely *B.* 'Masokui', is registered by No. 512 / PVHP / 2017. The new variety has been registered in the PPVTPP office of the Ministry of Agriculture in accordance with the prevailing laws and regulations.

ACKNOWLEGEMENTS

Our gratitude goes to the Director of the Centre for Plant Conservation Botanic Gardens, LIPI, who through the In-House Research Program 2013–2016 provided funding for the research. We would like to express our gratitude to Wisnu H. Ardi for his excellent horticultural skill and also to all colleagues in the nursery of Building IX Bogor Botanic Gardens who were very helpful during the research.

REFERENCES

Chen, Y-Ming and M. Mii. 2012. Interspecific hibridaization of *Begonia semperflorens* (section*Begonia*) with *B. pearcei* (section *Eupetalum*) for introducing yellow flower color. *Plant Biotechnology* 29: 77–85.

Hartutiningsih-M.Siregar. 2016. Four new variety of *Begonia* from interspecific hybridization *Begonia natunaensis* C.W. Lin x *Begonia puspitae* Ardi. *Biodiversitas* 17 (2): 776–782.

Hindarwati. 2006. Guidebook, Ministry of Agriculture of the Republic Indonesia, Center for Plant Variety Protection.General guidelines for the conduct of novelity, distictness, uniformity and stability.PVT/PP/1/1.http://www.scribd.com/doc/54017888/Panduan-Umum-BUSS. (accesssed 10 Sept 2016).

Kiew, R. 2005. *Begonias of Peninsular Malaysia*. Natural History Publications (Borneo). Sdn. Bhd.A 913, Wisma Merdeka. Kota Kinabalu, Sabah, Malaysia.

Kiew, R., J. Sang, R. Repin and J.A. Ahmad. 2015. *A guide to Begonias of Borneo*. Natural History Publications (Borneo). Kota Kinabalu. 293 p.

Krempin, J. 1993. *Know your Begonias*. Krempin Books 25 Beverley Crescent Broadbeach Waters, Queensland 4218 Australia. 119 p.

Maff. 2010. Rhizomatous *Begonia* (*Begonia* L.): Guidelines for the conduct of test for distinctness, uniformity and stability. Japan. http://www.hinsyu.maff.go.jp/info/sinsakijun/kijun/1097.pdf .(accesssed 10 Sept 2016).

Maff. 2011. *Begonia* L: Guidelines for the conduct of test for Distinctness, Uniformity and Stability. Japan

Ministry of Agriculture of the Republic Indonesia, Center for Plant Variety Protection. 2007. Decree of the Minister of Agriculture and Regulation of the Minister of Agriculture.148 p.

Ministry of Agriculture of the Republic Indonesia, Center for Plant Variety Protection (2014). Guidelines for The Conduct of Test for Distinctness, Uniformity and Stability. Begonia. (not publish).

Peng, C.I, T.Y. Hsieh and Q.H. Ngyuen. 2007. Begonia kui (Sect. Coelocentrum, Begoniaceae), A new species from Vietnam. *Botanical Studies* 48: 127–132.

Syukur, M., S. Sujiprihati S and R. Yuniaanti. 2012. *Plant breeding technique*. Jakarta. Penebar Swadaya.

Salisbury, G. 2008. New cultivar Begonia "Tuti Siregar" (♀ *Begonia listada* Smith & Wasshausen x ♂ *Begonia acetosa* Vellozo), Official International. Registration number 1001. *The Begonian* 2008. *The American Begonia Society*: 212–213.

Tebbitt, M.C. 2005. *Begonias, Cultivation, Identification, and Natural History*, 273 p.

Plant Exploration in North Sumatra To Enrich the Plant Collections of Samosir Botanic Garden

Hendra Helmanto*, D.M. Puspitaningtyas, Mujahidin, Saripudin, & Slamet
Center for Plant Conservation Botanic Garden, Indonesian Institute of Science
Jl. Ir. H. Djuanda No.13 Bogor, Indonesia
*Email: hendra31.lipi@gmail.com

ABSTRACT

Establishing an ex-situ conservation area is an alternative action for decreasing risk of extinction and protecting plant biodiversity in Indonesia. Samosir Botanic Garden (SBG) is proposed to be made North Sumatra's native plant conservation center. The aim of this study is to collect living plant materials from North Sumatra to enrich plant collections in SBG. Exploration activities were conducted at three locations: PT. Toba Pulp Lestari protected forest, Toba-Samosir protected forest, and Partukoan protected forest from April 11-30, 2016. The exploration was done based on the plants to be collected by purposive random sampling to record the plant diversity in this area. Results of this study recorded 180 plant collections, consisting of 64 families, 118 genera, 180 species, and 700 specimens, including *Casuarina sumatrana* and *Nepenthes tobaica* as North Sumatran native plants. Twenty two of those collected plants are recorded as new collections for Samosir Botanic Garden.

Keywords: botanic garden, exploration, ex-situ conservation, North Sumatra, Samosir.

INTRODUCTION

Indonesia is a biodiversity hotspot country, which has great but also big risk for biodiversity (Mittermeier *et al*., 1998). Indonesia is also the largest archipelagic country in the world with ± 17,508 islands. More than 10,000 of them are small islands, which have high diversity of plants. Plants in island ecosystems are more sensitive to threats because of small population sizes and ranges as well as unique characteristics resulting from prolonged evolutionary isolation (Frankham, 1998). Utilization of biodiversity for human welfare has been known traditionally and historically through the modern technology application, but there are many untapped forest potentials to be developed as a source of phytopharmaca or modern medicine (Ohlstein *et al*., 2000). Developing ex-situ conservation areas is one of the efforts to reduce degradation and protect biodiversity. One kind of ex-situ conservation areas in Indonesia is botanic garden.

Samosir administrative area covers all of Samosir Island surrounded by Lake Toba and a little part of the area in Sumatra mainland. Lake Toba stretches for ± 120 km and with a width of ± 45 km. Lake Toba is a large and deep caldera, which occurred due to volcanic eruptions around 75,000-100,000 years ago (Hartini & Puspitaningtyas, 2005). In general, the soil around Lake Toba is fertile volcanic soil coming from volcanic eruptions. Samosir is located at 900-1900 m above sea level, with high rainfall in the upper area and slightly reduced rainfall on the edges of Lake Toba. The existing natural forests around this area are characterized by highland rainy tropical forests dominated by *Pinus* spp. The topography is generally flat/sloping and slightly undulating, with altitude locations explored between 1,580 and1,858 m above sea level.

Samosir Botanic Garden (SBG) is part of Indonesia's Botanic Garden which can potentially be made as a conservation center for North Sumatra's plants. SBG located in Palilit, Tomok Village, Simanindo District, Samosir (2037'54 "LU and 98052'07" BT). The aim of developing SBG is for research and conservation center of North Sumatra's highland endemic plants (Junaedi *et al*., 2013). The future of the plants is unpredictable, so it is necessary to take preventive measures against extinction. The high growth population, deforestation, land use changes, and ecosystem degradation cases in Indonesia threaten the sustainability of Indonesian plants. Biodiversity is directly disrupted, which can lead to extinction of certain species (Subiandono & Heriyanto, 2009; Groom, 2016; Hartemik et. al., 2008). More recent concerns about relationship between climate change, deforestation (van der Werf *et al*., 2008) and ecosystem services loss have brought a new focus on forest conservation issues

(Laumonier *et al*., 2010). Therefore, we need conservation action and strategy to prevent our biodiversity from extinction in the future.

This exploration aims to collect plant materials from North Sumatra to be included in the plant collection in SBG as one kind of ex-situ conservation actions.

MATERIAL AND METHODS

The exploration was conducted in 2016 at several locations in North Sumatera. The first location is IUPHHK Protected Area of PT. Toba Pulp Lestari Tbk., which includes Samosir and Humbang Hasundutan administrative areas. The coordinates of this location are N 02°31'45,1"-E 098°37'55,5" and N 02°22'47,8"-E 098°37'18,4". The second location is Toba-Samosir protected forest. This place consists of two points i.e. Mt. Simanuk-manuk (N 02 ° 26'31,0 "- E 098 ° 38'28.6" and N 02 ° 33'29.2 "- E 099 ° 05'29.2") and Permaksian, Pintupohan Meranti Forest (N 02° 33'32,4 " - E 099° 05'39,5" and N 02° 33'32,4 " - E 099° 05'39,5 "). The third location is Samosir Protected Forest located in the coordinates of N 02 ° 37'05,7 " - E 098 ° 51'09,1" and N 02 ° 33'32,4 " - E 099 ° 05'39,5". The locations of exploration are shown in Figure 1.

Figure 1. Location map of explorations

The methods used in this exploration include collecting and researching, employing exploratory method with representative samples to collect plant information within a region. The exploratory method was carried out by tracing the forests through existing paths or by creating new paths. The locations were determined based on the preliminary literature review and field study. The chosen locations were forest areas around Lake Toba with the aim of obtaining plant species which have natural distribution around Lake Toba. The collected plants are expected to have high survival rate because the climate and conditions are not too different.

The plants were subsequently identified in detail, recorded and collected. The priority in collecting the plants was determined by whether the plants are rare, endemic, distinctive, unique, economic potential, ornamental or beneficial species for science. The materials were collected by seeds, cuttings, seedling, or herbarium specimens. The species were named based on their morphological characteristics. Unknown species would be made herbarium collections for further identification (Hartini & Sahromi, 2016). The plants found were identified by type and recorded for their specific morphological characteristics. The environment data recorded included: habitat, altitude, location coordinate, soil pH, water temperature, and humidity.

RESULTS AND DISCUSSION

As a whole this exploration resulted in 180 collection plants. The collected plants consist of 64 families, 118 genera, 180 species, and 700 specimens. The collected plants would be cultivated in Samosir Botanic Garden (SBG). More details are presented in Table 1.

Table 1. Exploration areas ranked based on total number of collections

Location	Specimen number	Collection total number	Family	Genus	Identified collection at the level of		
					Family	Genus	Species
Protected forest KPHL Toba-Samosir	382	86	46	63	3	47	36
Protected area IUPHHK PT. Toba Pulp Lestari	252	75	40	62	3	56	16
Partukoan Protected forest Kab. Samosir	66	19	15	19	0	11	8
Total	700	180	64	118	6	114	60

Note: Protected forest KPHL Toba-Samosir had the highest number of plant collections

Table 1 shows the number of plant collections found in each location. The location from which most of the plants were collected was KPHL Toba-Samosir Protected Forest Area with 86 collections, belonging to 46 families and 63 genera. In the protected area of IUPHHK PT. Toba Pulp Lestari, 75 plants were obtained, belonging to 40 families and 62 genera. In Samosir Protected Forest Area, 19 collections of plants were obtained, belonging to 15 families and 19 genera.

The three locations of exploration were the forests around Lake Toba, North Sumatra. The criteria for selecting the locations included highland forest which has an average height of 1,000 m above sea level. Samosir Botanic Garden is located at an altitude of 900-1,000 m above sea level. It was expected that the plant collections would not have too much difficulty in adapting to new environments because of the almost similar height and location ranges. Detail information and conditions of each location can be seen in Table 2. Environmental conditions of the three locations are almost similar, so there are similarities in forest stands. The temperature is quite cold (20-25°C), with quite high humidity, normal soil pH (6-7), and high soil moisture.

Lake Toba has fairly cold climate. According to Nasution & Damanik (2009), wet season there can occur for 3-9 months, while dry season (rainfall > 100 mm/month) only for 2-3 months/year. Based on climate classification according to Schmidt and Fergusson, Lake Toba area is included in the climate types A, B, and C. The average temperature in the areas around Lake Toba is around 20-23 °C. According to ITB Research Institute (2001), the mean temperature from 1992-1996 at Balige Station (Tobasa) and in Sidamanik (Simalungun District) was 19-21 °C. The increasing temperature was likely to occur due to global warming and reduced amount of forest in this area.

Table 2. Descriptions of each location around Lake Toba

Location	Altitude	Environmental description	Vegetation
Toba-Samosir protected forest	(Mt. Simanuk-manuk forest) 1,115 - 1,237 m above sea level.	Bumpy, hilly with various slopes; flowing river; approximate temperature 22° C; humidity 82%; soil pH 6.2; soil moisture 70%. Soil type consists of sandy rocks; large and small rocky soil; brown, clay and slippery soil when it rains.	Vegetation types are very diverse and not only dominated by 1-2 species. Vegetation consists of *Luvunga* sp. *Syzygium* sp. and *Arthocarpus* sp. Site is dominated by shrubs such as Begoniaceae, Araceae, *Homalanthus* sp, *Piper* sp, and *Hibiscus* sp.
	Permaksian and Pintupohan Meranti forest 1.110 - 1.140 m above sea level	Hilly protected forest area at a gradient ± 50%, temperature 22° C, air moisture 72%, soil pH 6.4, soil moisture 45%. Soil type consists of sandy rocks; large and small rocky soil; brown, clay, and slippery soil when it rains.	Species found are *Casuarina sumatrana* (native species of Sumatra) and *Nepenthes tobaica.* (native of the surrounding areas of Lake Toba)
PT Toba Pulp Lestari protected forest area	1.580 - 1.858 m above sea level	Topography is generally flat and slightly bumpy, temperature 21°C; humidity 87%; Soil pH 6.2; soil moisture 90%. Protected forest is located along the river. The types of soil consist of clay soil, sandy rocks, large and small rocky soil, reddish brown and tough.	Dominated by *Eucalyptus* sp. Prunes are quite dominated by ferns such as *Selaginela* sp., *Pterospermum* sp. and Begoniaceae. Common shrubs are *Medinilla* sp. and *Schefflera* sp. In some areas, rare and interesting plants are still found, such as *Rafflesia meijeri* Wiriad. & Sari.
Partukoan protected forest, Kab. Samosir	989 - 1,631 m above sea level.	General topography is bumpy, hilly with various slopes from steep to sloping. Exploration ranged from highland forest (23° C, 66%) (5.5, 78%), calcareous soil (30° C, 60%) (5.6, 50%), and natural forest stands (25° C; 65%) (6.5; 50%). Soil types in this area consist of clay; clay soil; sandy rock; large and small rocky soil; reddish brown, clay and slippery soil while it rains.	The dominant tree species are *Macadamia* sp., and *Pinus* sp. Some locations are quite dominated by shrubs, including *Rhododendron* sp., *Rodhomyrtus* sp. and ferns.

The soil types are almost similar in three locations, namely large sandy rocks, large and small rocky soil, and brown soil. The soil types of the ecosystems in the east area are litosol and regosol. This complex soil type is very sensitive to erosion. The southeast area has brown podsolic soil (sensitive to erosion) and a complex type of mountainous soil. In the western part of the ecosystem the soils are brown podsolic, while Samosir Island mostly consists of Toba brown forest (Balai I Rehabilitation and Soil Conservation Area I Medan 1987 in Nasution & Damanik 2009). Vegetation is almost similar, but there are only several species found in one location. This condition has caused the high altitude areas to have a limited amount of diversity.

Out of the sixty four plant families resulted from the exploration, the families of *Anacardiaceae, Annonaceae, Apocynaceae, Juglandaceae, Lauraceae etc*. *Lauraceae* had the highest number of total species with 16 species (Figure 2). The species belonging to Lauraceae that were discovered included *Actinodaphne* sp., *Cryptocarya* sp., *Cryptocarya crassinervia* Miq., *Cinnamomum iners* Renw. Ex Blume., *Cinnamomum porrectum* (Robx.) Kosterm, *Cinnamomum* sp., *Dehaasia* sp., *Litsea firma*

(Blume) Hook.f., *Litsea* sp., *Lindera* sp., *Persea* sp., *Beilschmiedia* sp., *Nothaphoebe umbelliflora* Blume, *Endiandra* sp., *Nectandra angustifolia* Nees, and Martelli ex Nees.

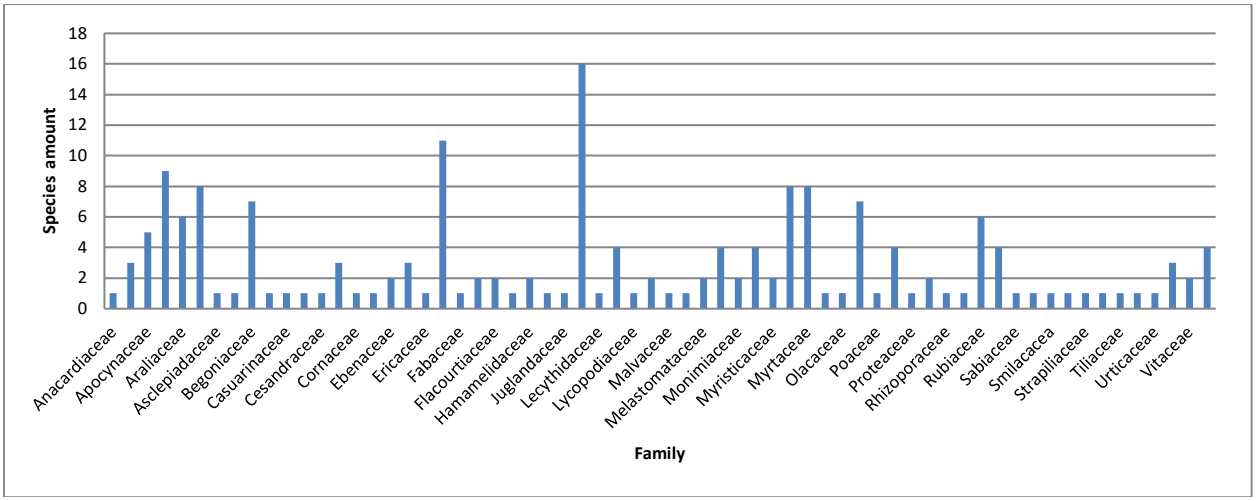


Figure 2. Total number of species classified by family (*Lauraceae* had the highest number of species collected by 16 species and *Euphorbiaceae* by 11 species).

Some species found are endemic, unique, and useful plants. The endemic species found include *Casuarina sumatrana* which is endemic to Sumatra and *Nephentes tobaica*, which is only spread around Lake Toba. Some unique begoniaceae plants have a potential use as ornamental plants. *Begonia longifolia* cf. Blume. plants were found in Mt. Simanuk-manuk forest around the river with an altitude of 1130 masl. This species is spread in Himalayas (India), China. South, Vietnam, Thailand, Malaysia, Sumatra, Java, Bali, West Nusa Tenggara (Girmansyah, 2008). This plant also has a potential use as ornamental plant, food, and medicine (Girmansyah, 2008). *Begonia robusta* Blume was found in the same location with an altitude of 1133 masl and near the river. This species is naturally distributed in Java and Sumatra. Tebbitt Begonia Scotii was found at an altitude of 1133 meters above sea level. The plant has white and yellow flowers with two beautiful petals and has a potential use as ornamental plants.

Another plant found was *Dacrycarpus imbricatus* (Blume) de Laubenf. (Podocarpaceae). This plant has a local name *sampinur bunga*. Its habitat is in the primary forest which is disrupted at an altitude of 500-1,500 masl. Distribution of this plant includes southern China, Indo-china, Burma, Thailand, Malaysia, Vanuata and Fiji. Its wood is useful for household utensils, building poles, ships, carvings, crates, and crafts. Another interesting plant is *Rhododendron jasminiflorum* Hook. (Ericaceae). Its flower crowns have a variety of colors (white, red, pink), scaly, and short haired on the outside. The plant lives in open and rocky areas, often found near volcanic vents and epiphytes which grow on tall trees in sub-montane forests. It grows at an altitude of 750-1,500 m above sea level. Its natural distribution includes Aceh and North Sumatra (Hartini & Puspitaningtyas 2005).

Table 3. List of new plant collections of Samosir Botanic Garden

No	Species	Family	Hab.
1.	*Cryptocarya crassinervia* Miq.	*Lauraceae*	T
2.	*Fagraea racemosa* Jack ex Wall.	*Loganiaceae*	T
3.	*Schefflera scandens* Dryand.	*Araliaceae*	WCl.
4.	*Rhodamnia cinnerea* Jack.	*Myrtaceae*	T
5.	*Kadsura scandens* (Blume) Blume.	*Cesandraceae*	Cl.

No	*Species*	*Family*	Hab.
6.	*Elmerrillia ovalis* Dandy	*Magnoliaceae*	T
7.	*Vitex cofassus* Reinw. Ex Blume	*Verbenaceae*	T
8.	*Luvunga sarmentosa* (Blume) Kurz	*Rutaceae*	WCl
9.	*Homalanthus populneus* (Giesel.) Pax.	*Euphorbiaceae*	S
10.	*Elaeocarpus angustifolius* Blume	*Elaeocarpaceae*	T
11.	*Alstonia scholaris* (L.) R.Br.	*Apocynaceae*	T
12.	*Eunymous javanicus* Blume	*Celastraceae*	T
13.	*Bischofia javanica* Blume	*Euphorbiaceae*	T
14.	*Prunus javanica* (Teijsm. & Binn.) Miq.	*Rosaceae*	Cl.
15.	*Angiopteris evecta* (G. Forst.) Hoffm.	*Marattiaceae*	Fn.
16.	*Maesa perlarius* (Lour.) Merr.	*Myrsinaceae*	S
17.	*Tacca chantrieri* Andre	*Taccaceae*	Ph.
18.	*Stemonurus scorpioides* Becc.	*Icacinaceea*	T
19.	*Ziziphus angustifolius* (Miq.) Hatus ex Steen.	*Ramnaceae*	S
20.	*Cinnamomum iners* Renw. Ex Blume.	*Lauraceae*	T
21.	*Kibara coriacea* (Blume). Tul	*Monimiaceae*	T.
22.	*Nothaphoebe umbelliflora* Blume	*Lauraceae*	S

Samosir Botanic Garden (SBG) is a new botanic garden in Indonesia. SBG is still in development and has a small number of plant collections. Therefore, more plants from North Sumatera are needed to add the collections in the garden. Table 3 shows the list of new plant collections of Samosir Botanic Garden. There are 22 new species categories in Samosir botanic garden, in which 3 of them are *Lauraceae*.

CONCLUSION

The results of this study recorded 180 plants collected in all exploration areas, consisting of 64 families, 118 genera, 180 species, and 700 specimens. The plant collections include *Casuarina sumatrana* and *Nepenthes tobaica* as North Sumatra native plants. Twenty two of those collected plants are recorded as new collections for Samosir Botanic Garden.

ACKNOWLEDGEMENTS

This study was a part of botanic exploration to North Sumatra carried out in April 2016 and funded by Center for Plant Conservation Botanic Garden – Indonesian Institute of Sciences. We would like to convey our gratitude to Saripudin, Slamet, Han Prasetya Adhi and Apri Wijaya from Bogor Botanic Garden. Our gratitude also goes to Sotar Siadari, Emerzon Siadari, Lokner Hutagaul, Leranzo Sinaga, Rommel Situmorang and Eva Hutagalung from Samosir Botanic Garden, North Sumatra.

REFERENCES

Frankham, R. 1998. Inbreeding and extinction: island populations. Conserv. Biol. **12**: 665-675, doi.org/10.1111/j.1523-1739.1998.96456.x.

Girmansyah, D. 2008. A taxonomix study of Bali and Lombok Begonia (Geboniaceae). Reinwardtia. **Vol. 12**, pp 419-434.

Groom, M.J. 2016. *Threat to Biodiversity in Principle of Conservation Biology*: 3rded. SinauerAssociates Inc. Sunderland. M.A.

Hartemik, A.E., T. Veldkamp, & Z. Bai. 2008. Land cover change and soil fertility decline in tropical region. Turkish Journal of Agricultural and Forest. **32(3)**: 195:213.

Hartini, S. & D.M. Puspitaningtyas. 2005. *Flora Sumatera Utara: Eksotik dan Berpotensi*. Pusat Konservasi Tumbuhan Kebun Raya Bogor-LIPI. Bogor.

Hartini, S. Sahromi. 2016. Samosir Botanic Garden: Study on flora resource and its potential. Masyarakat Biodiversitas Indonesia Proceeding. **Vol 2:2** page: 243-249. ISSN: 2407-8050. DOI: 10.13057/psnmbi/m020221.

Junaedi, D.I., T. Usin, Rosita. & R. Simarmata. 2013. Laporan Eksplorasi Tumbuhan di Kawasan Sekitar Danau Toba Sumatera Utara Dalam Rangka Pengayaan Koleksi Kebun Raya Samosir. Bogor. KRI-LIPI. (Unpublished)

Laumonier Y, Uryu Y, Stuwe M *et al*. 2010. Eco-floristic sectors and deforestation threats in Sumatra: identifying new conservation area network priorities for ecosystem-based land use planning. Biodivers Conserv **19**: 1153-1174.

Lembaga Penelitian ITB. 2001. *Pengkajian Teknis Pemanfaatan Sumberdaya Alam dan Pengelolaan Lingkungan Hidup Kawasan Danau Toba*. Institut Teknologi Bandung, Bandung.

Mittermeier, R.A., P.R. Gill & C.G. Mittermeier. 1998. *Megadiversity: Earth's Biologically Weatlthiest Nations*. CEMEX Corp, Canada.

Nasution, Z. & B.S.J. Damanik. 2009. *Ekologi ekosistem kawasan Danau Toba*. Fakultas Pertanian Universitas Sumatera Utara. Medan.

Ohlstein, E.H., R.R. Ruffolo Jr., & J.D. Elliot. 2000. Drug discovery in the next millennium. Annual Review Pharmacology and Toxicology. **40**: 177- 191.

Subiandono, Endro, Heriyanto NM. 2009. Kajian tumbuhan obat akar kuning di kelompok hutan Gelawan, Kabupaten Kampar, Riau. Buletin Plasma Nutfah **15 (1)**: 43.

Van der Werf GR, Dempewolf J, Trigg SN *et al*. 2008. Climate regulation of fire emissions and deforestation in equatorial Asia. Proc Natl Acad Sci USA **105(51)**: 20350-20355.

Orchid Diversity in the North Part of Batulanteh Mountain, Sumbawa, Nusa Tenggara Barat

***I Gede Tirta, I Putu Agus Hendra Wibawa dan Aninda Retno Utami Wibowo**
Balai Konservasi Tumbuhan Kebun Raya'Eka Karya' Bali-LIPI
Candikuning, Baturiti, Tabanan Bali 82191
E-mail : *igtirta170859@gmail.com

ABSTRACT

Orchid has a high diversity especially in tropical rain forest and they can found naturally grow as epiphyte or terrestrial habit. However, habitat change and over exploitation have decline orchid diversity on its natural habitat. Mount Batulanteh at 1,730 m above sea level, located in Sumbawa, Nusa Tenggara Barat (NTB) is one of the natural habitat for Orchid. The study was conducted with random exploration method and aimed to understand the richness and diversity of Orchid in Mount Batulanteh. Orchids collected from Mount Batulanteh were 112 specimens from 55 species (40 epiphyte species and 15 terrestrial species) and belongs to 29 genera. Genera with the highest number of species are *Dendrobium* (7 species), *Bulbophyllum* (5 species), and *Eria* (4 species). The genera of *Dendrobium* and *Eria* have highest probability as a new record species.

Keywords: Diversity,Mount Batulanteh, Orchid, Orchidaceae, NTB.

INTRODUCTION

Orchid is belonging to Orchidaceae and widely known as an ornamental plant with unique characteristic on each species. Indonesia as a tropical country has ±4.000 species (Puspitaningtyas *et al.*, 1994). Unevenly distribution and many of endemic species had recorded from Sumatra and Java as two of five big islands in Indonesia. Sumatra has 1118 species with 458 of them are endemic (Comber, 2001), while Java has 731 species with 231 of them are endemic (Comber, 1990). Kalimantan also recognises as hotspot area of Orchid diversity and many studies still held at the time.

In the other hand, the eastern parts of Indonesia have lack of concern of its biodiversity including in Lesser Sunda Islands (LSI). Lesser Sunda Islands consist of many small islands namely Bali, Lombok, Sumbawa, Flores, Sumba, Timor and Alor. Only few Orchid studies from LSI were recorded. Sulistyarini (2000) has reported the Orchid diversity in Bali from Herbarium Collection in Bogor (BO) which is most of the old collection came from early 18's century, Silveira *et al.* (2008) have report a checklist of Timor Orchid with 66 species and 32 of them were recognise as new species, Astuti *et al.* (2010) have report the terrestrial Orchid from Lemor protected forest in Lombok – Nusa Tenggara Barat. The recent study of Orchid seems to be focused in Bali as Sulistyarini *et al.* (2016). With these few records about Orchid distribution in Lesser Sunda Islands, new Orchid diversity study in another part of LSI is needed.

Sumbawa Island is a regency and belonging to Nusa Tenggara Barat Province. The island also a part of LSI and located at 8°8' - 9°7' S and 116°42' - 118°22' E on the east side from Lombok Island. Mount Batulanteh is located in Sumbawa, Nusa Tenggara Barat with elevation 1,730 m above sea level (asl). Mount Batulanteh covers 31,571 ha of protected and production forest. The Orchid diversity from south valley of Mt. Batulanteh is firstly reported in this study, complemented with short description, habitat and ecology, distribution, photos, and map.

MATERIALS AND METHODS

The Orchid inventory was conducted with random exploration method (Rugayah *et al.*, 2005) in two different locations in the north part of Mount Batulanteh, Sumbawa, Nusa Tenggara Barat on 16 April – 6 May 2015 (Figure 1). The first track was in Dusun Punik – Desa Batudulang with elevation 440 – 1,146 m asl. The second track was in Dusun Pusu – Desa Tepal with elevation 912 – 1,157 m asl.

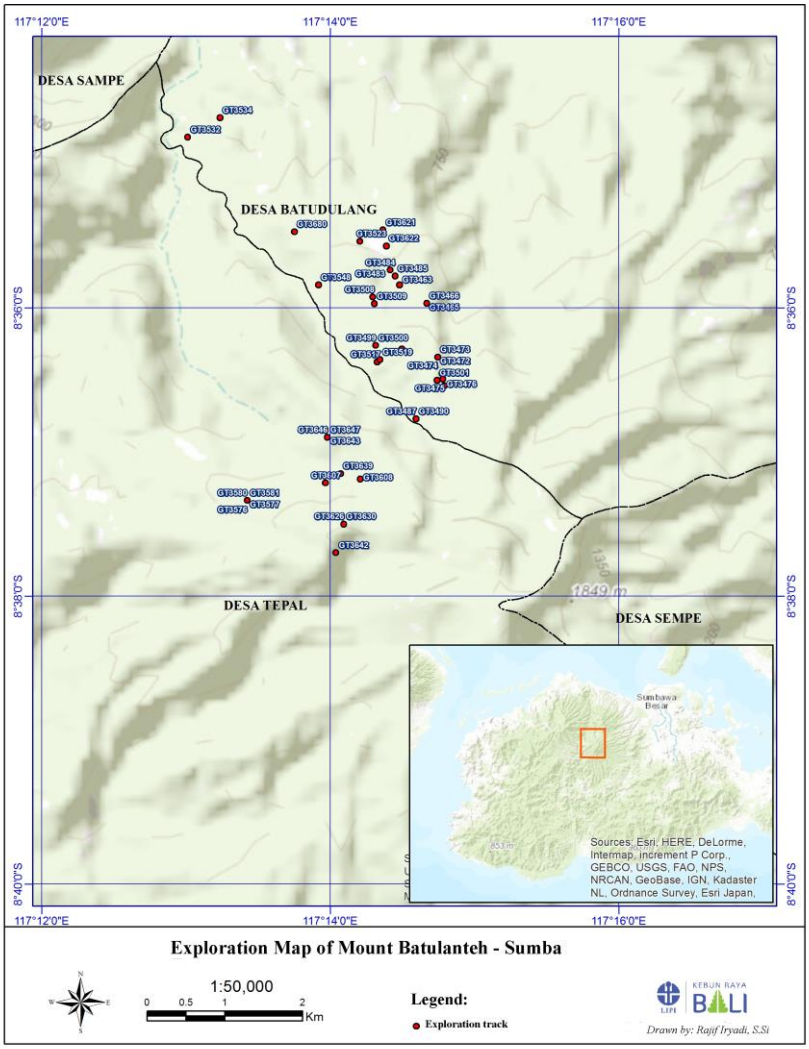


Figure 1. Orchids Exploration Map in Mount Batulanteh, Sumbawa, Nusa Tenggara Barat

Morphological characters were examined in the field and living plant collections were taken to be cultivated in Bali Botanic Garden (Bali BG). Main references for identification are Orchids of Java and Sumatra (Comber, 1990 & 2001), The Orchids of Peninsular Malaysia and Singapore (Seidenfaden & Wood, 1992). The resulted list is ordered alphabetically by genus and species.

RESULT AND DISCUSSION

Flora Vegetation

Several types of trees species found in the Punik and Pusu Forest Areas are: *Dipterocarpus retusus* Blume (keruing gunung), *Duabanga moluccana* Blume, *Magnolia* sp. (Treg mayong)., *Garcinia* sp. (Tengar*), Calophyllum soualattri* Burm. f. (slatri), *Litsea* sp., *Ficus* spp. and *Neonauclea* sp. In the community garden, many species of *Aleurites moluccana* (L.) Willd are found (Figure 7), *Coffea arabica* L., and *Coffea robusta* L. Linden, these three species of plants are the mainstay commodities of Punik Hamlet, Batudulang Village, Batulanteh District. *Oryza sativa* f *montana* (upland rice) and *Zea mays* L. (corn) are also planted on plantations, as the main food. Besides that, people have cultivated fruits like *Durio zibethinus* Murr. (durian), *Garcinia mangostana* L. (mangosteen), *Persea americana* Miller (avocado), *Musa* spp., *Carica papaya* L., and species of vegetables such as *Amaranthus* sp. (spinach), *Capsicum annum* L. (chili), *Cucurbita pepo* L. (waluh), *Cucumis sativus* L. (cucumber), *Luffa acutangula* (L.) Roxb. (pare). Planted tubers such as *Manihot*

utilissima Pohl (cassava), *Ipomoea batatas* (L.) L. (sweet potato), *Colocasia esculenta* (L.) Schott (taro), *Amorphophallus paeoniifolius* (suweg).

Area Conditions

The exploration area includes Punik Hamlet, Batudulang Village (440-1146 m asl) and Pusu Hamlet, Tepal Village (912-1157 m asl). In general, the forest area is still good, the type of soil is rocky clay with partially decomposed organic matter. Land tofography is mostly wavy and hilly, the weather for 20 days (April 16 to May 6, 2015) is always rainy and cloudy during the day. Data from the recording of soil and air temperatures are listed in Table 1.

Table 1. Temperature and humidity recording in the Punik and Pusu forests, Sumbawa, Nusa Tenggara Barat (April 16 to May 6, 2015)

Forest area	Soil		Air		Altitude m asl
	pH	RH (%)	Temperature (ºC)	RH (%)	
Punik : (at noon)	2-6,5	3-6	22-28	70-89	440-1146
(at night)	-	-	15-20	80-89	910
Pusu	4-6	3-5	21-25	70-86	912-1157

Although 55 species were found (Table 2), we couldn't able to identify all of the specimens because of the lack from generative materials. With the total of 24 species were clearly identified, all of them have widespread distribution area and none of them are endemic. In the other hand, many species are remains unidentified to species level which probably result as a new record. The genera of *Dendrobium* and *Eria* are needed to get further examination in cultivation, since many of the species being separate and move to another genus based on Chase *et al.* (2015).

Table 2. Orchid species found in the Batulanteh Mountain, Sumbawa, Nusa Tenggara Barat

No.	Number Collector	Plant Name	Habit	Number of specimen	Location & altitude
1	GT3460	*Parapteroceras odoratissimum* (J.J.Sm) J.J.Wood.	Epiphyte	4	Punik Forest 930 m asl
2	GT3461	*Trixspermum subulatum* (Blume) Rchb.f.	Epiphyte	2	Punik Forest 930 m asl
3	GT3462	*Dendrobium* sp.	Epiphyte	3	Punik Forest 930 m asl
4	GT3463	*Luisia javanica* J.J.Sm.	Epiphyte	3	Punik Forest 930 m asl
5	GT3465	*Habenaria* sp.1	Terrestrial	1	Punik Forest 1029 m asl
6	GT3466	*Habenaria* sp. 2	Terrestrial	1	Punik Forest 1029 m asl
7	GT3467	*Bryobium hyacinthoides* (Blume) Y.P.Ng & P.J.Crib	Epiphyte	2	Punik Forest 1029 m asl
8	GT3468	*Bryobium retusum* (Blume) Y.P.Ng & P.J.Cribb.	Epiphyte	2	Punik Forest 1099 m asl
9	GT3469	*Pinalia multiflora* (Blume) Kuntze.	Epiphyte	2	Punik Forest 1099 m asl
10	GT3470	*Eria* sp.	Epiphyte	6	Punik Forest 1099 m asl
11	GT3471	*Bulbophyllum* sp.	Epiphyte	3	Punik Forest 1099 m asl
12	GT3472	*Oberonia* sp.	Epiphyte	1	Punik Forest 1099 m asl

No.	Number Collector	*Plant Name*	Habit	Number of specimen	Location & altitude
13	GT3473	*Bulbophyllum lemniscatoides* Rolfe	Epiphyte	1	Punik Forest 1099 m asl
14	GT3474	*Vanda tricolor* Lindl.	Epiphyte	2	Punik Forest 1108 m asl
15	GT3475	*Appendicula elegans* Rchb.f.	Epiphyte	2	Punik Forest 1108 m asl
16	GT3476	*Polystachya concreta* (Jacq.) Garay & H.R.Sweet. J.J.Sm.	Epiphyte	3	Punik Forest 1104 m asl
17	GT3483	*Trichoglottis* sp.	Epiphyte	2	Punik Forest 910 m asl
18	GT3484	*Pholidota imbricata* Lindl.	Epiphyte	3	Punik Forest 910 m asl
19	GT3485	*Dendrobium macrostachyum* Lindl.	Epiphyte	2	Punik Forest 928 m asl
20	GT3487	*Malaxis* sp.	Terestrial	1	Punik Forest 928 m asl
21	GT3490	*Tuberolabium* sp.	Epiphyte	2	Punik Forest 1034 m asl
22	GT3494	*Calanthe* sp.	Terrestrial	4	Punik Forest 1146 m asl
23	GT3497	*Dendrobium linearifolium* Teijsm. & Binn.	Epiphyte	1	Punik Forest 1034 m asl
24	GT3498	*Eria* sp.	Epiphyte	2	Punik Forest 1034 m asl
25	GT3499	*Pinalia bractescens* (Lind.) Kuntze. Revis.	Epiphyte	2	Punik Forest 1034 m asl
26	GT3500	*Dendrobium convexum* (Blume) Lindl.	Epiphyte	4	Punik Forest 1034 m asl
27	GT3501	*Bulbophyllum* sp.	Epiphyte	1	Punik Forest 1037 m asl
28	GT3508	*Phaius* sp.	Terestrial	3	Punik Forest 1015 m asl
29	GT3509	*Malaxis* sp.	Terrestrial	2	Punik Forest 1037 m asl
30	GT3517	*Corymborchis veratrifolia* Blume	Terrestrial	2	Punik Forest 1000 m asl
31	GT3519	*Nervilia punctata (Blume) Makino.*	Terrestrial	1	Punik Forest 1004 m asl
32	GT3523	*Flickingeria sp.*	Epiphyte	2	Punik Forest 910 m asl
33	GT3532	*Nervilia concolor* (Blume) Schltr.	Terrestrial	4	Punik Forest 441 m asl
34	GT3534	*Nervilia* sp.	Terrestrial	2	Punik Forest 507 m asl
35	GT3548	*Oberonia* sp.	Epiphyte	1	Punik Forest 725 m asl
36	GT3567	*Mycaranthes latifolia* Blume	Epiphyte	1	Pusu Forest 1100 m asl
37	GT3568	*Eria* sp.	Epiphyte	1	Pusu Forest 1100 m asl
38	GT3569	*Bulbophyllum biflorum* Teijsm. & Binn.	Epiphyte	1	Pusu Forest 1100 m asl
39	GT3571	*Eria* sp.	Epiphyte	2	Pusu Forest 1100 m asl

No.	Number Collector	*Plant Name*	Habit	Number of specimen	Location & altitude
40	GT3576	*Trichotosia pauciflora* Blume	Epiphyte	4	Pusu Forest 1100 m asl
41	GT3577	*Mycaranthes oblitterata Blume*	Epiphyte	2	Pusu Forest 1100 m asl
42	GT3580	*Dendrobium sp.**	Epiphyte	4	Pusu Forest 1100 m asl
43	GT3581	*Dendrobium salaccense (Blume) Lindl.*	Epiphyte	1	Pusu Forest 1100 m asl
44	GT3607	*Habenaria* sp.	Terrestrial	1	Pusu Forest 1077 m asl
45	GT3608	*Spathoglottis plicata Blume*	Terrestrial	2	Pusu Forest 1099 m asl
46	GT3621	*Vanda* sp.	Epiphyte	1	Pusu Forest 881m asl
47	GT3622	*Trichoglottis* sp.*	Epiphyte	1	Pusu Forest 888 m asl
48	GT3626	*Appendicula imbricata* J.J. Sm.	Epiphyte	3	Pusu Forest 1135 m asl
49	GT3630	*Plocoglottis* sp.	Terrestrial	1	Pusu Forest 1135 m asl
50	GT3639	*Goodyera* sp.	Terrestrial	1	Pusu Forest 1157m asl
51	GT3642	*Ceratostylis* sp.	Epiphyte	2	Pusu Forest 1128 m asl
52	GT3643	*Bulbophyllum* sp.	Epiphyte	1	Pusu Forest 1142 m asl
53	GT3646	*Dendrochillum* sp.	Epiphyte	1	Pusu Forest 1142 m asl
54	GT3647	*Liparis* sp.	Epiphyte	2	Pusu Forest 1142 m asl
55	GT3680	*Malaxis* sp.	Terrestrial	1	Punik Forest 852 m asl

The results of the study carried out 112 orchid specimens consisting of 55 species (40 epiphytic species and 15 terrestrial species) (Figure 2) and belonging to 28 genera. Genera with the highest number of species are Dendrobium (7 species), Bulbophyllum (5 species), and Eria (4 species) (Figure 3).

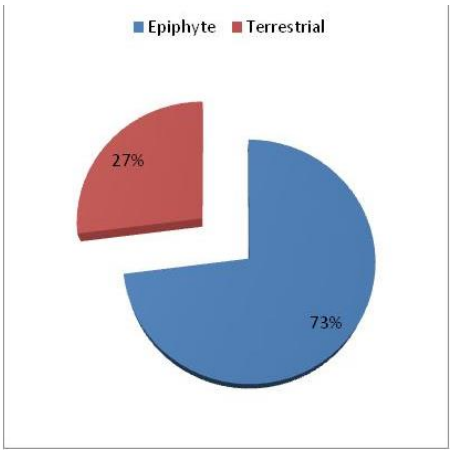


Figure 2. Comparison of Orchids habitus found in the Batulanteh Mountain, Sumbawa, Nusa Tenggara Bara

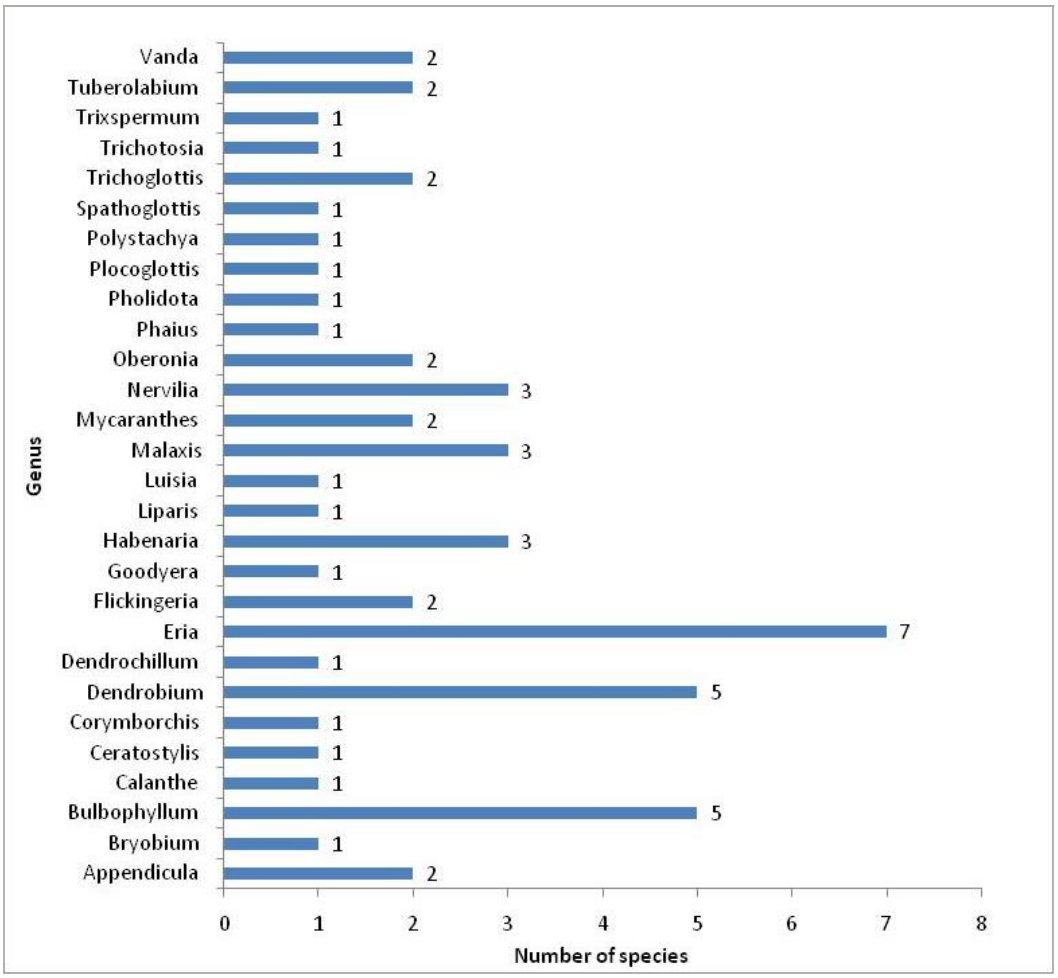


Figure 3. Number of Orchid spesies and its genera found in the Batulanteh Mountain, Sumbawa, Nusa Tenggara Barat

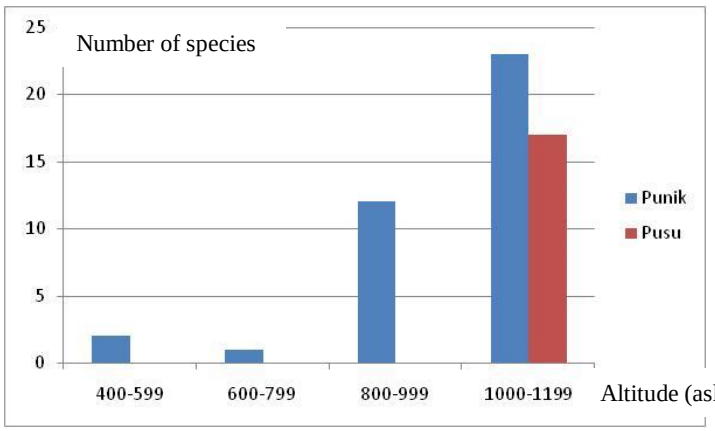


Figure 4. Number of Orchid species found in the Batulanteh Mountain, Sumbawa, Nusa Tenggara Barat based on their growing location (m asl)

Orchids were found more to grow at an altitude of 1000-1199 m asl. and only a few were found to grow in the range 400-799 m asl. 23 types of orchids were found at altitudes of 1000-1199 m

asl and 12 species were found at an altitude of 800-999 m asl and only three species were found growing at an altitude of 400-799 m asl (Figure 4).

Synonyms and general distribution

Like other plants, orchids also have synonyms names, the following is presented the accepted name and synonyms of the orchid that found in this research, and the distribution that has been recorded :

**Bryobium hyacinthoides* (Blume) Y.P.Ng & P.J.Cribb, Orchid Rev. 113 (1265): 272 (2005).

Synonyms: *Dendrolirium ebulbe* Blume. Bijdr. Fl. Ned. Ind. 7: 343 (1825); *Dendrolirium hyacinthoides* Blume. Bijdr. Fl. Ned. Ind. 7:346 (1825); *Eria ebulbis* (Blume) Lindl. Gen. Sp. Orchid. Pl. 69. (1830); *Eris endymion* Ridl. J. Linn. Soc., Bot. 32:295 (1896); *Eria hyacinthoides* Lindl. Gen. Sp. Orchid. Pl. 66 (1830); *Pinalia ebulbis* (Blume) Kuntze. Revis. Gen. Pl. 2:679 (1891); *Pinalia hyacinthodes* (Blume) Kuntze. Revis. Gen. Pl. 2:678 (1891).

General distribution: Peninsular Malaysia, Sumatra, Java, Borneo, Lesser Sunda Islands

**Bryobium retusum* (Blume) Y.P.Ng & P.J.Cribb. Orchid Rev. 113(1265): 272 (2005).

Synonyms: *Bryobium pubescens* Lindl. Edwards's Bot. Reg. 24(Misc.):79 (1838); *Dendrolirium retusum* Blume. Bijdr. Fl. Ned. Ind. 7: 351 (1825); *Eria retusa* (Bume) Rchb.f. Bonlandia 5: 54 (1857); *Phreatia congesta* Rolfe ex Hemsl. J.Linn.Soc., Bot. XXV. 358; *Phreatia retusa* (Blume) Lindl. Gen. Sp. Orchid. Pl. 64 (1830); *Pinalia retusa* (Blume) Kuntze. Revis. Gen. Pl. 2:679 (1891).

General distribution: Java, Borneo, Lesser Sunda Islands, Sulawesi, New Guinea, Christmas Island, New Caledonia

Figure 2. Some Orchids collection from Mount Batulanteh, Sumbawa, Nusa Tenggara Barat A) *Dendrobium convexum*; B) *Dendrobium linearifolium*; C) *Dendrobium salaccense*; D) *Nervilia concolor*; E) *Polystachya concreta.*

**Bulbophyllum biflorum* Teijsm. & Binn. Natuurk. Tijdschr. Ned. Indie 5:48 (1854).

Synonyms: *Bulbophyllum geminatum* Carr. Gard. Bull. Starits Settlem. 5: 12 (1929); *Cirrhopetalum biflorum* (Teijsm. & Binn.) J.J.Sm. Icon. Bogor. [Boerlage] 2: t.120 B (1930); *Phyllorkis biflora* (Teijsm. & Binn.) Kuntze. Revis. Gen. Pl. 2: 678 (1891).

General distribution: Peninsular Malaysia, Thailand, Philippines, Sumatra, Java, Borneo, Lesser Sunda Islands.

**Dendrobium convexum* (Blume) Lindl. Gen. Sp. Orchid. Pl. 76 (1830).

Synonyms: *Abaxianthus convexus* (Blume) M.A. Clem. & D.L. Jones. Orchadian 13(11): 485 (2002); *Callista convexa* (Blume) Kuntze. Revis. Gen. Pl. 2: 654 (1891); *Desmotrichum convexum* Blume. Bijdr. Fl. Ned. Ind. 7: 329, t. 331 (1825); *Ephemerantha convexa* (Blume) P.F. Hunt & Summerh. Taxon X. 103 (1961); *Flickingeria convexa* (Blume) A.D. Hawkes. Orchid Weekly 2(46): 454 (1961).

General distribution: Thailand, Malaysia, Indonesia, North Australia

**Dendrobium macrostachyum* Lindl. Gen. Sp. Orchid. Pl. 78 (1830).

Synonyms: *Callista macrostachya* (Lindl.) Kuntze. Revis. Gen. Pl. 2: 655 (1891); *Callista stuartii* (F.M.Bailey) Kuntze. Revis. Gen. Pl. 2: 655 (1891); *Callista tetrodon* (Rchb.f. ex Lindl.) Kuntze. Revis. Gen. Pl. 2: 655 (1891); *Dendrobium gamblei* King & Pantl. J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 66: 584 (1897); *Dendrobium stuartii* F.M. Bailey. Proc. Roy. Soc. Queensland 1: 12 (1884); *Dendrobium tetrodon* Rchb.f. ex Lindl. J. Proc. Linn. Soc., Bot. 3: 10 (1858); *Dendrobium viridicatum* Ridl. J. Straits Branch Roy. Asiat. Soc. xxxix. 72; *Dendrobium whiteanum* T.E.Hunt. Queensland Naturalist xiv. 60 (1951).

Distribution: Wide distribution from Assam, Bangladesh, Eastern Himalayas to Srilanka, all of the Peninsular Malaya, Borneo, Sumatra, Java, Lesser Sunda Islands, Queensland Australia

**Dendrobium salaccense* (Blume) Lindl. Gen. Sp. Orchid. Pl. 86 (1830).

Synonyms: *Callista bambusifolia* (E.C. Parish & Rchb.f.) Kuntze. Revis. Gen. Pl. 2: 654 (1891); *Callista cathcarthii* (Hook.f.) Kuntze. Revis. Gen. Pl. 2:654 (1891); *Callista haemoglossa* (Thwaites) Kuntze. Revis. Gen. Pl. 2:654 (1891); *Callista intermedia* (Teijsm. & Binn.) Kuntze. Revis. Gen. Pl. 2:655 (1891); *Callista salacense* (Blume) Kuntze. Revis. Gen. Pl. 2:655 (1891); *Dendrobium bambusifolium* E.C.Parish & Rchb.f. Trans. Linn. Soc. Londong 30(1): 149 (1874); *Dendrobium cathcartii* Hook.f. Fl. Brit. India [J.D. Hooker] 5(16): 727 (1890); *Dendrobium haemoglossum* Thwaites. Enum. Pl. Zeyl. [Thwaites] 429 (1864); *Dendrobium intermedium* Teijsm.

& Binn. Natuurk. Tijdschr. Ned. Indie. 5: 490 (1854); *Grastidium bambusifolium* (E.C.Parish & Rchb.f.) Brieger. Orchideen (Schlecter) 1(11-12): 653 (1981); *Grastidium cathcartii* (Hook.f.) M.A.Clem. & D.L.Jones. Lasianthera 1(2): 67 (1997); *Grastidium haemoglossum* (Thwaites) M.A.Clem. & D.L. Jones. Lasianthera 1(2): 78 (1997); *Grastidium salaccense* Blume. Bijdr. Fl. Ned. Ind. 7: 333 (1825).
General distribution: Cina, Myanmar, Thailand, Laos, Vietnam, Peninsular Malaysia, Sumatra, Java, Borneo, Lesser Sunda Island

**Mycaranthes latifolia* Blume. Bijdr. Fl. Ned. Ind. 7:352, t. 353 (1825).

Synonyms: *Eria bidens* Ridl. J. Linn. Soc., Bot. 32: 289 (1896); *Eria iridifolia* Hook.f. Fl. Brit. India [J.D. Hooker] 5(16): 790 (1890); *Eria latifolia* (Blume) Rchb.f. Bonplandia 5: 55 (1857); *Eria longispica* Rolfe. Bot. Mag. 133: t. 8171 (1907); *Eria validissima* Kraenzl. Bot. Jahrb. Syst. 44(2-3, Beibl. 101): 27 (1910); *Pinalia iridifolia* (Hook.f.) Kuntze. Revis. Gen. Pl. 2: 679 (1891); *Pinalia latifolia* (Blume) Kuntze. Revis. Gen. Pl. 2: 679 (1891).
General distribution: Peninsular Malaysia, Sumatra, Java, Borneo, Sulawesi, Lesser Sunda Islands.

**Mycaranthes oblitterata* Blume. Bijdr. Fl. Ned. Ind. 7:352, t. 353 (1825).

Synonyms: *Eria callosa* M.R.Hend. J. Fed. Malay States Mus. 13: 225 (1927); *Eria javensis* Zoll. & Moritzi. Syst. Verz. Zoll. 86; *Eria kingii* Hook.f. Fl. Brit. India [J. D. Hooker] 5(16): 790 (1890); *Eria larutensis* Ridl. J. Straits Branch Roy. Asiat. Soc. 82:198 (1920); *Eria major* Ridl. Ex Stapf. Trans. Linn. Soc. London, Bot. 4(2): 237 (1894); *Eria oblitterata* (Blume) Rchb.f. Bonplandia 5: 55 (1857); *Eria ridleyi* Rolfe. J. Linn. Soc., Bot. xiii. 150 (1914); *Eria sclerophylla* Lindl. J. Proc. Linn. Soc., Bot. 3: 56 (1858); *Eria tahanensis* Ridl. J. Fed. Malay States Mus. 6: 177 (1915); *Eria tomentella* Rchb.f. Ann. Bot. Syst. (Walpers) 6(2): 271 (1861); *Mycaranthes abbreviata* Blume ex Kraenzl. Pflanzenr. (Engler) Orch.-Dendrob. Pars 2, 42 (1911); *Mycaranthes ridleyi* (Rolfe) Rauschert. Feddes Repert. 94(7-8): 456 (1983); *Pinalia javensis* (Zoll. & Moritzi) Kuntze. Revis. Gen. Pl. 2: 679 (1891); *Pinalia obliterata* (Blume) Kuntze. Revis. Gen. Pl. 2: 678 (1891); *Pinalia sclerophylla* (Lindl.) Kuntze. Revis. Gen. Pl. 2: 679 (1891).
General distribution: From Vietnam downwards to the south and east to the South Pasific Ocean

**Nervilia concolor* (Blume) Schltr. Bot. Jahrb. Syst. 45(3): 404 (1911).

Synonyms: *Aplostellis flabelliformis* (Lindl.) Ridl. Fl. Malay Penins. iv. 203 (1924); *Cordyla concolor* Blume. Bijdr. Fl. Ned. Ind. 8:416 (1825); *Epipactis carinata* Roxb. Hort. Bengal. 63, Fl. Ind. iii. 454 (1832); *Nervilia aragoana* Gaudich. Voy. Uranie, Bot. 422, t. 35 (1829); *Nervilia carinata* (Roxb.) Schltr. Bot. Jahrb. Syst. 45(3): 404 (1911); *Nervilia flabelliformis* (Lindl.) Tang & F.T.Wang. Acta Phytotaxa. Sin. i. 68 (1951); *Nervilia scottii* (Rchb.f.) Schltr. Bot. Jahrb. Syst. 45(3): 404 (1911); *Nervilia tibetensis* Rolfe. Notes Roy. Bot. Gard. Edinburgh 8: 128 (1913); *Nervilia yaeyamensis* Hayata. Icon. Pl. Formosan. 2: 140 (1912); *Pogonia carinata* (Roxb.) Lindl. Gen. Sp. Orchid. Pl. 414 (1840); *Pogonia concolor* Blume. Mus. Bot. 1(2): 32 (1849); *Pogonia flabelliformis* Lindl. Gen. Sp. Orchid. Pl. 415 (1840); *Pogonia gracilis* Blume. In rev. Engenh. 74, ex ej. Orch. Nov. ii. 267; *Pogonia nervilia* Blume. Mus. Bot. 1(2): 32 (1894); *Pogonia scottii* Rchb.f. Flora 55: 276 (1872); *Roptrostemon concolor* (Blume) Lindl. Gen. Sp. Orchid. Pl. 453 (1840)
General distribution: Widespread species from eastern Himalayan to the North and South Pasific Ocean.

**Nervilia punctata* (Blume) Makino. Bot. Mag. (Tokyo) xvi. 199

Synonyms: *Aplostellis punctata* (Blume) Ridl. Fl. Malay Penins. iv. 204 (1924); *Nervilia punctata* (Blume) Schltr. Bot. Jahrb. Syst. 45(3): 402 (1911); *Pogonia punctata* Blume. Mus. Bot. 1(2): 32 (1849).
General distribution: From India downwards to the south east to Peninsular Malaysia, New Guinea, and Fiji

**Pinalia bractescens* (Lind.) Kuntze. Revis. Gen. Pl. 2: 679 (1891).

Synonyms: *Dendrobium subterrestre* Gagnep. Bull. Mus. Natl. Hist. Nat (1930); *Eria bractescens* Lindl. Edwards's Bot. Reg. 27(Misc.): 18 (1841); *Eria dillwynii* Hook. Bot. Mag. 71: t.

4163 (1845); *Eria griffithii* Rchb.f. Xenia Orchid. ii. 162, 163; *Eria kurzii* Anderson ex Hook.f. Fl. Brit. India [J.D. Hooker] 5(16): 797 (1890); *Eria littoralis* Teijsm. & Binn. Natuurk. Tijdschr. Ned. Indie xxiv (1862); *Eria pulchella* Griff. Not. Pl. Asiat. 3: 297 (1851); *Pinalia pulchella* Kuntze. Revis. Gen. Pl. 2:679 (1891); *Tropilis subterrestris* (Gagnep) Rauschert. Feddes Repert. 94(7-8): 471 (1983).
General distribution: Wide distribution in Assam, Bangladesh, Himalayan area, andSouth East Asia

**Pinalia multiflora* (Blume) Kuntze. Revis. Gen. Pl. 2: 679 (1891).

Synonyms: *Dendrolirium micranthum* Blume. Bijdr. Fl. Ned. Ind. 7: 349 (1825); *Dendrolirium multiflorum* Blume. Bijdr. Fl. Ned. Ind. 7: 349 (1825); *Eria micrantha* (Blume) Lindl. Gen. Sp. Orchid. Pl. 68 (1830); *Eria multiflora* (Blume) Lindl. Gen. Sp. Orchid. Pl. 68 (1830); *Octomeria racemosa* Breda. Gen. Sp. Orchid. Asclep. t: 12 (1827); *Pinalia micrantha* (Blume) Kuntze. Revis. Gen. Pl. 2: 679 (1891); *Urostachya multiflora* (Blume) Rauschert. Feddes Repert. 94(7-8): 471 (1983).
General distribution: Sumatra, Java, Lesser Sunda Islands

Pholidota imbricata Lindl. Exot. Fl. 2: t. 138 (1825).

Synonyms: *Coelogyne conchoidea* (Lindl.) Rchb.f. Ann. Bot. Syst. (Walpers) 6(2): 237 (1861); *Coelogyne crotalina* (Rcbh.f.) Rchb.f. Ann. Bot. Syst. (Walpers) 6(2): 237 (1861); *Coelogyne imbricata* (Lindl.) Rchb.f. Ann. Bot. Syst. (Walpers) 6(2): 238 (1861); *Coelogyne loricata* (Rchb.f.) Rchb.f. Ann. Bot. Syst. (Walpers) 6(2): 238 (1861); *Coelogyne triotos* (Rchb.f.) Rchb.f. Hamb. Gartenz. xvii (1862); *Cymbidium imbricatum* (Lindl.) Roxb. Hort. Bengal. 63, Fl. Ind. iii. 460; *Ornithidium imbricatum* (Lindl.) Wall. ex Hook.f. Fl. Brit. India [J.D.Hooker] 5(16): 846 (1890); *Pholidota beccarii* Schltr. Notizbl. Bot. Gart. Berlin-Dahlem 8: 17 (1921); *Pholidota bracteata* (D.Don) Seidenf. Opera Bot. 89: 100 (1986); *Pholidota calceata* Rchb.f. Bonplandia 4: 329 (1856); *Pholidota conchoidea* Lindl. Edwards's Bot. Reg. 26(Misc.): 84 (1840); *Pholidota crotalina* Rchb.f. Allg. Gartenzeitung (Otto & Dietrich) 24: 218 (1856); *Pholidota henryi* Kraenzl. Vierteljahrsschr. Naturf. Ges. Zürich ix. 427 (1915); *Pholidota loricata* Rchb.f. Bonplandia 5: 43 (1857); *Pholidota spectabilis* Kraenzl. ex Guillaumin. Ann. Mus. Colon. Marseille sér. 6, 5-6: 15 (1948); *Pholidota triotos* Rchb.f. Ann. Bot. Syst. (Walpers) 6(2): 238 (1861); *Ptilocnema bracteata* D.Don. Prodr. Fl. Nepal. 33 (1825).
General distribution: Wide distribution in Central China, Assam, Tibet, Himalayan area, India, Sri Lanka, South East Asia

Polystachya concreta (Jacq.) Garay & H.R.Sweet. Fl. Lesser Antilles [R.A. Howard] 178 (1974)

Synonyms: *Callista flavescens* (Blume) Kuntze. Revis. Gen. Pl. 2: 654 (1891); *Cranichis luteola* Sw. Fl. Ind. Occid. iii. 1433 (1806); *Dendrobium flavescens* (Blume) Lindl. Gen. Sp. Orchid. Pl. 85 (1830); *Dendrorkis extinctoria* (Rchb.f.) Kuntze. Revis. Gen. Pl. 2: 659 (1891); *Epidendrum concretum* Jacq. Enum. Syst. Pl. 30 (1760); *Onychium flavescens* Blume. Bijdr. Fl. Ned. Ind. 7: 325 (1825); *Polystachya flavescens* (Blume) J.J. Sm. Orchid. Java 284 (1905); *Polystachya luteola* Hook. Exot. Fl. 2(10): t. 103 (1824); *Polystachya penangensis* Ridl. J. Linn. Soc., Bot. 32: 344 (1896).
General distribution: Wide distribution on tropical hemispheres country

Parapteroceras odoratissimum (J.J.Sm) J.J.Wood. Nordic J. Bot. 10(5): 485 (1990).

Synonyms: *Saccolabium odoratissimum* J.J. Sm. Orchid. java 645 (1905); *Tuberolabium odoratissimum* (J.J.Sm.) Garay. Bot. Mus. Leafl. 23(4): 210 (1972).
Genral distribution: Java, Lesser Sunda Island

Spathoglottis plicata Blume. Bijdr. Fl. Ned. Ind. 8: 401, t. 76 (1825).
General distribution: Wide distribution in Bangladesh, Himalaya, India, Peninsular Malaysia, Indonesia and Pacific Ocean Islands

Thrixspermum subulatum (Blume) Rchb.f. Xenia Orchid. ii. 122 (1868).

Synonyms: *Aerides subulata* (Blume) Lindl. Gen. Sp. Orchid. Pl. 241 (1833); *Dendrocolla subulata* Blume. Bijdr. Fl. Ned. Ind. 7: 291 (1825); *Sarcanthus timorensis* Decne. Nouv. Ann. Mus.

Par. iii: 365 (1834); *Sarcochilus croceus* Lindl. Edwards's Bot. reg. 32: t. 19 (1846); *Sarcochilus subulatus* (Blume) Rchb.f. Ann. Bot. Syst. (Walpers) 6(4): 500 (1863); *Thrixspermum croceum* (Lindl.) Rchb.f. Xenia Orchid. ii. 122 (1868); *Thrixspermum falcilobum* Schltr. Bull. Herb. Boissier Ser. II. vi. 469 (1906).
General distribution: Taiwan, Thailand, Peninsular Malaysia, Sumatra, Java, Lesser Sunda Islands, Sulawesi, Moluccas, Philippines

Trichotosia pauciflora Blume. Bijdr. Fl. Ned. Ind. 7: 343 (1825).
Synonyms: *Eria biflora* (Griff.) Lindl. J. Proc. Linn. Soc., Bot. 3: 56 (1858); *Eria monticola* Hook.f. Fl. Brit. India [J.D.Hooker] 5(16): 806 (1890); *Eria pauciflora* (Blume) Blume. Mus. Bot. 2(9-12): 183 (1856); *Eria tuberosa* Hook.f. Fl. Brit. India [J.D.Hooker] 5(16): 807 (1890); *Eria vrieseana* Rchb.f. C. Koch, Allg. Gartenx. 379 (1858); *Pinalia monticola* (Hook.f.) Kuntze. Revis. Gen. Pl. 2: 679 (1891); *Pinalia pauciflora* (Blume) Kuntze. Revis. Gen. Pl. 2: 679 (1891); *Pinalia tuberosa* (Hook.f.) Kuntze. Revis. Gen. Pl. 2: 679 (1891); *Trichotosia biflora* Griff. Not. Pl. Asiat. 3: 331 (1851); *Trichotosia tuberosa* (Hook.f.) Kraenzl. Pflanzenr. (Engler) Orch.-Dendrob. Pars 143 (1911).
General distribution: Thailand, Peninsular Malaysia, Borneo, Sumatra, Java, Lesser Sunda Islands

CONCLUSION

Orchids of Batulanteh Sumbawa, Nusa Tenggara Barat were recorded as widespread species and needed to be studied further. Orchids collected were 112 specimens from 55 species (40 epiphyte species and 15 terrestrial species) and belongs to 28 genera. Genera with the highest number of species are *Dendrobium* (7 species), *Bulbophyllum* (5 species), and *Eria* (4 species). The genera of *Dendrobium* and *Eria* have highest probability as a new record species.

ACKNOWLEDGEMENT

This study was funded by DIPA 2015 of BKT Kebun Raya "Eka Karya" Bali – LIPI. We thanks to Director of BKT Kebun Raya "Eka Karya" Bali – LIPI, Head of Sumbawa Besar District Forestry and Plantation Service -NTB for the permission.

REFERENCES

Astuti, I.P., and I.D.P. Darma. 2010. Keanekaragaman Anggrek Tanah di Kawasan Hutan Lindung Lemor, Lombok Timur Nusa Tenggara Barat. *Berkala Penelitian Hayati* 15: 187-190.

Chase, M.W., K.M. Cameron, J.V. Freudenstein, A.M. Pridgeon, G. Salazar, C.van Den Berg, and A. Schuiteman. 2015. An updated classification of Orchidaceae. *Botanical Journal of the Linnean Society* 177: 151-174.

Comber, J.B. 1990. *Orchid of Java*. Bentham Moxon Trust, Royal Botanic Garden, Kew.

Comber, J. B. 2001. *Orchids of Sumatra*. Natural History Publications, Kinabalu. p. 524-548.

Rugayah, E.A.Widjaja and Praptiwi. 2005. Pedoman Pengumpulan Keanekaragaman Flora. Pusat Penelitian Biologi Lembaga Ilmu Pengetahuan Indonesia. Bogor. 145 p.

Seidenfaden, G. and J.J. Wood. 1992. *The Orchids of Peninsular Malaysia and Singapore*. Olsen & Olsen, Fredensborg/Royal Botanic Gardens, Kew.

Silveira, P., A. Schuiteman, J.J. Vermeulen, A.J. Sousa, H. Silva, J. Paiva, and E.D. Vogel. 2008. The Orchids of Timor: checklist and conservation status. *Botanical Journal of the Linnean Society* 157: 197-215.

Sulistiarini, D. 2000. Anggrek dari Kepulauan Sunda Kecil. Prosiding Seminar Hari Cinta Puspa dan Satwa Nasional. Kebun Raya Bogor, Bogor. p 255-260

Sulistiarini, D., D. Arifiani, and Y. Santika. 2016. New records of Orchidaceae from Bali, Indonesia. *Gardens' Bulletin Singapore* 68(1): 87-95.

Pollen Morphology and Viability of Pengo (*Sarcotheca macrophylla* Blume, Oxalidaceae)

Inggit Puji Astuti, Sudarmono and Frisca Damayanti
Center for Plant Conservation - Botanic Gardens, Indonesian Institute of Sciences
Jl. Ir. H Juanda No. 13, Bogor 16003, West Java. Tel./fax: +62-251-8322187
Email: inggit.pa@gmail.com

ABSTRACT

Sarcotheca macrophylla Blume (Oxalidaceae) is an endemic plant to Borneo island, except for the Southern part. Most all of the species in Oxalidaceae are heterostylous (distylous). *S. macrophylla* have a long inflorescence of up to 1.5 m, which consists of more than 100 clusters of flowers. Each cluster consists of 1-7(8) flowers. The flower is small, red, and blooms in one day. This species produces 10 to 100 parthenocarphic fruits. There are four tubes of fruit in each ovule. The present study observed the structure of the stamen and pistil, pollen morphology, and viability of *S.microphylla*. The pistil, stamen, and pollen were observed using a microscope stained by aniline blue dye and 20% sucrose solution for the pollen viability test. Stamen of *S. macrophylla* is heterostyly; it consists of 10 stamens (i.e. 5 stamens are longer than the other 5). The pollen grains of *S. macrophylla* were stained with aniline blue dye solution and observed at 400x microscope magnification, and the results showed that the pollen grains were rounded in shape. The pollen grains of *S. macrophylla* are of tricolpate with three apertures, and the surface of the pollen grains is coarse. The morphology of the pollen grains shaped by SEM is rounded. The viability test of fresh pollen grains of *S.macrophylla* recorded by staining with aniline blue showed that the pollen germinated after 5 minutes. The viability of *S. macrophylla* pollen was recorded 80% with 20% sucrose solution.

Keywords: Oxalidaceae, Pengo, Pollen, *Sarcotheca macrophylla*, Viability.

INTRODUCTION

Sarcotheca is one of the genera belonging to Oxalidaceae or "belimbing-belimbingan", which are naturally distributed in Sumatera, Malaya, Borneo and Sulawesi. In West Malaysian regions, there are 11 species of *Sarcotheca*. Eight out of the eleven species are found in Indonesia, i.e., Sumatera, West Kalimantan, Central Kalimantan, East Kalimantan and Sulawesi (Chung, 1995; Mabberley, 1997; Veldkamp, 1967). The eight species of *Sarcotheca* which grow in Indonesia are *S. ferrugenea, S. griffitii, S. laxa, S. diversifolia, S. glauca, S. macrophylla, S. rubrinervis*and *S. celebica*. Four of them are found in Kalimantan: *S. diversifolia, S. glauca, S. macrophylla,* and *S. Rubrinervis* (Astuti, Sari, Agustin 2009). *S. macrophylla* (pengo) is an endemic species of Borneo. Its natural distribution in Kalimantan includes West, Central, and East Kalimantan. There is no record in South Kalimantan (Astuti *et al*. 2014).

Sarcotheca macrophylla has been a plant collection of Bogor Botanic Gardens, LIPI, since May 2015. The plant originated from Katingan, Central Kalimantan. As a collection plant, its life cycles must be observed, such as the flowering and the fruiting season in the garden. The flowering season of this species is all year round. During the observation, it was found that the plant produced parthenocarphic fruits. Parthenocarpy is the production of fruits without fertilization (Polito, 1999).

The flowering and fruiting phenology of *S. macrophylla* had been conducted by Adi Darmawan (2017, unpublished), so the morphological character of the budding phase, blooming phase and anthesis phase have been known. Darmawan (2017) mentioned that the flower of *S. macrophylla* is small, dark purplish red with 9–10 stamens and 4 anthers without pistils. The flower only blooms for 8–12 hours, and the anthesis phase takes place for 2 days. Based on this result, Darmawan recommended that further study be conducted, especially on pollen viability test for plants such as the *Averrhoa* species and *S. macrophylla* which has heterostylous flowers.

Many species in the Oxalidaceae are heterostylous (Lack & Kevan, 1987). Heterostylous plants are composed of two (distyly) or three (tristyly) morphs that differ in the heights of stigmas and anthers in flowers. The different arrangement of stigmas and anthers promotes intermorph pollen transfer (Sakai

& Wright, 2008). All of the heterostylous taxa are self-incompatible; besides, most of the heterostylous taxa are distylous, and the others are tristylous. Heterostyly is found in *Sarcotheca*, *Dapania*, *Oxalis*, *Biophytum* and *Averrhoa carambola* (Ornduff 1964; Mulcahy 1964). Based on the position of the stigma and pistil and the parthenocarpic fruits found in this species, the present study was conducted on the morphology and pollen viability test of *S. microphylla*.

MATERIAL AND METHODS

Observation of the structure of stamens and pistils

The flowers of *S. microphylla* were taken from five plants collection of Bogor Botanic Gardens. Two blooming flowers from each plant were used as sample in this observation.. The corolla was removed from the flowers, so the stamen and pistil could be visible. The flowers without corolla were placed on a glass object. After that, they were observed under a microscope with 40x magnification.

Observation of pollen morphology

Pollens were taken from the blooming flowers and placed on a glass object. The pollens were observed directly under a microscope with 400x magnification. The method used in the staining of pollen was by soaking them in Aniline Blue dye for various durations. The purpose of coloring with Aniline Blue dye dissolved in distilled water was to examine which pollen grains were fertile and which were unfertile. The quality of stored pollen grains can be tested by staining with Aniline Blue 0.1% (w/v) in 108 mM K3PO4 (pH ~11) (Mori *et al*. 2006).

The stained pollen grains were covered with a glass lid after previously getting poured with glycerin jelly as adhesives. Every corner of the glass was covered with a tiny piece of paraffin. Closure was done carefully to minimize the possibility of bubbles arising, then mounted on a microscope slide and examined using a light microscope, namely the Olympus BX53M series in the Treub laboratory at Bogor Botanic Gardens, Indonesian Institute of Sciences (LIPI). The pollen grains of *S. microphylla* were observed by SEM with 1500x magnification. The pollen grains were placed into a glass and observed under SEM.

Test of viability with the Coloring and Sucrose Solution Methods

The pollens taken from the flower were placed on a glass object which has been poured with 1% aniline blue dye solution. The observations were performed under a microscope with 100x magnification after a few minutes.

Pollen grains were taken from the flowers then placed on the preparation glass. To the grains was added 20% sucrose solution and waited for two hours at room temperature (Sudarmono *et al*. 2016). The pollen grains were taken and placed on the glass object, closed with glass cover. After that, they were observed under a microscope with 10x magnification properly. The microscope was equipped with Optica Vision Lite 2.1 software. Germination of the pollen occurs if the tube length is equal to or more than the diameter of the pollen. The percentage of pollen viability was calculated using the following formula:

$$\text{Viability: } \frac{\text{The amount of pollen that is stained in the field of view}}{\text{Total pollen in the field of view}} \times 100\%$$ (Ulfah *et al*., 2016)

RESULTS AND DISCUSSION

Structure of Flowers

S. macrophylla flower is small, approximately 0.5 cm in length and 0.6 cm in width. The petal color is red, with green calyx and corolla tube. The structure inside of the petal is composed of stamens with slender stalk called filament and the top of the stamen called anther. The shape of the anther is ovoid, and the color is yellowish. There are 10 yellow stamens. Pistil is located at the center of stigmas (Figure 1A). Adi Darmawan (2017) mentioned that *S. macrophylla* has four anthers without pistil, but there was one pistil found in our observation.

S. macrophylla stamens are heterostylous because the five stamens are long and the other five stamens are short (Figure 1B). One stigma is located in the middle of the stamens. The stigma ripened earlier than the stamen. The stigma ripened when the stamen was still developing. After the stamen developed, the stigma was reduced. Therefore, the pollination type of *S. macrophylla* is self-incompatible.

Figure 1. A. The pistil of *Sarcotheca macrophylla*, B. the structure of *Sarcotheca macrophylla* heterostylous stamen (40x)

Pollen Grains

Observation of the morphology of *S. macrophylla* pollen grains with aniline blue dye solution shows that the pollen is rounded, ovoid in shape. The pollen of *S. macrophylla* belongs to the tricolpate type with three apertures and the surfaces of the pollen grain are coarse. The shape of pollen grains observed by SEM is rounded and tricolpate (Figure 2). The shape of pollen grains of *S. macrophylla* is similar to that of other species of Oxalidaceae. Erdtman (1972) mentioned that the shape of pollen grain of Oxalidaceae family is rounded with 3 apertures (tricolpate). Tricolpate means that the surface of the pollen grain has a groove. According to Perveen & Qaiser (2003), the pollen grain character of Oxalidaceae family is tricolpate or pantacolpate. This result is also supported by Kapsah *et al*. (2016) who reported that the shape of the pollen grains is *Averrhoa dolicocarpa*. Rugayah & Sunarti and *A. leucopetala* Rugayah & Sunarti are rounded and belong to the tricolpate type.

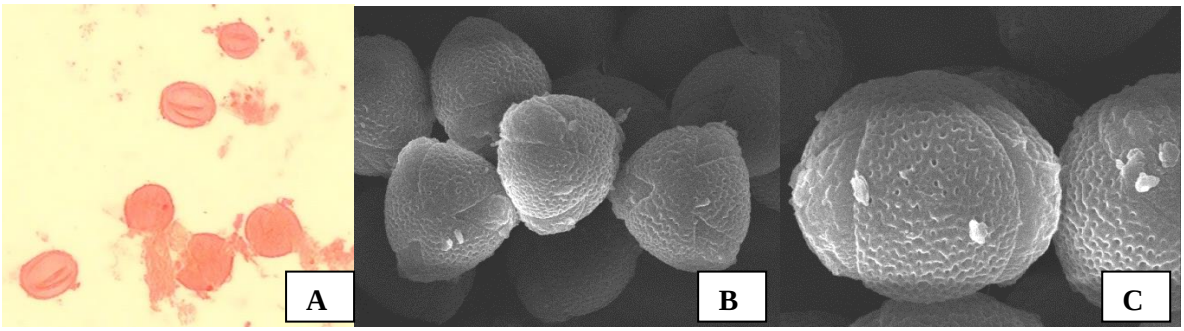


Figure 2. Morphology of *Sarcotheca macrophylla* pollen grains A. 40x, B, and C.1500x magnification

Pollen Viability

Pollen viability indicates the ability of the pollen grains to perform their function of delivering the sperm cells to the embryo sac following compatible pollination (Shivanna *et al*., 1991). Pollen viability is measured as the percentage of pollen grains produced that are viable. Pollen viability has been evaluated by various staining techniques; for example, aniline blue. The function of aniline blue

is to detect callose in pollen walls and pollen tubes (Adhikari, 1998). In this study, the pollen germinated after 5 minutes in the aniline blue (Figure 3). Therefore, the pollen grain of *S. macrophylla* tested in this study is in good quality (Figure 4). It is because the pollen grain was mature when harvested.

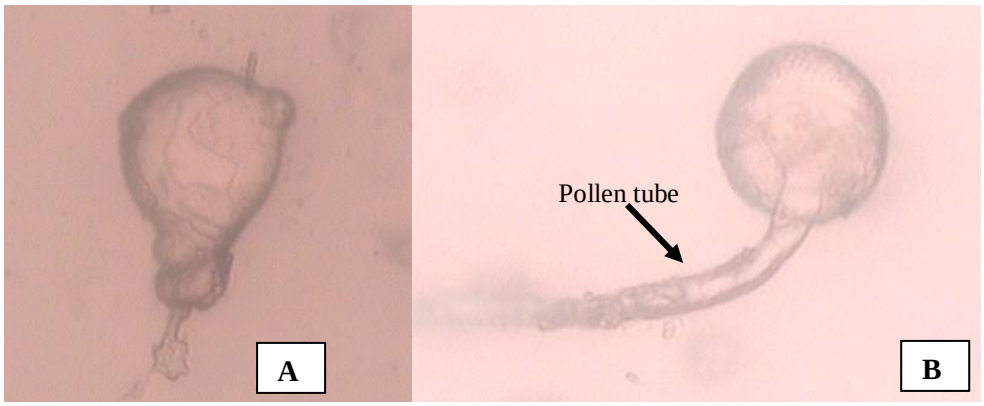


Figure 3. The process of *Sarcotheca macrophylla* pollen germination (100x) in aniline blue dye after 2 minutes (A) and 5 minutes (B)

Based on the results, 20% sucrose solution is suitable for *S. macrophylla* pollen germination. Sucrose is a common medium used in pollen germination test. Germination of pollen requires sucrose for its energy (Patel & Mankad, 2015). The pollen is said to be germinated when the tube's length is equal to or greater than the size of the pollen diameter (Sari *et al*., 2013). From the observation, it was found that the viability of *S.macrophylla* pollen was recorded 80%. According to Hersuroso *et al*., (1984), viability of >30% is a good viability for pollen. High viability can be caused by the ability to germinate well. It is also because pollen sampling was conducted in the morning at around 09.00 a.m, so the flowers taken had bloomed perfectly, and the research was conducted in a lab with a room temperature of about 20^0C. According to Perveen (2007), temperature and humidity are factors influencing the behavior and viability of pollen. In addition, the samples were immediately treated and tested because the storage of pollen can reduce its viability value (Ulfah *et al*., 2016).

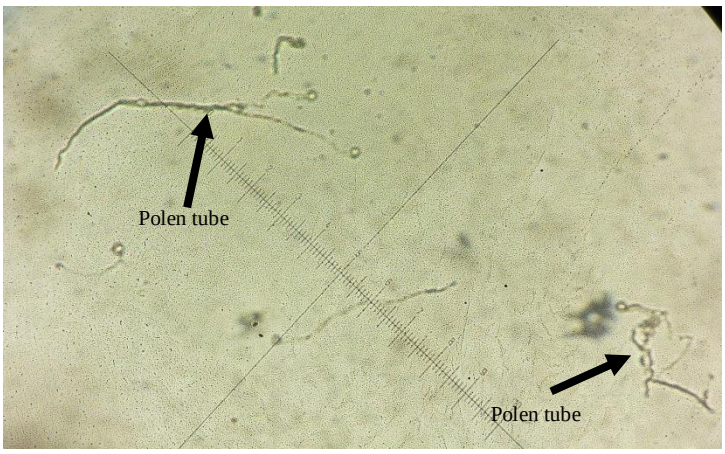


Figure 4. *Sarcotheca macrophylla* pollen germination after 4 hours in 20% sucrose solution (10x).

S. macrophylla pollen has an excellent viability; however, it was found that the pollen could not fertilize the pistil because the pollen and pistil had different maturation times. This is still a problem

of this plant. The fruit of *S. macrophylla* planted in the Bogor Botanic Garden is seedless, as result of parthenocarpy process. The fruit of *S. macrophylla* in Bogor Botanic Gardens will produce seeds if only one fruit develops in one inflorescence. The existence of parthenocarpic fruits in this species is possibly caused by the different positions of the stigma and pistil, the number of pollen grains produced, and the difference in maturity time of the pollen and pistil.

CONCLUSION

S. macrophylla has heterostylous stamens: 5 long stamens and 5 short stamens. Its pollen grains are rounded and ovoid in shape. The pollen grains belong the tricolpate type with three apertures and the surface is rough. In this study, *S. macrophylla* fresh pollen grains germinated after 5 minutes in aniline blue and 80% of the pollens germinated in 20% sucrose solution.

REFERENCES

Adhikari K. N. and C. G. Campbell. 1998. In vitro germination and viability of buckwheat (*Fagopyrum esculentum* Moench) pollen. *Euphytica* 102*:* 87-*92*

Adi Darmawan. 2017. Fenologi Perbungaan Kerumbai Merah (*Sarcotheca macrophylla*) di Kebun Raya Bogor. Skripsi. Departemen Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Pertanian Bogor.

Astuti, I.P**.,** R. Sari dan E.K. Agustin. 2009. Pemanfaatan Asam Kalimbawan (*Sarcotheca diversifolia* (Miq.) Hallier. f.) dan Kalimbawan Tikus (*S. glauca* (Hook.f.) Hallier.f.) di Kalimantan Barat. Prosiding Seminar Etnobotani IV. LIPI Press. P 449-453

Chung R.K. 1995. Oxalidaceae. In: E. Soepadmo and K.M. Wong (Eds). Flora of Sabah and Serawak Volume I . FRIM, Kuala Lumpur. p.287-296.

Erdtman, G. 1972. Pollen Morphology and Plant Taxonomy – Angiosperms (An Introduction to Polynology I). Hafner Publishing Company. New York

Hersuroso, I., Suharyo, Santoso, S. Hastjarjo, and Y. Faswani. 1984. Panduan Kebun Induk Kelapa Hibrida. Pusat Penelitian Kelapa. Medan

Kapsah, Dorly dan I.P. Astuti. 2016. Morfologi dan Viabilitas Polen Dua Spesies Belimbing Hutan (*Averrhoa dolicocarpa* dan *A.leucopetala*). Buletin Kebun Raya 19(2): 79-90

Lack AJ, Kevan PG. 1987. The reproductive biology of a distylous tree, *Sarcotheca celebica* (Oxalidaceae) in Sulawesi, Indonesia. *Botanic Journal of the Linnean Society* 95: 1–8.

Mabberley, D.J., 1997. The Plant-Book. 2nd Eds., Cambridge University Press, Cambridge.

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

Mulcahy, D. 1964. The reproductive biology of *Oxalis priceae*. *American Journal of Botany* 51: 1045-1050.

Ornduff, R.1964. The breeding system of *Oxalis suksdorfii*. *American Journal of Botany* 51:307-314.

Patel E., and Mankad. 2015. Sucrose Needs for Pollen Germination of *Impatiens balsamina* L. *International Journal of Innovative Research in Science, Enginering and Technology* 4(10): 10242-10244

Perveen, A & M. Qaiser. 2003. Pollen Flora of Pakistan – XXII. Oxalidaceae. *Pakistan Journal of Botany* 35 (1) : 3-6

Perveen, A. 2007. Pollen germination capacity, viability and maintanence of *Pisium sativum* L (Papilionaceae). *Middle-East Journal of Scientific Research* 2:79-81

Polito, V.S. 1999. Seedlessness and parthenocarpy in *Pistacia vera* L. (Anacardiaceae): temporal changes in patterns of vascular transport to ovules. *Annals of Botany* 83: 363-368.

Sakai, S., and S.J. Wright. 2008. Reproductive ecology of 21 coexisting *Psychotria* species (Rubiaceae): when is heterostyle lost? *Biological Journal of the Linnean* Society 93:125-134

Sari N.L.G.C.T., E. Kriswiyanti, and N.N. Darsini. 2013. Perkembangan mikrogametofit dan uji viabilitas serbuk sari kelapa (*Cocos nucifera* L. "Ancak"). *Jurnal Simbiosis* 1(2): 51-58

Sudarmono, D. Latifah, S. Hartini, Hary Wawangningrum. 2016. Hand-Pollination of the Giant Corpse Flower in The Bogor Botanic Gardens. *International Journal Of Conservation Science* 7(4): 1153-1160

Ulfah, S.M., Dorly., and S. Rahayu. 2015. Perkembangan Bunga dan Uji Viabilitas Serbuk Sari Bunga Lipstik *Aeschynanthus radicans* var. 'Monalisa' di Kebun Raya Bogor. *Buletin Kebun Raya* 19(1): 21-32

Veldkamp JF 1967. A revision of *Sarcotheca* Bl. & *Dapania* Korth. (Oxalidaceae). Blumea 15: 519–543.

Diversity Studies of *Piper* spp. in Bali Botanical Garden Area, The Essential Oils Yields and Antifungal Activities against *Fusarium oxysforum*

I Putu Agus Hendra Wibawa, Putri Sri Andila, Putri Kesuma Wardhani dan I Gede Tirta
BKT Kebun Raya'Eka Karya' Bali-LIPI Candikuning, Baturiti, Tabanan Bali 82191,
E-mail: agus.hen9@gmail.com

ABSTRACT

Piperaceae is a genus of plants that are commonly used by communities in many cultures for medicinal purposes and health for thousands of years. One of the beneficial components found in Piperaceae is an essential oil. Essential oils can be used as aromatherapy and herbal medicine because it has the ability as an antioxidant, antimicrobial and anti-inflammatory. This study aims to determine the variation of the essential oil content of several species of Piper that grow of Bali Botanical Gardens area, and to know its ability as an antifungal against *Fusarium oxysporum*. To find out the essential oil content of each Piper by the Distillation method using *Steam Distillation Duran*. Antifungal assay by agar diffusion method. The results showed that there are six types of piper found growing around the area of the Bali Botanical Garden, such as: *Piper aduncum*, *P. betle*, *P. capense*, *P. pendulispicum*, *P. sarmentosum* and *Piper sp*. Essential oil contained by *Piper* spp. varies between 0.412% - 0.1762%. The specific gravity of the essential oil produced is between 1,130 g/ml - 0,888 g/ml. Antifungal assay showed that only *P. betle* and *Piper* sp. has actively inhibits the *F. oxysporum*.

Keywords: essential oils, piper, Bali Botanical Gardens, plant utilization

INTRODUCTION

Piper consist of about 1,000 species, distributed widely in the tropical and subtropical regions of Asia and America (Nunes *et. al*., 2007). Plants from this genus are commonly used by communities around the world. The traditional communities in Brazil used decoction of *Piper* spp. against rheumatism, bronchitis, and sexually transmitted diseases (Agra *et al*. 2007). The Piper was used as fold medicine in India (Atal *et. al*., 1974, Nakatani, 1986). *Piper betel*. Linn has been used as a religious, recreational and medicinal plant in Southeast Asia (Fazal *et. al.*, 2013).

Piper spp. is also potentially as medicinal plant. Some studies have reported about Piper potential as antioxidant (Nakatani, 1986; Agbor *et. al*., 2007; Abrahim *et. al*., 2012; Salleh *et. al*., 2014), antimicrobial (Nakatani, 1986; Regasini *et. al*., 2009; Kumar *et. al*., 2015; Salleh *et. al*., 2015), anti-inflammatory properties (Tasleem *et. al*., 2014; Iwamoto *et. al*., 2015; Kumar *et. al*., 2015; Salleh *et. al*., 2015), anti cancer (Iwamoto *et. al*., 2015), and others.

Like other medicinal plants, most of the *Piper* spp. members content essential oil (Basak and Guha, 2015, Rath and Mohapatra, 2015, Shah *et. al*., 2016). Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. Essential oils were largely employed for their properties had already been observed in nature. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially in the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (Bakkali *et al*., 2008). These healing oils are rapidly growing in popularity because they act as herbal medicine without any side effects. This study was conducted to detect the essential oil of *Piper* spp. that grow at Bali Botanic Garden area and measure the essential oils yields and their density of each species. The antifungal assay of the essential oils against *Fusarium oxysforum* was also done.

MATERIAL AND METHODS

Plant collection

The material used in this study is leaves form several Piper species in Bali Botanical Garden area. The leaves used in this experiment are not too young and not too old (fresh). Each sample is taken as 3 x 100 gr.

Isolation of Essential Oil

To know the essential oil content, each leaf is distilled with steam system using "Laboratory Scale Distillation Schott Duran" for 5 hours (Harborne, 1987). Each type of distilled sample is repeated three times then the result is averaged to ensure its oil content. The distillation product in the form of oil and aerosol separated by a separating funnel (Schott Duran Sparator), the oil obtained is stored for further analysis. The specific gravity of the oil is calculated on an analytical scale and compared to the specific gravity of water.

Determination of Extraction Yield (% yield*)*

The essential oil yields (%, w/w) from all fresh materials were calculated according to modification of Negreiros *et al.* (2015) method, as:

$$Yield\ (\%) = \frac{W1}{W2} \times 100\%$$

Where W1 is the weight of the essential oil after hydro distillation, and W2 is the weight of the fresh plant material.

Antifungal Assay

Plant pathogenic fungi in the study were *Fusarium oxysforum*. Antifungal assay were using the disc diffusion assay method (Pratiwi, 2008). Observation of antifungal activity is carried out by observing the inhibitory zones and measuring the diameter of the inhibitory zone formed. Observations are carried out every 24 hours for 3-4 days.

RESULTS AND DISCUSSION

The diversity of *Piper* spp. used in the study were showed in table 1. There are six types of piper that are found growing at Eka Karya Botanic Gardens, Bali area. Three of them (*Piper capense*, *P. pendulispicum*, and *P. sarmentosum*) grow wildly in the Bali Botanic Garden area. *P. aduncum*, *P. betle* and *Piper* sp. is plant collection of Bali Botanic Gardens.

Tabel 1. The Plant Material that were Used in Study

No.	Species	Pictures	Origin
1	*Piper aduncum*		Bali Botanical Garden plant collection

No.	*Species*	Pictures	Origin
2	*Piper betle*		Bali Botanical Garden plant collection
3	*Piper capense*	 	Wild type grow at Bali Botanic Garden area
4	*Piper pendulispicum*		Wild type grow at Bali Botanic Garden area

No.	*Species*	Pictures	Origin
5	*Piper sarmentosum*		Wild type grow at Bali Botanic Garden area
6	*Piper* sp.		Bali Botanical Garden plant collection

An analysis of essential oil yields, density and antifungal acitivity of *Piper* spp. were conducted in this reseach, and the result was showed in Tabel 2. The result revealted that the *Piper* spp. essential oils yields vary between 0.412%- 0.162% with the highest yield is *P. capense* oil (0.412%), followed by *P. sarmentosum* (0.371%), *P. pendulispicum* (0.330%), *Piper* sp. (0.298%), *P. aduncum* (0.275%), and *P. betle* (0.165%). In comparison, a such result is similar to some previous study which reported about variation essential oil yields obtained from *Piper* spp. Navickiene at. al. (2006) reported that the hydrodistillation of *Piper aduncum* gave yields 2.0% for leaves, 2.5% for fruits, and 0.5%, for stems, calculated on basis a dry weight.

Table 2. An analysis of essential oil yields, density and antifungal acitivity of *Piper* spp.

No	Species	Plant Material	Yields of Essential oil (Fresh material) % (v/w)	Density (g/ml)	Antifungal Activities against *Fusarium oxysforum*
1	*Piper aduncum*	Leaf essential oil	0,275	1.069	-
2	*Piper betle*	Leaf essential oil	0,162	1.034	+
3	*Piper capense*	Leaf essential oil	0,412	1.122	-
4	*Piper pendulispicum*	Leaf essential oil	0,330	0.947	-
5	*Piper sarmentosum*	Leaf essential oil	0,371	1.130	-
6	*Piper* sp.	Leaf essential oil	0,298	0.888	+

The density of *Piper* spp. essential oil included in Table 2. showed that the density is between 1.130 g/ml – 0.888 g/ml. The highest leaf essential oil density is *P. sarmentosum* (1.130 g/ml), followed by *P. capense* (1.122 g/ml), *P. aduncum* (1.069 g/ml), *P. betle* (1.034 g/ml), *P. pendulispicum* (0.947 g/ml), and *Piper* sp. (0.888 g/ml).

The results showed that the density of the leaf volatile oil from several species of *Piper* spp. were larger (> 1 g / ml) than the fresh water density (1 g/ml), among others: *P. sarmentosum* (1,130 g / ml), *P. capense* (1.122 g / ml), *P. aduncum* (1.069 g / ml), and *P. betle* (1.034 g / ml), while the leaf

oil dencity of *P. pendulispicum* (0.947 g / ml), and *Piper* sp. (0.888 g / ml) werelower than the fresh water density. The case in this reseach showed that, *Piper* spp that contain higher leaf oil density (*P. aduncum*, *P. betle*, *P. capense* and *P. sarmentosum*) than the fresh water density (1 mg/ml), they were drowning at the bottom of fluid during the hydrodistilation, whereas the leaf oil of *P. pendulispicum* and *Piper* sp. (their density were less than the fresh water density) were found floating on top layer.

Figure 1. Essential oil that is partially submerged and partially floating

The antifungal activity from essential oil of *Piper* spp. against *Fusarium oxysforum* had also been studied in this study. As shown in table 2, *P. betle* and *Piper* sp. performed antifungal activity against plant phatogen fungi *F. oxysforum*, but inactive for *P. aduncum*, *P. capense*, *P. pendulispicum*, and *P. sarmentosum*. As a comparison, this result is corresponding to previous studies which have reported anti-fungal activities of *Piper* spp. essential oil. The previous data about antifungal activities of Essential oil of *Piper* spp. againts Human and Plant Phatogen Fungus were shown in Table 3.

Table 3. The previous data about antifungal activities of Essential oil of *Piper* spp. againts Human and Plant Phatogen Fungus

No.	Species	Main Compound	Antifungal Activities	Literatures
1.	*Piper betle* (The leaf essential oil)	Chavibetol (22%), estragole (15.8%), β-cubebene (13.6%), chavicol (11.8%) and caryophyllene (11.3%) as major contain (most of them was phenolic compound)	*Penicillium expansum*	Basak and Guha, 2015.
2.	*Piper betle* (The leaf essential oil)	Not Observed	Human pathogen species of *Candida viz*, *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis.*	Rath and Mohapatra, 2015.
3	*Piper betle* (The leaf essential oil)	Not Observed	*Colletotrichum* sp., *Fusarium oxysporium*, *Corynospora cassicola*, and *Rigidoporous* sp.	Shah et. al., 2016

No.	*Species*	*Main Compound*	*Antifungal Activities*	Literatures
4	*P. aduncum* (The fruit essential oils)	*Piper aduncum* was rich of monoterpene and sesquiterpene as major compound	*Cladosporium cladosporioides* and *C. sphaerospermum*	Navickiene et. al., 2006

As shown in Table 3. (The previous data about antifungal activities of Essential oil of *Piper* spp. againts Human and Plant Phatogen Fungus), there are two species of *Piper* spp. which had been reported for giving antifungal acitivies, among other: *Piper betle* (Basak and Guha, 2015, Rath and Mohapatra, 2015, Shah *et. al.*, 2016), *P. aduncum* (Navickiene *et. al.*, 2006). They active against several human and plant phatogen fungus, comprise of *Penicillium expansum* (Basak and Guha, 2015), *C. albicans* (Tirillini *et. al.*, 1996, Rath and Mohapatra, 2015) *Candida viz, C. tropicalis, G. glabrata, C. parapsilosis* (Rath and Mohapatra, 2015), *Colletotrichum* sp., *Fusarium oxysporium*, *Corynospora cassicola*, *Rigidoporous* sp. (Shah *et. al.*, 2016), *Cladosporium cladosporioides*, *C. sphaerospermum* (Navickiene *et. al*., 2006). Piper spp. essential oil containa number of chemical compounds that have capability to inhibite the phatogen fungi growth. *Piper betle* leaf oil contained Chavibetol (22%), estragole (15.8%), β-cubebene (13.6%), chavicol (11.8%) and caryophyllene (11.3%) as major chemical compound (most of them was phenolic), and predicted that this chemical content plays a important role in antifungal activity (Basak and Guha, 2015). *Piper aduncum* leaf oil contain monoterpene and sesquiterpene as major compound, the chemical compound of the species showed significantly antifungal acivities for *Cladosporium cladosporioides* and *C. sphaerospermum* (Navickiene *et. al*., 2006; Hosana, 2006).

CONCLUSION

There are six species of piper found growing around the area of the Bali Botanical Garden, such as: *Piper aduncum*, *P. betle*, *P. capense*, *P. pendulispicum*, *P. sarmentosum* and *Piper sp*. Essential oil contained by *Piper* spp. varies between 0.412% - 0.1762%. The specific gravity of the essential oil produced is between 1,130 g/ml - 0,888 g/ml. Antifungal assay showed that only *P. betle* and *Piper* sp. has actively inhibits the *F. oxysporum*.

REFERENCES

Abrahim, N. Nazirahanie , M S Kanthimathi and A. Abdul-Aziz. 2012. *Piper betle* shows antioxidant activities, inhibits MCF-7 cell proliferation and increases activities of catalase and superoxide dismutase. Journal of the International Society for Complementary Medicine Research (ISCMR)12:220. DOI: 10.1186/1472-6882-12-220

Agbor, GA, Vinson JA, Oben JE, Ngogang JY.2007. In vitro antioxidant activity of three Piper species. J Herb Pharmacother.7(2):49-64.

Agra, M. F., Freitas, P. F., Barbosa-Filho, J. M. 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Brazilian Journal of Pharmacognosy 17, 114–140.

Atal, C. K., Dhar, K. L., and Singh, J. 1974. The chemistry of Indian Piper species. Lloydia 38: 256-264.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils – a review. Food Chem. Toxicol. 46, 446–475.

Basak, S. Guha P. 2015. Modelling the effect of essential oil of betel leaf (*Piper betle* L.) on germination, growth, and apparent lag time of Penicilliumexpansum on semi-synthetic media. Int J Food Microbiol215: 171-178 doi : 10.1016/j.ijfoodmicro.2015.09.019.

Fazal F., P. P. Mane, M.P. Rai, K. R. Thilakchand, H. P. Bhat, P. S. Kamble, P. L. Palatty, M. S. Baliga. 2013. The phytochemistry, traditional uses and pharmacology of *Piper Betel*. linn (Betel Leaf): A pan-asiatic medicinal plant. Chinese Journal of Integrative Medicine: 1–11.

Harborne, J.B. 1987. Metode Fitokimia. Penuntun Cara Moderen Menganalisis Tumbuhan. Terjemahan Kosasih P. dan Iwang S.J. Penerbit ITB Bandung.

Hosana M. D. Navickiene; Andréia de A. Morandim; A. C. Alécio; L. O. Regasini; D. C. B. Bergamo; M. Telascrea; A. J. Cavalheiro, M. N. Lopes, Vanderlan da S. Bolzani, M. Furlan; M. O. M. Marques, M. C. M. Young; M. J. Kato. 2006. Composition and antifungal activity of essential oils from Piper aduncum, Piper arboreum and Piper tuberculatum. Quím. Nova. 29(3): 467-470. DOI : http://dx.doi.org/10.1590/S0100-40422006000300012

Iwamoto, L. Hespporte, D. B. Vendramini-Costa, P. A. Monteiro, A. L. T. G. Ruiz, I. M. O. Sousa, M. A. Foglio, J. E. Carvalho, and R. A. F. Rodrigues. 2015. Research Article : Anticancer and Anti-Inflammatory Activities of a Standardized Dichloromethane Extract from Piper umbellatum L. Leaves. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Volume 2015, Article ID 948737, 8 pages. DOI: http://dx.doi.org/10.1155/2015/948737

Kumar S, Malhotra S, Prasad AK, Van der Eycken EV, Bracke ME, Stetler-Stevenson WG, Parmar VS, Ghosh B. 2015. Anti-inflammatory and antioxidant properties of Piper species: a perspective from screening to molecular mechanisms. Curr Top Med Chem. 15(9):886-893.

Nakatani, N. Nakatani, R. Inatani, H. Ohta, and A. Nishioka. 1986. Chemical Constituents of Peppers (Piper spp.) and Application to Food Preservation: Naturally Occurring Antioxidative Compounds. Environmental Health Perspectives 67:135-142.

Navickiene, H.M.D.; Morandim, A.A.; Alécio, A.C; Regasini, L.O.; Bergamo, D.C.B.; Telascrea, M.; Cavalheiro, A.J.; Lopes, M.N.; Bolzani, V.S.; Furlan, M.; Marques, M.O.M; Young, M.C.M.; Kato, M.J. 2006. Composition and antifungal activity of essential oils from Piper aduncum, Piper arboreum and Piper tuberculatum. Quim. Nova 29(3) , 467-470. DOI :http://dx.doi.org/10.1590/S0100-40422006000300012

Negreiros JRS, Miqueloni DP, Cartaxo CBC. 2015. Yield of essential oil and safrole content based on fresh and dry biomass of long pepper in the Brazilian Amazon. Acta Amazonica 45: 75-80.

Nunes, J. D., Torres, G. A., Davide, L. C., Salgado, C. C., 2007: Citogenética de Piper hispidinervum e Piper aduncum. Pesquisa Agropecuária Brasileira 42, 1049–1052.

Pratiwi, S.T. 2008. Mikrobiologi Farmasi.Yogyakarta: Penerbit Erlangga. Halaman 176.

Rath CC and Mohapatra S.2015. Susceptibility characterization of Candida spp. to four essential oils.Indian J. Med Microbiol. 33 Suppl:93-96.DOI: 10.4103/0255-0857.150903

Regasini, L. O., Cotinguiba,F.,Morandim,A.A.,Kato,M.J.,Scorzoni,L.,Mendes,M. J., Bolzani, V. S., Furlan, M., 2009: Antimicrobial activity of Piper arboreum and Piper tuberculatum (Piperaceae) against opportunistic yeasts. African Journal of Biotechnology 12, 2866–2870.

Salleh WM, Kammil MF, Ahmad F, Sirat HM. 2015. Antioxidant and Anti-inflammatory Activities of Essential Oil and Extracts of Piper miniatum. Nat Prod Commun. 10(11):2005-2008.

Salleh, Hakimi Wan, Wan MohdNuzul; AHMAD, Farediah; KHONG, Heng Yen. 2014. Chemical composition of Piper stylosum Miq. and Piper ribesioides Wall. essential oils, and their antioxidant, antimicrobial and tyrosinase inhibition activities Boletín Latinoamericano y del Caribe de PlantasMedicinales y Aromáticas, 13(5):488-497.

Shah, S. Kumar, G. Garg, D., N. Patel. 2016. Article Review: Piper betle: Phytochemical, Pharmacological and Nutritional Value in Health Management. Int. J. Pharm. Sci. Rev. Res., 38(2), Pages: 181-189

Tasleem F, Azhar I, Ali SN, Perveen S, Mahmood ZA. 2014. Analgesic and anti-inflammatory activities of Piper nigrum L. Asian Pacific Journal of Tropical Medicine 7(1): S461-S468.

Tirillini B, Velasquez ER, Pellegrino R.1996.Chemical composition and antimicrobial activity of essential oil of Piper angustifolium. Planta Med. 62(4):372-3. DOI: 10.1055/s-2006-957911

Leaf, Flower, and Pollen Micromorphology: A Case Study of *Orthospihon,* Lamiaceae

SUDARMONO
Centre for Plant Conservation Botanic Gardens – LIPI
Jalan Ir. H. Juanda 13, Bogor
email: s_darmono@yahoo.com

ABSTRACT

Pollen as a part of plant reproduction plays a role in the closeness of its relationship. Leaf stomata, trichomes, and mericarp structures are also useful for limiting taxon and specific to *Orthosiphon*. This study aims to determine the relationship of four taxa on *Orthosiphon* spp. (Lamiaceae family) through the morphology of the leaves, flowers, pollen, and mericarps. Samples used were from the species of *Orthosiphon* species. The morphology of trichomes, stomata, pollen, and mericarps was observed by scanning electron microscope (SEM). to determine whether the plant belongs to *Orthosiphon aristatus* (Blume) Miq. or Cat's whisker and *Orthosiphon* spp. The leaf, flower, and pollen morphology of the Lamiaceae are similar. The peltate glands are 4-celled, stomatas are diacytic (Caryophyllaceae type), and cross-celled, similar for all *Orthosiphon* taxa. The pollen has 6 or more apertures on the surface called hexacolpate, and the pollen surface is reticulate. Mericarps: oval, brown, globous, except for small hairs at apex of *Orthosiphon* sp. "West Java".

Keywords: Orthosiphon, micromorphology, stomata, trichomes, pollen, mericarps

INTRODUCTION

Orthosiphon is a genus of the Lamiaceae. The generic name is derived from the Latin, *Iorthos* and *Siphon*; *Iorthos* means straight and *siphon* means like a tube or cylindrical, referring to the shape of the straight tube-like corolla (Keng & Siong 2006). Only *Orthosiphon aristatus* (Blume) Miq. is found in Indonesia, namely *Orthosiphon stamineus*. Benth is a synonym of this species (Plantlist 2018). This species is known locally as Kumis Kucing or Remujung (Javanese) and in English "Cat's whiskers or Java tea". This plant is a famous medicinal herb in Southeast Asia (Han, Hussin, and Ismail, 2008). It is believed that the leaves of *O.aristatus* have diuretic properties and have been used to remove uric acid stones from the kidneys (Affendy et al. 2010). It is also widely applied in traditional medicine to cure rheumatism, fever, hepatitis, gallstones, hypertension, diabetes, epilepsy and eruption (Awale et al. 2003). The dried water extract from the leaves is used for kidney disease and urinary tract treatment in Myanmar (Awale et al. 2001). On the other hand, some compounds that have anti-proliferation activity against liver-metastatic colon 26-L5 cancer cells have been detected in methanol extract from the aerial part of this plant (Awale et al. 1999). It is also rich in active chemical compounds such as steroids, oleanolic acid, polyphenols, flavonoids, and terpenoids (Awale et al. 2003).

In terms of diversity, currently only two species of *Orthosiphon* are recognized in Indonesia, namely *O. aristatus* and *O. Grandiflorus* (Backer, Bakhuizen van den Brink 1965). Morphology of the species of *Orthosiphon* is still used as a classification in plant taxonomy and there is limited information of its DNA. However, the leaf stomata and trichomes, palynological, and mericarp structures of two species of *Orthosiphon* in Indonesia have not been investigated yet. The characteristics of leaf, flower, pollen, and mericarp morphology are useful for limiting a taxon specific to *Orthosiphon*. In the Lamiaceae family, there is little information about the relationship to *Orthosiphon* spp., although some studies indicate the closeness of the relationship.

This study hence aims to determine the relationship of the Lamiaceae family through the morphology of the leaves, flowers, pollen and mericarps.

MATERIALS AND METHODS

The research was carried out from 2014 to 2017 at the Bogor Botanical Gardens, Bogor, and then continued at the SEM Laboratory, Research Center for Biology, Cibinong Science Center, Bogor and Kyung Hee University (KHU), Seoul, South Korea. Observations were done to four taxa (*Orthosiphon aristatus* collected from Central Kalimantan BO-0017329), *Orthosiphon aristatus* "pink flower" (*Orthosiphon aristatus* collected from Bogor, West Java), *Orthosiphon aristatus* "white flower" (*Orthosiphon* sp. collected from Waigeo Island, Raja Ampat Regency, West Papua (BO-18 82360), 50-66 m.27/04/2008. *Sudarmono,s.n., H. Okada, H. Tsukaya*), and *Orthosiphon* "Waigeo Island" and *Orthosiphon* sp. collected from Bogor Regency, West Java, *Orthosiphon* sp. "West Java". The observations were focused on the leaf, flower, and fruit features (Figure 1). In addition, other prominent characteristics from observations were taken into consideration. Micromorphology was observed in the leaves, flowers, pollen grains, and mericarps using Hitachi SEM (Scanning Electron Microscope). The leaves were taken from the stem, and observations of stomata, leaf glands, pollen and mericarps were made using a scanning electron microscope (SEM). Samples were cleaned with 95% Ethanol fixation, vacuum dried, coated with gold, and observed with SEM. The sizes of pollen and mericarps were determined based on the scale in the image. The stomata was classified based on Metcalfe and Chalk (1950), using the terms "anomocytic" for Ranunculaceous, "anisocytic" for Cruciferous, "diacytic" for Caryophyllaceousor, "labiateous" and "paracytic" for Rubiaceous stomata. Leaf trichomes were distinguished and classified according to Singh et al. (1974), Cantino (1990) and Salmaki et al. (2009). Pollen observations used the parameters of size, type, and ornamentation (Punt et al. 2007; Erdtman G. 1945)). Mericarp size was also observed as well as the presence or absence of hair on the mericarp coat. The mericarps were categorized based on Barthlott (1981) and Stearn (1983). Herbarium vouchers were held at Herbarium Bogoriense (BO).

Figure 1. Left to right, four taxa in this study, i.e. *Orthosiphon aristatus* "pink flower", *Orthosiphon aristatus* "whiteflower", *Orthosiphon* sp. nov (collected from Waigeo island, West papua), and *Orthosiphon* sp. nov. (collected from West Java).

RESULTS AND DISCUSSION

The results of the study on Orthosiphon morphology are as follows:

Orthosiphon aristatus "pink flower" (Figure 2A). Habit: shrub 1–1.5m high. Leaves: simple, obovate, acute-rhomboid–ovate, crenate – serrate, 2X7 cm, bifacial, amphistomatic. Stomata type: diacytic (Caryophyllaceae type), cross-celled, 15X25 µm. Trichomes: peltate gland trichomes with 4 cell heads. Inflorescences: terminal, 13-19 nodes, 5 or 6 flowers per node; Inflorescence 14 – 29.4cm; color of corolla pink (1.5-2.2 cm); Stamen 3-5 cm and style length 4-5.2 cm. Petiole 5-7 mm and calyx 3-5 mm. Pollen (Figure 3): monadic, medium sized, and hexacolpate. Polar axis (P) 53µm, equatorial

axis (E) 74µm, and P/E level 0.71. In equatorial view, the pollen shape is oblate. Its ornamentation is reticulate. Mericarps: oval, bald, and brown, with no hair.

Orthosiphon aristatus "white flower" (Figure 2B). Shrub: 1.0 – 1.5 m high, simple, obovate, acute-rhomboid–ovate, crenate–serrated, 2.5X7cm. Leaf: bifacial, amphistomatic. Stomata type: diacytic (Caryophyllaceae type), cross-celled, size 12X20 m. Trichomes: peltate gland trichomes with 4 cell heads. Inflorescence: terminal, 13-15 nodes, 5-6 flowers per node 12.4 m, pinkish white corolla 2.2-2.5 cm; Stamen 0.8 cm and long style. Petiole 4.5 mm and calyx 5.7 mm. Pollen in Figure 3: monad, medium sized, and hexacolpate. Polar axis (P) 50µm, equatorial axis (E) 70µm, and P/E level 0.71, equatorial side, pollen shape oblate; its ornamentation reticulate. Mericarps: ovate, bald, and brown, with no hair.

Orthosiphon sp "Waigeo island" (Figure 2C). Shrubs: 60-120 cm high, simple, ovate, acute, serrate–sub serrate 1.4X3.3 (3.8X8) cm. Leaf: bifacial, amphistomatic. Stomata type: diacytic (Caryophyllaceae type), cross-celled, size 15-20X17-26 µm. Trichomes: peltate gland trichomes with 4 cell heads. Inflorescence: terminal, 5-12 nodes, 5 or 6 flowers per node, pinkish white corolla. Pollen (Figure 3): monad, medium sized, and hexacolpate. Polar axis (P) 43 µm, from the equator axis (E) 57µm, and P/E level 0.75; in equatorial view, the shape of the pollen is suboblate. Mericarps: ovoid, bare, and brown, no hair.

Orthosiphon sp. "West Java" (Figure 2D). Mericarps: ovoid, surface prominent reticular pattern and brown; no hair. Leaf: bifacial, amphistomatic. Stomata type: diacytic (Caryophyllaceae type), cross-celled, size 11.1X22.2 µm. Trichomes: peltate gland trichomes with 4 cell heads. Inflorescence: 9–14.7 cm, terminal, 14–16 nodes, 5 or 6 flowers per node, pinkish white corolla. Pollen (Figure 3): monad, medium sized, and hexacolpate. Polar axis (P) 25 µm, from the equator axis (E) 30µm, and P/E level 0.83, equatorially the shape of the pollen is suboblate. Mericarps: ovate, mericarp surface with a prominent reticulate pattern and brown. No hairs or few hairs in apex.

Both *O. Aristatus* "white and pink flowers" produce the same green leaves, but the leaf veins on pink flowers are darker. However, both of them can be distinguished because of the pink flower stem and branches and the dark pink leaf veins. Meanwhile, in white flowers, the leaves are bright green. Keng & Siong (2006) concluded that both the white variety and the pink variety have leaves, anthers, stigma, and pollen grains which are similar. The leaves of pink *Orthosiphon*is (7X2 cm) are larger than those of the white flowers (7X2.5cm). The size is strongly influenced by environmental conditions, such as soil fertility. According to Keng & Siong (2006), the length and width of the leaves on white flowers were 5.7X2.5cm (length to width ratio 2: 1) in the shape of rhomboid and on pink flowers 4.5X2.7cm (ratio 3: 2) in ovate shape. However, the size and shape of the leaves of both are relatively the same, only the color of the stem and leaves are darker or purplish. The species will of course be easier to distinguish if they are flowering because of the different colors of the flowers. Another factor other than flowering to be observed is the leaf section, i.e. the stomata. The stomata on all four taxa have the same type, namely diacytic (Caryophyllaceen type) and cross-celled. According to Naidu & Shah (1981), the structure and ontogeny of cotyledonary stomata and trichomes are described in 34 species of *Lamiaceae.* Stomata can be diacytic, anomocytic, haplocytic, transitional, tetracytic, paracytic, and tricytic. Different types often occur side by side, and even on the leaf. The most frequent type is diacytic on both surfaces in most taxa, but it is anomocytic on adaxial surface or both surfaces in some. Anisocytic is rarely found. Transitional form or haplocyticstomata are also dominant on the adaxial surface. Naid. & Shah (1981) also described that trichomes were present on both surfaces. Trichomes can be glandular or non-glandular. The taxonomic significance of stomata and trichomes to delineate genera and species is also suggested. According to Celep et al. (2014), three main types of *Salvia quezelii* trichomes are peltate, glandular, and non-glandular. Head-shaped trichomes and non-glandular glands are further divided into several types. Trichome glands are present in abundance in all vegetative parts, especially in the inflorescence axis. Some studies of pollen are also associated with the micromorphology of the leaves and mericarps. Research by Eshratifar, Attar, & Mahdigholi (2010) shows that the micromorphology of the pollen can be seen in polar and equatorial axes. Polyad pollen grains are known to have five tribes, namely: Annonaceae, Leguminosae, Hippocrateaceae (in the Hippocraea clan), Asclepiadaceae, and Orchidaceae (Septina 2004). The pollens on *Orthosiphon aristatus* and *O*. sp. nov are categorized as medium pollen. However, the shapes in all four taxa are the same, namely hexacolpate, and this shape is common in the family Lamiaceae (Gençay, et al. 2008).

The mericarps of all four taxa are almost ovate and hairless. Only on *O.* sp. nov. from West Papua, little hair was found on the edges. Tarimcilar et al. (2013) reported that the mericarp morphology of the 11 taxa of *Mentha s*pp. distributed throughout Turkey was broadly oblong or ovoid. Based on the data of trichomes, stomata, palynology and mericarps, the systematic value of the four taxa of *Orthosiphon* morphology features in Lamiaceae can be used to evaluate the relationship of family members of Lamiaceae. Plant systematics generally uses morphological characteristics to classify its diversity into its taxonomic subunits, while plant morphology uses these variations to deduce the basic shapes without regard to its systematic relationships. Characterization of certain plant organs can be found in other organs which are usually smaller or finer called micromorphology or additional complementary organs (organa accessoria), such as hair or trichomes, stomata, and pollen. Together with stem morphological features, leaf stalks and leaf anatomy, the type and distribution of trichomes, pollen and mericarp micromorphology became useful in Salvia systematics (Kahraman et al. 2009a, b)

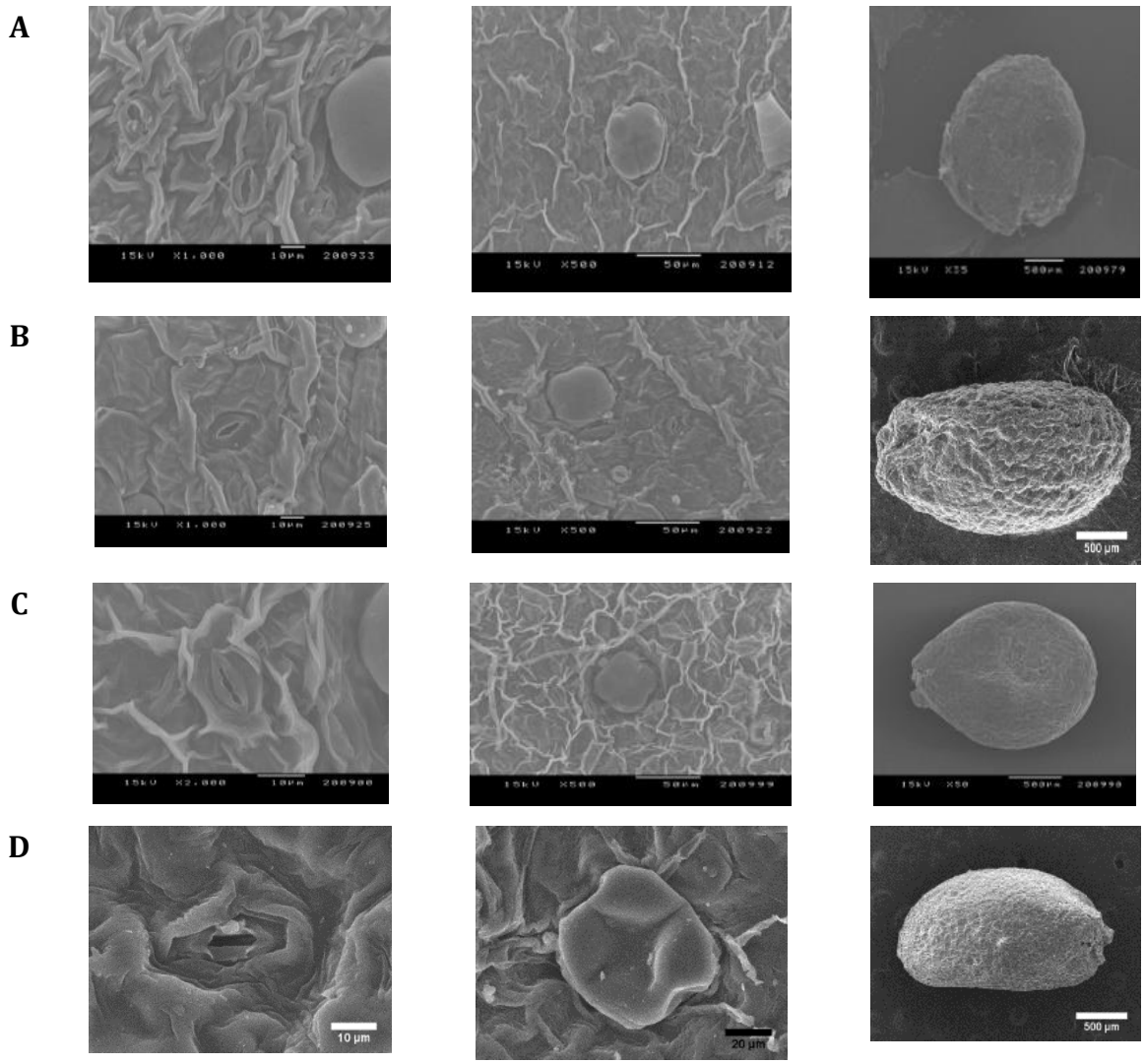


Figure 2. Stomata, trichomes, and mericarps on *Orthosiphon aristatus* "pink flower" (A), .*Orthosiphon aristatus* "pink flower" (B), *Orthosiphon* sp. " Waigeo island" (C) and *Orthosiphon* sp. "West Java". (D). The size is listed below the scale line of each of the images.

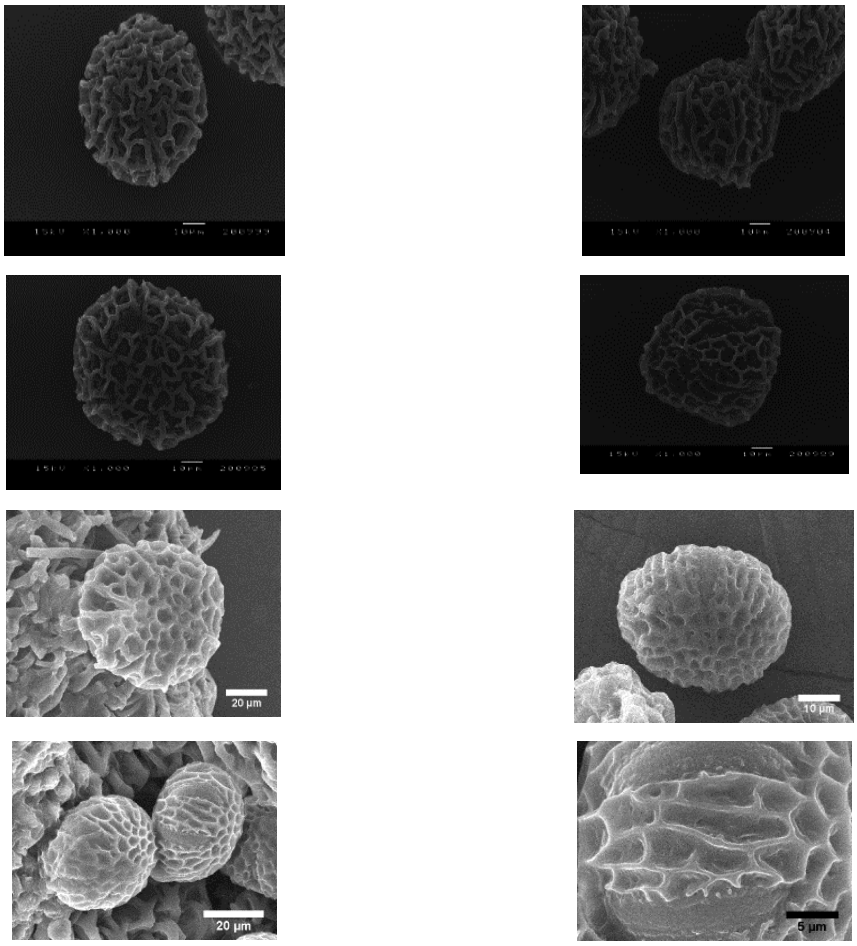


Figure 3. Figure of pollen morphology of *Orthosiphon aristatus* "pink flower", *Orthosiphon aristatus* "pink flower", *Orthosiphon* sp." Waigeo island" and *Orthosiphon* sp." West Java". The size of the pollen is listed below the scale line of each of the images.

CONCLUSION

The leaf stomata, trichomes, pollen, and mericarp micromorphology of the four taxa of *Orthosiphon* (*Orthosiphon aristatus* "pink flower", *Orthosiphon aristatus* "white flower", *Orthosiphon* "Waigeo island", and *Orthosiphon* sp. "West Java", a genus of the Lamiaceae members in leaves (stomata and trichomes), flowers (pollen), and mericarps are similar to each other. The stomata have a relatively similar size and the same type, namely diacytic (Caryophyllaceen type) and cross-celled. Trichomes: peltate gland trichomes with 4 cell heads. Pollen: hexacolpate, suboblate shaped. Mericarp is ovoid-ovate shaped and with no hair, except for small hairs on the apex of *Orthosiphon* sp. "West Java".

REFERENCES

Affendy, H., Aminuddin, M., Arifin, A., Mandy, M., Julius, K. and Tamer, A.T. 2010 Effects of light intensity on *Orthosiphon stamineus* Benth. nutlets lings treated with different organic fertilizers. *International Journal of Agricultural Research*, 2010;5(4): 201-207.

Awale S, Tezuka Y, Banskota AH, Adnyana IK, Kadota S 2003 Nitric oxide inhibitory isopimarane-type diterpenes from *Orthosiphon stamineus* of Indonesia. *Journal of Natural Products*, 2003; 66(2): 255-258.

Awale S, Y. Tezuka, A. H. Banskota, K. Kouda, K. M. Tun, and S. Kadota. 2001 Five novel highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar. *Journal of Natural Products*, 2001; 64(5): 592-596.

Awale S**,** Tezuka Y, Shimoji S, Taira K, Kadota S. 1999 Secoorthosiphols A-C: Three highly oxygenated secoisopimarane-type diterpenes from *Orthosiphon stamineus*. *Tetrahedron Letters*, 2002; 43(8):1473- 1475.

Awale S, Y. Tezuka, A. H. Banskota, and S. Kadota, 2003 Inhibition of NO production by highly oxygenated diterpenes of *Orthosiphon stamineus* and their structure-activity relationship. *Biological and Pharmaceutical Bulletin*, 2003; 26(4): 468-473.

Backer CA, Bakhuizen van den Brink RC 1965 *Lamiaceae*, *Flora of Java*. NVP Noordhoff, Groningen, pp 614–640

Celep, F., Kahraman, A., Atalay, Z. & Doğan, M. (2014) Morphology, anatomy, palynology, mericarp and trichomemicromorphology of the rediscovered Turkish endemic *Salvia quezelii* (Lamiaceae) and their taxonomic implications. *Plant Systematics and Evolution* 300: 1945–1958.

Eshratifar M., Attar F. & Mahdigholi K. 2010. Micromorphological studies on nutlets and leaf indumentum of genus *Teucrium* L. (Lamiaceae) in Iran. Turk J. Bot. **35**: 25–35.

Erdtman G 1945 Pollen morphology and plant taxonomy IV. Labiatae, Verbenaceae and Avicenniaceae. Svensk Botanisk Tidskrift 39:279–285

Han, C.J., A.H. Hussin, and S. Ismail, 2008 Toxicity study of *Orthosiphon stamineus* Benth (misai kucing) on Sprague dawley rats. *Tropical Biomedicine*, 2008; 25(1): 9–16.

Stampoulis, P., Y. Tezuka 1999Staminol A, a novel diterpene from *Orthosiphon stamineus. Tetrahedron Letters*,; 40(22): 4239-4242.

Kahraman A, Celep F, Dogan M 2009a Comparative morphology, anatomy and palynology of two Salvia L. species (Lamiaceae).Bangl J Plant Taxon 16:73–82

Kahraman A, Celep F, Dogan M 2009b Morphology, anatomy and palynology of Salvia indica L. (Labiatae). World App Sci J 6:289–296

Keng C.L. & L.P. Siong , 2006. Morphological Similarities and Differences between the Two Varieties of Cat`s Whiskers (*Orthosiphon stamineus* Benth.) grown in Malaysia. *International Journal of Botany, 2: 1-6.)*

Lee, Sang-Myoung ; Lee, Hwa-Yuon 2012. Pollen Morphology of the Family Lamiaceae in Mongolia; Journal of Asia-Pacific Biodiversity ▪Volume 5, Issue ,2, 2012, pp.169-179 ▪Publisher : National Science Museum of Korea ▪DOI : 10.7229/jkn.2012.5.2.169).

MetCalfe, C. R. & L. Chalk. 1950. Anatomy of the Dicotyledons I + IL ClarendonPress, Oxford.

Naidu A. C. & Shah G. L. 1981. Observations on the cotyledonary stomataand trichomes and their ontogeny in some genera of *Lamiaceae.* — Phyton(Austria) 21 (1): 137—152

Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Thomas, A.L., 2007. Glossary of pollen and spore terminology. Rev. Palaeobot. Palynol. 143, 1–81.

Tarimcilar, G., Yilmaz O., Daskin R and Kaynak, G. 2013. Nutlets morphological and its Taxonomic Significance in theGenus*Mentha* L. (Lamiaceae) from Turkey. Bangladesh J. Plant Taxon. **20**(1): 9-18

Lygodium spp. of Bukit Bungkuk Nature Reserve and Its Growth in Bogor Botanical Gardens

Sri Hartini and Sahromi*
Center for Plant Conservation- Botanical Gardens, Indonesian Institute of Sciences (LIPI)
Jl. Ir. H. Juanda 13 P.O. BOX 309 Bogor 16003. Ph. (+62251) 8352518, Fax. +62 (251) 322187.
*Email: ssahromi@yahoo.co.id

ABSTRACT

Lygodium is a group of potential terrestrial fern in the family Schizaeaceae. *Lygodium* commonly has an important economic value, but information concerning its cultivation and conservation is still limited. The economic values of *Lygodium* spp. include its benefits for medicinal plants, crafts and vegetables/food. Bogor Botanical Gardens, as an *ex-situ* conservation institution, can play important roles in the conservation of the genus. This research was aimed to reveal the diversity of *Lygodium* species in Bukit Bungkuk Nature Reserve, Riau Province and to observe the growth of the collected specimens in Bogor Botanical Gardens. The method used for the inventory of *Lygodium* spp. in Bukit Bungkuk Nature Reserve was exploratory, in which researchers randomly explore an area . The observation of the growth of *Lygodium* spp. collection, employing a descriptive method, was conducted at Bogor Botanical Gardens Nursery. It was reported that there were three species of *Lygodium* in Bukit Bungkuk Nature Reserve, i.e *L .microphyllum* (Cav.) R.Br., *L. flexuosum* (L.) Sw. and *L .circinatum* (Burm.f.) Sw. The species grew up well in the garden.

Keywords: *Lygodium* spp., growth, Bogor Botanical Gardens, Bukit Bungkuk Nature Reserve

INTRODUCTION

Lygodium is one of the genera in the family Schizaeaceae. Species that are included in this genus have long rachis that reach up to 10 m. In the world, there are about 41 species of *Lygodium* (Hoshizaki & Moran, 2001), mostly in the tropics and subtropics.

Some species of *Lygodium* have been known for their benefits, but there are also species which, based on previous research results, were proved to be invasive plants that could disrupt the existing plants in the vicinity. Some of the species can be used in traditional medicine such as *L. circinatum* and *L. flexuosum*; the young leaves can be consumed as a vegetable (all species *Lygodium*); and the plants can also be made into craft such as *L. circinatum* and for other uses. The species of *Lygodium* included in the list of invasive plants are *L. microphyllum* and *L. japonicum*.

Bogor Botanical Gardens as an ex-situ conservation institution has a major role in the protection of plant species, including the species of *Lygodium*. Bogor Botanical Gardens has collected *Lygodium* since 1920, starting with *L .circinatum* from Sumatera (Wigman, 1920). Until now, the attempt of collecting more plants from this genus has been continuously made. The species collected were not only those that have been known for their benefits, but also other species of unknown utility. Based on the data of the registration of Bogor Botanical Gardens, the species of *Lygodium* that have been collected in Bogor Botanical Gardens were *L. circinatum*, *L. flexuosum*, *L. salicifolium*, *L. scandens* (*L. microphyllum*) and *L. bifurcatum*. According to Astuti *et al*. (2001), there were three surviving species of *Lygodium* collected in the gardens, *L. circinatum*, *L. japonicum* and *L.salicifolium*. However, based on observations in the garden, there was only one specimen of *L ygodium* that remained, namely *L. circinatum*. For this reason, it is necessary to add more species to the collection through exploration.

In an effort to fulfill their mission of ex-situ conservation, botanic gardens in Indonesia have carried out explorations and plant inventory to study plant diversity. One of the interesting topics in Bogor Botanic Garden is the research on ferns. The research aims to create an inventory of fern diversity, especially *L ygodium* spp. in Bukit Bungkuk Nature Reserve, to enrich the *Lygodium* collection of Bogor Botanical Gardens, and also to find out the growth of the collection at Bogor Botanical Gardens. The results are expected to be used as an initial step to determine their conservation strategies in the future. Although Bogor Botanical Gardens has long had a collection of species from this genus, the species

have never been observed for their growth since they get collected from the forest until they are ready to be planted in the garden.

MATERIALS AND METHODS

Study Site

The research was conducted in the area of Bukit Bungkuk Nature Reserve, located in the Sub-District of Koto Kampar XIII and West Bangkinang Sub-District, Kampar Regency, Riau Province, Indonesia, from April 19 to May 18, 2008.

Bukit Bungkuk Nature Reserve is one of the in-situ conservations in Riau Province. Administratively, it is included in the Sub-District of Koto Kampar XIII and Sub-District of West Bangkinang, Kampar Regency. This area is located at an altitude of 100–200 m above sea level, with a lowland rain forest ecosystem. In general, the condition of the forests of Bukit Bungkuk Nature Reserve is still quite good, both as primary and secondary forests. The degree of soil acidity (pH) measured in some areas explored ranged from 4.5 to 6.8. The average air temperature from morning, noon to evening ranged for 22°C, 28°C and 23°C, respectively. Meanwhile, the average air humidity from morning, noon till night ranged for 100%, 65% and 90%, respectively. Forest conditions varied, from closed to very open. Closed forests were still relatively intact, while very open forests have been cleared by the local people who intended to plant the cleared land with tree crops such as rubber, gambier or food crops. The opening of the forests in this region was quite extensive, probably due to the area's position by the river that is accessible and the weak supervision.

Procedures

The exploration of *Lygodium* spp. was based on plant collection and carried out through purposive random sampling (Partomihardjo & Rahajoe, 2005). The living materials collected were mostly planted in Bogor Botanical Gardens for ex-situ conservation purposes. Identification methods were used by reference to herbarium specimens in the Herbarium Bogoriense and or living collections in the Bogor Botanical Gardens (Hidayat *et al*., 2017). Literature reviews (Andrews, 1990; Hoshizaki & Moran, 2001) were also deployed to support identification. Valid names were attributed based on the current listings in "The Plant List, A Working List of All Plant Species" (www.theplantlist.org).

The collection of sporelings was done by rotating the sporelings along with their soil in the surrounding areas. The soil attached to the roots were cleaned or reduced and then the roots were soaked in a solution of growth stimulant such as Rootone-f, coupled with humus, and then wrapped with colored (black) plastic and labeled. To reduce excessive evaporation, reduction or defoliation of all leaves, except for the new emerging shoots, was carried out. Subsequently, the collections were covered with plastic herbarium, slightly puffed and tied.

Observations in the field were conducted to describe the morphological characteristics and habitat of the species found. Some ecological data such as altitude, soil pH, average daily temperatures and average daily humidity at the location in which *Lygodium* plants were found were noted to obtain more complete information about the natural conditions of the collected species.

The growth observations of *Lygodium* collections were made in the greenhouse for ferns collection of Bogor Botanical Gardens. There were three collections of *Lygodium* with different numbers of specimens among the species. The specimens were then planted in polybags with a mixture of soil and compost media with a ratio of 1: 1 and a pH of about 5. They were placed under a paranet shade (40%) and observed every two weeks for six months to determine the growth and development of the collection. The observation results recorded included the addition of the length of the plant and the freshness of the collections.

RESULTS AND DISCUSSION

Results of the inventory and identification reveal that in the area of Bukit Bungkuk Nature Reserve, there were three species of *Lygodium* (Table 1.), namely *L. microphyllum, L. flexuosum* and *L. circinatum* (Fig. 1).

Table 1. Collections of *Lygodium* from Bukit Bungkuk Nature Reserve

No.	Species	Collection Number	Access Number	Number of Specimens
1	*Lygodium circinatum* (Burm. f.) Sw.	TT 1198	B200712222	2
2	*Lygodium flexuosum* (L.) Sw.	TT 1188	B200712211	3
3	*Lygodium microphyllum* (Cav.) R. Br.	TT 1187	B200712212	4

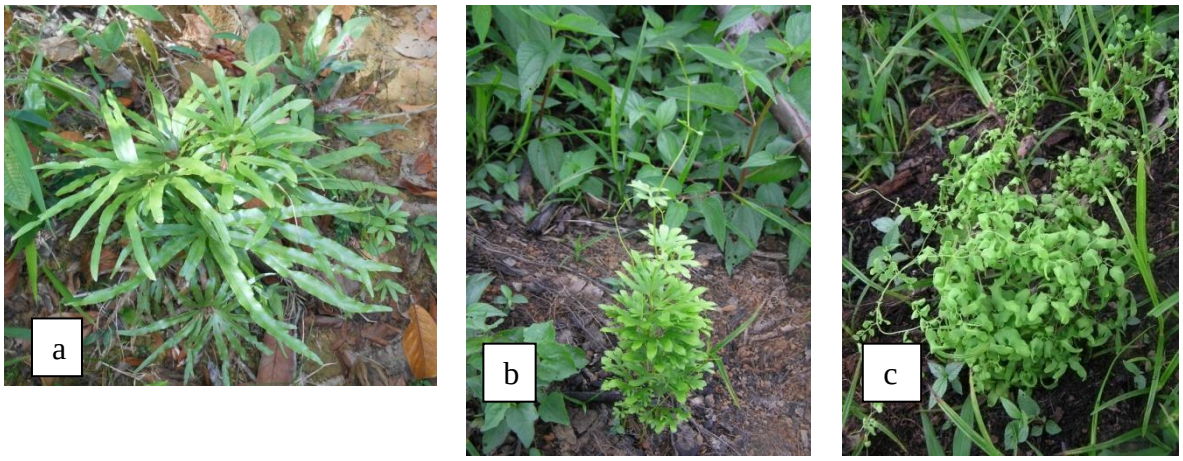


Figure 1. (a)*Lygodium circinatum*, (b) *Lygodium flexuosum*, (c) *Lygodium microphyllum*

***Lygodium circinatum* (Burm. f.) Sw.**

This species is also known as *akarkawek* (wire roots), although the size of the main rachis was larger than the main rachis of *L. microphyllum* and *L. flexuosum*. *L. circinatum* is also a fern that can be large in size (its length can reach 10 m) with short-creeping rhizomes. This plant also has two kinds of leaves, sterile and fertile leaves. The leaflets on sterile leaves can reach 25 cm in length. Leaflets are actually leaf lobes with lines and oblongs and very deep in dentations similar to leaflets arranged in pinnate. This species is a native plant of India, South China, Southeast Asia, Malaysia and Melanesia (Hoshizaki & Moran, 2001). It is alternatively called *Hydroglossum circinnatum* (Burm. f.) Willd.and *Ophioglossum circinnatum* Burm. f. (The plant list, 2017).

At Bukit Bungkuk Nature Reserve, this species was found several times in a shaded area on the edge of the forest, not inside the forest. As was the case with the species of *Lygodium flexuosum*, this species was not included in the List of Invasive Plant Species in Florida Exotic of Pest Plant Council's 2001.

***Lygodium flexuosum* (L.) Sw.**

Local communities of Bukit Bungkuk Nature Reserve area also know this species as *akarkawek* (wire roots). This species has alternative names *Hydroglossum circinnatum* (Burm. f.) Willd.and *Ophioglossum circinnatum* Burm. f. (The plant list, 2017). It has a strong main rachis like a twining frond and is larger than *L. microphyllum*. *L. flexuosum* is also a large fern that can enlarge its size with short creeping rhizomes. This plant also has two kinds of leaves, sterile and fertile leaves. The leaflets on sterile leaves are the elongated lateral portion consisting of only one or two lobes, while those on the tip consist of two or three lobes and 3–10 cm long leaflets.

This species is a native plant of India, South China, Japan and Malaysia and also Australia and Melanesia (Hoshizaki & Moran, 2001). In the Nature Reserve of Bukit Bungkuk, the species is also commonly found in a very open area, usually growing together with *L. microphyllum* at the logged and burned areas of the forest. But the number of plants found was not as many as that of *L. microphyllum*.

According to Florida Exotic of Pest Plant Council's 2001, this species is not included in the List of Invasive Plant Species. In the area of primary forests that are shaded, this species was also not found. However, this species is known to dislike places that are too open.

***Lygodium microphyllum* (Cav.) R. Br.**

Local communities of the surrounding area know this species as *akar kawek* (wire roots) because it has a strong globular main rachis and stems like wire. Communities in West Java know this species as *hataleutik* because this plant has small leaflets. This species is also called rope fern because its stem (actually a leaf spine) can grow very long; hence, it is widely used to make rope. This species has synonyms such as *Lygodium scandens* (L.) Swartz.,*Ugena microphylla* Cav. R.Br. (Andrews, 1990) and also *Ophioglossum filiforme* Roxb. (The plant list, 2017). Internationally, this species is known as the climbing maiden hair, snake fern (Andrews, 1990) or small-leaf climbing fern (Hoshizaki & Moran, 2001).

L. microphyllum is a fern which can be large in size with short creeping rhizomes. This plant has sterile and fertile leaves. Leaflets of sterile leaves are oval to elongated ovoid, up to 2.5 cm long, with heart-shaped base and jagged edges. Leaflets of fertile leaves are triangular-shaped, with flat base and rounded end portion, smaller than sterile leaves.

In Bukit Bungkuk Nature Reserve, this species was found in a very open place, at primary forest areas that had been logged and burned for planting. The degree of acidity of the soil at this location was on average about 5.4. *L. microphyllum* in this location grew in group and spread almost evenly across the open areas. Many of the plants were still little-stemmed plants beginning to creep. This species was found to grow up together with pioneer plants such as *Mallotus* seedlings that were very abundant in open locations and other shrubs species. In the primary forest area with shaded condition, this species was not found.

In the wild, *L. microphyllum* generally likes living in an open to very open place in unspoiled forests, riparian zones and fern wet places (Andrews, 1990) at an altitude of 1,500 m above sea level. The species is believed to originate from Africa, Southeast Asia, Australia and Polynesia and this species has many uses (Hoshizaki & Moran, 2001). The trunk has been used to make ropes and baskets. It has also been used as medicines for intestinal thrush, dysentery, fever, skin diseases, chicken pox and swelling. Its young leaves can also be consumed as vegetable.

In the United States, *L. microphyllum* has become an invasive plant that is difficult to be controlled. According to the Florida Exotic of Pest Plant Council, this species belongs to category I in the List of Invasive Plant Species (FLEPPC, 2001). The species of invasive plants included in Category I show high level of invasiveness. This species grows up and spread rapidly to form large colonies that will dominate a region. It is a climber and will cover other vegetation until the vegetation turns invisible (Pemberton & Ferriter, 1998).The mixed mating system observed *in L. microphyllum* appears to give this species the ability to invade distant habitats and then adapt to local conditions (Lott *et al*., 2003). Research results indicate that *L. microphyllum* will undergo global range expansion in concert with anthropogenic global warming and continued dispersal (Humphreys *et al*., 2017).

Lygodium has invaded many habitats in Florida, growing over trees and shrubs, smothering whole plant communities. It is difficult for other plants to grow through the thick fern mats. *L. microphyllum* is common in cypress stands, but also infests pine flatwoods, wet prairies, sawgrass marshes, mangrove communities, and everglades tree islands (Ferriter, 2001).

L. microphyllum dominates both understory and overstory native wetland habitats and has the ability to grow in varying hydrological (Gandiaga *et al*., 2009), nutrient (Volin *et al.*, 2010), soil pH (Soti *et al.,* 2014) and light gradients (Volin *et a*l., 2004). Soti *et al*. (2014) have shown that *L. microphyllum* is highly dependent on mycorrhizal fungi for growth and phosphorus uptake which can highly enhance its invasiveness.

The Growth of *Lygodium* spp. at Bogor Botanical Gardens

The acclimatization of *Lygodium* resulted from the exploration in the area of Bukit Bungkuk Nature Reserve was done in Bogor Botanical Garden Nursery. The growth and development of each species during the acclimatization period are as follows.

***Lygodium circinatum* (Burm. f.) Sw.**

The specimens were taken from two pieces of large clumps which had 5–6 fronds with a length of 1–10 m and a couple of small shoots that began to appear. However, the fronds were then cut to only about 15 cm long and the leaves were removed. The specimen collection was numbered TT 1198 and given access number B200712222.

The shoots contained in this specimen were observed for their growth during the first month of the acclimatization period. However, the growth of this species seemed to be slower than the previous two species of *Lygodium*. After a month in Bogor Botanical Gardens, the shoots only grew for about 5 cm. Nevertheless, the plant grew up better in the following months. In the second and third months, increased frond length and number of fronds were observed. In the fourth month, nearly all plants produced new fronds and the longest frond was 80 cm with four pairs of leaflets. Bamboo stakes were also fitted to support the fronds. Transplanting and switching the location of maintenance was performed on the sixth month and did not have much effect on the growth of *L. circinnatum*. The longest frond reached about 1.2 m and the shortest 0.25 m.

***Lygodium flexuosum* (L.) Sw.**

For this species, living specimens collected from the field were in the form of three small clumps, each with 3–4 fronds and 20–30 cm long. The collection was numbered TT 1188 and registered as a collection of Bogor Botanical Gardens with access number B200712211.

Based on observations, this species was shown to grow up well. In the first month, the plant stems grew up longer and new shoots appeared. The longest main rachis reached 40 cm and the shortest about 5 cm (the main rachis of new shoots). The growth of new shoots of this species appeared not as fast as that on *L. microphyllum*. In the following (second and third) months, the growth of the plant improved, characterized by an increasing length of the main rachis and number of leaves. By the fourth month, almost all of the specimens had produced new leaves and the longest main rachis reached 80 cm with four pairs of leaves. Bamboo stakes were also fitted for the main rachis of this plant to climb. In the fifth month, the frond length increased and young shoots also appeared.

Transplanting and transfer of maintenance locations as in *L. microphyllum* was also performed on this collection. As a result, the plant could grow up well, the growth of main rachis length and number of leaves also increased. The longest frond reached about 1.2 m and the shortest 0.25 m.

***Lygodium microphyllum* (Cav.) R. Br.**

Living specimens that were collected from the field were in the form of four small clumps, each having 3–4 fronds with a length of 10–20 cm. The collection was numbered TT 1187 and listed as a collection of Bogor Botanical Gardens with access number B200712212.

In the first period of acclimatization, four specimens grew up longer and produced new shoots. The longest main rachis reached a length of 30 cm and the shortest about 10 cm (stems from new shoots appeared). The plant growth seemed to be getting better in the second and third months. Plastic covering method used at the time of collection in the field and its delivery to the Bogor botanical Gardens was able to help the recovery process, so the plants became quickly adapted and could produce new shoots.

By the fourth month, almost all of the specimens had produced new leaves, although leaf development quite varied. In the fourth month, the longest frond reached a length of 1 m and had six pairs of leaflets. In the fifth month, the stem continued to grow and a few young shoots appeared again. This indicates that the acclimatization process occurred well and the growth medium used was quite adequate.

In the sixth month, transplanting (pot replacement and media additions) and switching of maintenance locations from the greenhouse to ferns collection of Bogor Botanical Gardens were carried out. Based on observations, switching locations and replacement of planting medium did not have much effect on the growth and development of this fern collection. During the sixth month, increased stem length and number of leaves persisted. The longest frond reached about 1.8 m and the shortest 0.75 m.

Based on the results of research conducted by Soti & Jayachandran (2016), *L. microphyllum* can grow and thrive in a wide range of soil types and the impact on the soil was site specific with effects being more prominent in sites with low nutrient status. Additionally, there were significant differences in the soil nutrient status and microbial population in the invaded and un-invaded sites. Sites with Old World climbing fern had significantly higher nutrient concentrations that correlated with higher soil

organic matter. Overall, the results indicate that this exotic pest plant can potentially alter its below ground environment to its own benefit by enhancing the soil nutrient status by adding soil organic matter.

At the end of the observations, although the specimens showed good growth, it was not sufficient for the plant to be planted in the garden. The new plant can be grown in the garden collection on conditions, among others, that: (a) the plants are strong and sturdy with a sufficient number offronds and (b) the plants have already adapted to the environment of the botanical gardens and have experienced various seasonal changes. The success of one-year acclimatization did not guarantee that the plant could be grown in the garden collections with good results. For example, some species of orchids that were collected from the highland showed good growth during the process of acclimatization in Bogor Botanical Gardens, but in subsequent years the growth quality was declining, even some of them, especially the terrestrial orchids, eventually died. The same thing can also occur to ferns, for the adaptation process of ferns in the nursery requires more than a year to wait until they are large enough and stronger before they can eventually be planted in the garden.

CONCLUSION

In the conservation area of Bukit Bungkuk Nature Reserve, Riau Province, there were three species of *Lygodium* namely *L. microphyllum*, *L. flexuosum* and *L. circinnatum*. *L. microphyllum* was the species most commonly found in this region. The growth of the three *Lygodium* species during the process of acclimatization in the Bogor Botanical Gardens was generally good. *L. microphyllum* showed the most rapid growth, while *L. circinnatum*t he slowest. Bogor Botanical Gardens has conserved species of *Lygodium* since 1920. The gardens currently has three species, so more serious efforts to collect the species and maintain the existing collection as well as possible are necessary

REFERENCES

Andrews, S.B. 1990. *Ferns of Queensland*. Brisbane. Queensland Department of Primary Industries.

Astuti, I.P., L.P. Soewilo, T.D. Said and R.N.A. Kosasih (Editors). 2001. *An Alphabetical List of Plants Species Cultivated in the Bogor Botanical Garden*. Botanic Gardens of Indonesia. Indonesian Institute of Sciences.

Ferriter, A. 2001. *Lygodium management plan for Florida*. Florisa Exotic Pest Plant Council Lygodium Task Force.

Florida Exotic Pest Plant Council (FLEPPC). 2001. *Lygodium Management Plan for Florida*. Ferriter, A. (Ed.).

Gandiaga, S., J. Volin, E. Kruger and K. Kitajima. 2009. Effects of hydrology on the growth and physiology of an invasive exotic, *Lygodium microphyllum* (old world climbing fern). *Weed Research*. 49, 283–290.

Hidayat, S., D.M. Puspitaningtyas, S. Hartini, E. Munawaroh, I.P. Astuti and H. Wawangningrum. 2017. *Flora exploration: 25 years of exploring the jungle in Indonesia*. LIPI Press. Jakarta. (Indonesian)

Hoshizaki, B.J. and R.C. Moran. 2001. *Fern Grower's Manual. Revised and Expanded* Edition. Portland, Oregon. Timber Press.

Humphreys J.M., J.B. Elsner, T.H. Jagger and S. Pau. 2017. A Bayesian geostatistical approach to modeling global distributions of *Lygodium microphyllum* under projected climate warming. *Ecological Modelling* 363: 192–216.

Lott, M.S., C.V. John, W.P. Robert and F.A. Daiel. 2003. The reproduktive biology of the invasive ferns *Lygodium microphyllum* and *L. javonicum* (Schizaeaceae): implications for invasive potential. *American Journal of Botany* 90 (8): 1144–1152.

Partomihardjo, T. & J.S. Rahajoe. 2005. *The data collection of plant ecology in flora biodiversity data collection guidelines* In: Rugayah, E.A. Widjaja, Praptiwi (eds). Research Center for Biology LIPI, Bogor. (Indonesian)

Pemberton R.W. and A.P. Ferriter. 1998. Old world climbing fern (*Lygodium microphyllum*), a dangerous invasive weed in Florida. *American Fern Journal* 88:165–175.

Soti, P.G., K. Jayachandran, M. Purcell, J.C. Volin and K. Kitajim. 2014. Mycorrhizal symbiosis and *Lygodium microphyllum* invasion in South Florida — a biogeographic comparison. *Symbiosis* 62 (2): Doi: 10.1007/513199-014-0272-4

Soti, P.G. and K. Jayachandran. 2016. Effect of exotic invasive old world climbing fern (*Lygodium microphyllum*) on soil properties. *Journal of Soil Science and Plant Nutrition*. 16 (4): 930–940.

Theplantlist. 2017. The Plant List, A Working List of All Plant Species Version 1.1. Published on the Internet; http://www.theplantlist.org/ (Accessed 01 February 2017)

Volin, J.C., E.L. Kruger, V.C. Volin, M.F. Tobin and K. Kitajima. 2010. Does release from natural below ground enemies help explain the invasiveness of *Lygodium microphyllum*? A cross-continental comparison. *Journal Plant Ecology*. 208: 223–234.

Volin, J.C., M.S. Lott, J.D. Muss and D. Owen. 2004. Predicting rapid invasion of the Florida Everglades by old world climbing fern (*Lygodium microphyllum*). *Journal of Diversity and Distribution* 10 (5-6): 439–446.

Wigman, H.J. 1920. *Catalogue de Graines qui peuvent etre fournies parle Jardin Botanique-de Buitenzorg*. Java.

The contribution of Cibodas Botanical Gardens to plant conservation based on material transfer agreement assessment

[1]Imawan W. Hidayat, [1*]Neneng I. Kurnita, [1]Yudi Suhendri,
[1]Dimas Ardiyanto, [1]Puji Soepriati, and [1]Agus Darmawan
[1]Cibodas Botanical Garden - Indonesian Institute of Sciences (LIPI).
Kebun Raya Cibodas Street, P.O. Box 19 SDL, Sindanglaya, Cianjur 43253 West Java, Indonesia. Ph/Fax: +62-263-512233.
*Email: inekurnita@gmail.com

ABSTRACT

Botanical gardens have been greatly contributing to ex-situ conservation efforts. One of the efforts is by extending cultivated plants from the gardens to be further utilized by public, especially for conservation, research, education, ecotourism, and environmental services. This extension can be conducted through seed and plant exchange for various users. Cibodas Botanical Gardens (CBG), as one of the Indonesian botanical gardens coordinated by the Indonesian Institute of Sciences (LIPI), has also carried out this activity. The plants needed for these purposes are recorded in the material transfer agreement (MTA). This MTA is a prudent action and also a list recording plant material transfers from CBG to users. This study aimed to assess the amount of plant materials (e.g. seedling, seed, fungi etc.) which are transferred to users based on the number of MTAs from 2013 to 2016. Based on the data of the MTAs during the four year-period, there were a total of 39 requests, from local institutions (92.3%) and foreign institutions, such as Japan, China, and the Netherlands (7.7%). The materials transferred are classified into collection plants (75.8%) and non-collection plants (24.2%). The results are expected to show some of the contribution of CBG in ex-situ conservation, both on the local and international scales.

Keywords: Cibodas Botanical Gardens (CBG); ex-situ conservation; material transfer agreement (MTA)

INTRODUCTION

Cibodas Botanical Gardens (CBG) is one of the government institutions which have the main duty and function in ex-situ conservation, research, education, eco-tourism, and environmental services (LIPI, 2016). The role of CBG as a plant conservation institution includes promoting cultivated plants from the gardens to be further utilized by its users. One of the things which distinguish botanical gardens (BG) from national parks, nature or wildlife reserve, and other institutions is the activity of plant data recording. Plant data recording of CBG is conducted by its "registration unit". It is one of the work units at CBG assigned to handle data management and history of each plant material that will and has become a collection of the gardens (CBG, 2012). In its early establishment, CBG was intended as a place of acclimatization of plant species from foreign countries with high value and economic importance, such as quinine tree (*Cinqueona calisaya*), tea (*Camellia sinensis*), pine (*Pinus merkusii*) and resin tree (*Agathis* spp.). Along with its development, CBG has also cultivated around 46 plant species from the garden that are categorized, according to International Union for Conservation of Nature (IUCN), as plants with a high-risk presence in the wild (Registration Unit-CBG, 2017a; IUCN, 2017). In recent years, CBG has conducted several *ex-situ* conservation activities with a focus on the typical Sumatran mountain plants. Moreover, *ex-situ* plant collections would help conserve threatened species (Richards et al., 2007), function as a source of traits for agricultural improvement (Brummer et al., 2011; Neale & Kremer, 2011), or have been used to propagate large numbers of individual plants for ecosystem restoration or assisted migration (Broadhurst et al., 2008; Vitt et al., 2010; Aitken & Whitlock, 2013).

A material transfer agreement (MTA) is a type of legally enforceable contract employed by research institutions and (or private) companies to set the terms under which their materials and

associated data may be obtained and used by others (Henderson, 2007; Ku & Henderson, 2007). These agreements provide a mechanism to protect the interests of the owners of discoveries and inventions while promoting data and material sharing in the research community. The latter is an admirable goal in an age where research is increasingly collaborative, multinational, and multidisciplinary. MTA may apply to anything, from materials that are simply under the control of the originator but have no formal intellectual property rights attached to them to proprietary materials protected by patents and trade secrets (Rodriguez, 2005). MTA commonly place limits on the use of materials, their physical handling, and distribution to third parties. For example, the use of materials may be limited to noncommercial or preclinical research, or to specific fields of use, such as research on a specific disease. Distribution to third parties may be prohibited or subject to permission from the provider (Bubela et al., 2015). On the other hand, MTA has a bad reputation among researchers for being overly complex and, in practice, hindering the exchange of research reagents (Mishra & Bubela, 2014: Bubela et al., 2015). Nevertheless, MTA remains necessary in order to protect the provider's biological resources, as well as taking responsibility for the development and utilization processes. In this context, CBG has also applied the MTA policy in the transfer of its biological resources.

CBG has routinely disseminated environmental and plant conservation activities and also the related information through electronic media (such as website, social media), printed media (such as leaflets, brochures, banners) and direct extension to the public. Based on Peterson et al. (2003), in order to achieve a greater success in conservation efforts CBG has to employ both active and passive approaches in the scenario planning of the conservation. A passive approach (Peterson et al., 2003) is implemented by CBG by serving the demand for plants from users for various activities such as education, research, and other needs (such as reforestation, plant restoration). Meanwhile, the active approach (Peterson et al., 2003) is conducted by CBG through public campaign to local communities or areas that require conservation efforts, especially for areas vulnerable to landslides, floods, bare lands, and others. Plant materials used in those activities are recorded in MTA. This MTA is a prudent action of plant data recording, as evidence that the utilization of any form of bio-resources material in CBG is well-documented and as a contribution of plant conservation (UN, 1992). Forms of materials which have been used consist of seedling, seeds, and parts of plant materials (such as leaves, roots, stems, and fruit), mushrooms, moss, and other biological resources.

The objectives of this study were to assess the amount of plant material transferred to users based on the number of MTAs from 2013 to 2016 and to describe the characteristics of the data. Through this study, the utilization of plant materials and users' characteristics and purposes of plant utilization can be analyzed. Indirectly, research results can be described as the contribution of CBG to plant conservation, especially for collection and non-collection plants.

METHODS

Study site

The study was conducted at CBG, Cianjur, West Java. CBG is located at the side of Mount Gede and Mount Pangrango at an altitude of approximately 1,300-1,425 meters above sea level, with an area of 84z99 hectares (Widyatmoko et al., 2010). The average temperature is 20°C, humidity of 80.82%, and an average rainfall of 2,950 mm per year (Registration Unit-CBG, 2017b). CBG is a comfortable place to rest while enjoying the beauty of the various types of plants that mostly originated from Indonesia. More than 60% (Registration Unit-CBG, 2017a) of the plants are especially from tropical wet highlands and other foreign countries. CBG is ± 100 Km from Jakarta and ± 80 Km from Bandung.

Study framework

In this study, MTA of CBG referred to document coded FR-7.5.1-RG-1.G4Ed.1Rev.0.04122012 (CBG document of ISO 9001:2008 section Form of Collection Registration Unit, 2012, unpublished data), which is related to the document with the following codes: (1) FR-7.5.1-RG-1.A6Ed.1Rev.1.01072014, delivery order of plant material to external party; (2) FR-7.5.1-RG-1.A7Ed.1Rev.1.01072014, order/request acceptance of plant material from external party; and, (3) FR-7.5.1-RG-1.A8Ed.1Rev.1.01072014, follow up to order/request of plant material. MTA intends to honor the letter and spirit of the 1992 Convention on Biological Diversity (CBD) (UN, 1992), Convention on

International Trade in Endangered Species of Wild Fauna and Flora (CITES), and other relevant conventions and laws regarding the use of biological diversity as well as benefit-sharing.

The materials explained in CBG's MTA include, but are not limited to plants, parts of plants or propagation materials (such as seeds, cuttings, roots, bulbs, corms, leaves, plant tissue culture, plant herbarium, or any other material of plant). Based on CBG's MTA agreement, materials and any progeny or derivatives thereof, modified or unmodified extracts, will not be used for commercial purposes. If at any point in the future the recipient wishes to commercialize the material and any progeny or derivatives, the recipient must first obtain the written permission of CBG. Any commercialization to which CBG agrees will be subject to a separate agreement between the recipient and the CBG consistent with the CBG's policy that benefits be shared fairly and equitably with the country of origin of the material. Commercialization means the use or exploitation of genetic resources, their progeny or derivatives, with the object of, or resulting in financial gain, and includes but not limited to the following activities: sale, applying for, obtaining or transferring intellectual property rights or other tangible or intangible rights by sale or license or any other manners, commencement of product development, conducting market research, and seeking pre-market approval (CBG document of ISO 9001:2008, section Form of Collection Registration Unit, 2012, unpublished data).

Data analysis

The subject of this study was CBG's MTAs which were established from 2013 to 2016. CBG's MTA is a contractual document between CBG and recipients/users regarding all material forms in CBG which are ordered, requested or transferred. MTA includes recipient/user personal data and type of materials transferred (collection or non-collection materials of the garden). The data were analyzed statistically for each period. The results included a description of the materials which have been utilized by recipients/users for education and research interests. The results can also reveal CBG's contribution to plant conservation as well as information and knowledge transfer about CBG's plant property.

RESULTS AND DISCUSSION

Based on the data from 2013 to 2016 period, there were 39 CBG's MTA documents (Table 1): 4 documents in 2013, 8 documents in 2014, 10 documents in 2015, and 17 documents in 2016. In total, the transferred materials included 22 packages (usually in the form of seeds), 2,995 specimens (usually in the form of plants or parts of plant), 29.12 kg (usually in the form of parts of plant such as leaves, branches, stems, shoots, etc.) and 4cm^2 materials (in the form of fungi on soil, leaves, roots and rotten fruits).

Table 1. Material types requested from 2013 to 2016

MTA Documents	Characteristics	Year			
		2013	2014	2015	2016
Number of documents		4	8	10	17
Origin of user	Local	4	7	10	15
	Foreign	-	1	-	2
Types of material	Plants (*n*-number)	-	27	212	70
	Leaves and/or Branches and/or stems	6 kg	10 kg; 7 stems	13.12 kg	67 stems
	Leaves (*n*-sheet)	-	3	58	284
	Roots (*n*-number)	-	-	2	-
	Root segments (*n*-number)	-	-	-	5
	Shoots (*n*-number)	-	235	-	2
	Flowers (stalks and sap) (*n*-number)	-	-	-	117
	Fruits (stalks, skin, sap) (*n*-number)	-	-	58	184
	Seeds (*n*-number)	40	920	20	330
	Packages of seeds* (very small to small size)	11	9	11	-

MTA Documents	Characteristics	Year			
		2013	2014	2015	2016
Gardens property	Cuttings (*n*-number)	-	11		-
	Grafting (*n*-number)	-	-	1	-
	Fungi	-	-	4 cm^2	-
	Mycorrhiza (*n*-colony)	-	15	-	92
	Lichens (*n*-colony)	-	-	4	235
	Rhizoid + thallus (*n*-number)	-	-	-	30
	Ascoma (*n*-number)	-	-	-	20
	Collection (*n*-specimens)	15	31	67	216
	Non-collection (*n*-specimens)	-	4	5	96

The data show that each year plant requests tended to rise in number (Figure 1). These results can be explained, among others, due to information disclosure by CBG in various media, especially in http://sindata.krcibodas.lipi.go.id/Cibodas-Botanic-Gardens-Record/CBGR/index.php regarding the richness of collections that can be accessed by anyone. This openness (especially for information) has attracted a broader range of potential users and allies and customers (Shapiro et al., 1999). The establishment of new botanic gardens has also contributed to the increasing requests. Enrichment of the plant collections through plant seed exchange is one of the botanic gardens' efforts to develop the conservation value of the garden (Davis, 2008; BGCI, 2012; Purnomo et al., 2015).

Local users still dominated plant requests, with 36 agreements, compared to foreign users with 3 agreements. It was recorded that education and research institutions mostly established the agreements. Local institution users recorded in the MTA were Samosir Botanic Gardens, Center of Plant Conservation-Bogor Botanic Gardens, Baturaden Botanic Gardens, Kuningan Botanic Gardens, Wamena Biological Garden, Forest Research and Development Center–Ministry of Environment and Forestry and Herbarium Bandungense. Moreover, some individuals have established the agreements for education and research interests. The local individuals were dominantly from a research institution, university and other educational institutions. Furthermore, three agreements from foreign countries were established: with South China Botanic Garden (2014) and two agreements in 2016 with a student of Mie University of Japan and a student of Leiden University of Netherlands. MTAs from foreign countries contributed 7.7% of the total plants contributed by CBG (Figure 1).

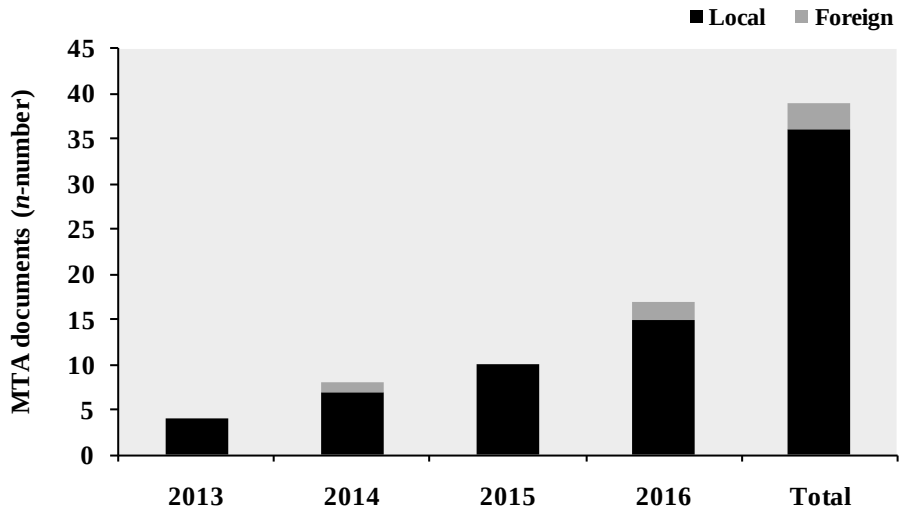


Figure1. Trends in the number of MTA documents from 2013 to 2016

The plant materials requested by foreign countries can be seen in Table 2. Parts of the plant materials most frequently requested were in the form of leaf sheets. Based on the permit stated in the MTA, most leaves were used for research purposes and educational interests. Leaves were allowed by CBG because of the lack of plant stocks in the nursery. In addition, leaves are easy to transport and do not require special treatments compared to whole plants (usually inserted in a plastic or large paper envelope). Based on the types of material, seeds and packages of seeds were the most frequently requested items by users. A total of 1,310 seeds and 31 seed packages were transferred (if each package contains more than 20 pieces, there were more than 620 pieces of seed in total).

Packages of seeds were established from very small to small size. Packaging was done because of the difficulty to count the seeds one by one in large numbers and because the stock at the gardens was abundant. High request for materials in the form of seeds can be understood because seeds can be wrapped, transferred and delivered easily, and do not require much space. In addition, seeds can be sown and cultivated into plants that have similar characteristics to their parents in the gardens.

Table 2. CBGs MTA information applied by foreign applicants

Institution/ individual	Species	Family	Garden non/collection	Type of materials	*n*-number	Purposes of the request
South China Botanic Garden	*Musa acuminata*	Musaceae	Collection	leaves	1	Research interest on mycorrhiza attaced to *Musa* spp.
	Musa uranoscopus	Musaceae	Collection	leaves	1	
	Musa sp.	Musaceae	Collection	leaves	1	
	Mycorrhiza in wild *Musa* spp.	-	Non collection	colony	10	
A student of Mie University of Japan	*Castanopsis argentea*	Fagaceae	Collection	leaves	30	Research interest on tropical highland plants species.
	Castanopsis javanica	Fagaceae	Collection	leaves	66	
	Codiaeum variegatum	Euphorbiaceae	Collection	leaves	20	
	Hibiscus indicus	Malvaceae	Collection	leaves	9	
	Acalypha amentacea	Euphorbiaceae	Collection	leaves	10	
	Breynia microphylla	Phyllanthaceae	Collection	leaves	8	
	Acanthus montanus	Acanthaceae	Collection	leaves	8	
	Acalypha × *cristata*	Euphorbiaceae	Collection	leaves	12	
	Morus alba	Moraceae	Collection	leaves	8	
	Aglaia rimosa	Meliaceae	Collection	leaves	20	
	Acalypha hispida	Euphorbiaceae	Collection	leaves	10	
	Solanum sp.	Solanaceae	Collection	leaves	5	
	Hibiscus sp.	Malvaceae	Collection	leaves	8	
	Euphorbia pulcherrima	Euphorbiaceae	Collection	leaves	8	
A student of Leiden University of	*Trigonostemon* sp.	Euphorbiaceae	Collection	leaves	2	Research interest on tropical
	Ostodes paniculata	Euphorbiaceae	Collection	leaves	2	
	Phyllanthus sp.	Phyllanthaceae	Collection	leaves	2	

According to the policy of the Head of CBG, transfers of living plants, especially whole living plants, are mostly granted to fellows of the botanical garden institutions in Indonesia, and the number of plants given depends on the number of the collections of CBG. Plant requests among fellow members are usually made in the context of enrichment of the collections and for research and education purposes. The second policy is if the number of plants was abundant in the nursery or and/or the technique of breeding is relatively easy, the request may be granted (especially for a whole living plant request). The next policy is if the number of plants is just a few (5 specimens or less) and/or difficult to breed, the number of plants granted will probably be reduced from the requested, or even denied.

However, personal requests for whole living plants are still possible, but must be approved by the Head of CBG, and will not necessarily be granted. The possibly granted plant requests exclude plant species protected by Indonesian law (*Government's Regulation Nomber 7 of 1999* and amended by the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia *Number P.92/MENLHK/SETJEN/KUM.1/8/2018*). The request for parts of a plant (such as leaves, branch, flower, fruit etc.) listed in the law is still possible to be granted; for example, *Castanopsis argentea* and *Castanopsis javanica*. However, it also depends on the stock and plant collection sensitivity. If the main plant is too vulnerable, then only limited number of leaves will be granted. Nevertheless, this granting is only valid if the user guarantees that the material is used for conservation or research and education purposes only.

Plant(s) and/or part(s) of the plant granted to the applicant are not charged because this is a part of the contribution of CBG in conservation, research, and education. After the request is granted, all responsibility for shipping and licensing is borne by the applicant, and CBG does not hold any responsibility. These policies are listed in CBG's Documents of ISO 9001:2008 Section Form of Collection Registration Unit: FR-7.5.1-RG-G4 Material Transfer Agreement. CBG has also carried out activities for commercial purposes. The activities are conducted by the Services and Information Section of CBG while still obeying the previous policy principles, but this service will not be further discussed in this paper.

Based on the MTA documents, it has been discovered that the most frequently requested whole plant species were *Crinum* sp. and *Brunfelsia* sp.; fruits: *Elaeagnus latifolia* and *Amorphophallus titanium*; leaves: *Castanopsis javanica* and *Castanopsis argentea*; and seeds: *Pittosporum moluccanum* and *Castanopsis argentea*. The details of most requested plant species based on CBGs MTA from 2013 to 2016 can be seen in Table 3.

Table 3. The most requested plant species based on CBGs MTA from 2013 to 2016

Species	Family	Plants (*n*-number)	Fruits (*n*-number)	Leaves (*n*-sheet)	Seeds (*n*-number)
Crinum sp.	Amaryllidaceae	35			
Brunfelsia sp.	Solanaceae	20			
Zinnia sp.	Compositae	15			
Neomarica longifolia	Iridaceae	12			
Podocarpus imbricatus	Podocarpaceae	10			
Elaeagnus latifolia	Elaeagnaceae		58		
Amorphophallus titanum	Araceae		50		
Ficus variegata	Moraceae		13		
Muehlenbeckia platyclados	Polygonaceae		7		
Solanum aligerum	Solanaceae		6		
Castanopsis javanica	Fagaceae			66	
Castanopsis argentea	Fagaceae			30	
Codiaeum variegatum	Euphorbiaceae			20	
Aglaia rimosa	Meliaceae			20	
Acalypha × cristata	Euphorbiaceae			12	
Pittosporum moluccanum	Pittosporaceae				500
Castanopsis argentea	Fagaceae				130
Platea latifolia	Icacinaceae				101
Symplocos costata	Symplocaceae				85
Lithocarpus pallidus	Fagaceae				68

The data in Table 3 indicate that the stock of these species was abundant, so CBG could comply with the request (Registration Unit-CBG, 2017a). Moreover, most of these species are wet-tropical highland plants, so they are appropriate to be planted and enrich the collections such as those in Baturraden and Kuningan Botanic Gardens with similar elevation and relative microclimate condition. This uniqueness and the native characteristics of some species such as *C. javanica*, *A. titanum* and *S.costata* made the species interesting objects for further study, and this interest has driven the request for these species even more.

These processes, mechanisms, and policy of MTA were also applied by other botanic gardens, especially those coordinated by Indonesian Institute of Sciences (LIPI), such as Center of Plant Conservation-Bogor Botanic Gardens, Cibodas Botanical Garden, Purwodadi Botanical Garden, and Eka Karya-Bali Botanical Garden. In general, these botanic gardens do not charge any cost in seed and plant exchange, excluding species for commercial use.

Furthermore, from 2013 to 2016, CBG denied five requests. Three requests were from local institutions and two from foreign institutions/individuals. This rejection was because the requests were incompatible with the CBG policies and/or indicated violation of the law. Some reasons included the requested plants were limited in stock, the plants belonged to protected plant(s), or the applicants cannot show the permit from the authorities (such as The Ministry of Environment and Forestry of Indonesia, Research Center of Biology-Indonesian Institute of Sciences (LIPI), Plant Conservation Center-Bogor Botanic Gardens (LIPI)). In order to maintain the credibility and reputation of the applicants, their names were not mentioned.

CBG's MTA is not to prevent researchers, students, or other subjects from obtaining plants or plant materials from CBG, but it is a prudent action and well-documented process of how plants or plant materials of CBGs are transferred outwards. As a conservation institution, directly or indirectly, CBG is partly responsible for its plants and materials. Unfortunately, in reality CBG has difficulty in controlling the development and utilization of plants and materials that have been transferred to users, although it is clearly stated in MTA that CBG should get benefit shares if the plants and materials can be developed and/or further utilized by users. Benefits arise from the utilization of such knowledge, innovations, and practices (UN, 1992; Streitz & Bennet, 2003; Rodriguez, 2005; Mirowsky, 2008). Benefits can also arise from the commercialization and other utilizations of genetic resources with the Contracting Party providing such resources. Such sharing shall be upon mutually agreed terms (Streitz & Bennet, 2003; Rodriguez, 2005; Mirowsky, 2008).

CBG's MTA document has been written by holding several principles, which are simple, with management of risks that should be proportionate to the type and likelihood of benefits and avoid reach-through claims (Mishra & Bubela, 2014; Bubela et al., 2015). MTAs should also be commensurate with a realistic assessment of the risks and benefits to the institutions, both in terms of legal liabilities and potential revenue generation. Institutional contract staff should evaluate the presence or likelihood of the following categories of risks relative to the likely benefits to accrue to the institution and/or its researchers and use MTAs or simpler agreements.

Through the MTA, CBG widely opens up space for anyone or institution that will and wants to engage in the activities, especially in the field of plant research and education, through mutually beneficial agreements. CBG adopts these MTA's simple agreements in cases where the risk is low and the benefits are for non-commercial interest. Nevertheless, CBG still concerns those cases where more complex agreements are warranted, especially in relations to industry and in contexts closer to commercial development and/or clinical application, and violation of the efforts of plants conservation.

CONCLUSION

CBG's MTA has provided an overview of the contribution of CBG to plant conservation, both locally and internationally. From 2013 to 2016, CBG has served 36 local requests and 3 international requests for plant materials. CBG has also disseminated 329 plant specimens of the garden collections and 105 specimens of the non-collection plants. This contribution is the real form of commitment of CBG to continuously encourage plant conservation efforts, both in local and the international scales. MTA is a prudent action and also an order of recording plant material transfer of CBG to user. MTA is also a tool to control the appropriate use of the materials transferred in the context of education and

research, and a fair benefit-sharing agreement between CBG and the user in the frame of commercialization as a further step.

ACKNOWLEDGEMENTS

Our gratitude goes to the Head of CBG, Mr. Agus Suhatman, and Head of Plant Exploration and Collection of CBG, Mr. Muhammad Imam Surya, Ph.D., for their support, advice, and communication. Our gratitude also goes towards all registration unit staffs of CBG, especially Mr. Didi Rasidi and Mr. Habibulloh for cooperation in data compilation. This paper is expected to serve our small role in contributing information and knowledge regarding the transfer of CBG's plant property.

REFERENCES

Aitken, S.N. and M.C. Whitlock. 2013. Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology and Systematics* 44: 367–388.

[BGCI] Botanic Gardens Conservation International. 2012. *International agenda for botanic gardens in conservation: 2nd edition*. Botanic Gardens Conservation International, Richmod-UK.

Broadhurst, L.M., A. Lowe, D.J. Coates, S.A. Cunningham, M. McDonald, P.A. Vesk and C. Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* 1(4): 587–597.

Brummer, E.C., W.T. Barber, S.M. Collier, T.S. Cox, R. Johnson, S.C. Murray, R.T. Olsen, R.C. Pratt and A.M. Thro. 2011. Plant breeding for harmony between agriculture and the environment. *Frontiers in Ecology and the Environment* 9(10): 561-568.

Bubela, T., J. Guebert and A. Mishra. 2015. Use and misuse of material transfer agreements: Lessons in proportionality from research, repositories, and litigation. *PLoS Biology* 13(2): 1002060.

[CBG] Cibodas Botanical Garden. 2012. *CBG's Documents of ISO 9001:2008 Section Form of Collection Registration Unit: FR-7.5.1-RG-G4 Material Transfer Agreement*. CBG, Cibodas. [unpublished].

[CITES] Convention on International Trade in Endangered Species of Wild Fauna and Flora. *https://cites.org/eng/app/appendices.php*. [Accessed: 11-05-2017]

Davis, K. 2008. *A CBD manual for botanic gardens*. Botanic Gardens Conservation International, Richmod-UK.

Henderson, J. 2007. Counterpoint: MTAs are a practical necessity. *Nature Biotechnology* 25(7): 722–724.

[IUCN] International Union for Conservation of Nature. 2017. *https://www.iucnredlist.org/* [Accessed: 25-04-2017]

Ku, K. and J. Henderson. 2007. The MTA—rip it up and start again? *Nature Biotechnology* 25(7): 721–722.

[LIPI] Lembaga Ilmu Pengetahuan Indonesia. 2016. *Peraturan Kepala LIPI Nomor 3 Tahun 2016 tentang Organisasi dan Tata Kerja Balai Konservasi Tumbuhan Kebun Raya Cibodas.* LIPI, Jakarta.

Mirowski, P. 2008. Livin' with the MTA. *Minerva* 46: 317–342.

Mishra, A. and T. Bubela. 2014. Legal agreements and the governance of research commons: Lessons frommaterials sharing in mouse genomics. *OMICS A Journal of Integrative Biology* 18(4): 254–273.

Neale, D.B. and A. Kremer. 2011. Forest tree genomics: growing resources and applications. *Nature Reviews Genetics* 12(2): 111–122.

Peraturan Pemerintah Republik Indonesia Nomor 7 Tahun 1999 tentang Pengawetan Jenis Tumbuhan dan Satwa.

Peraturan Menteri Lingkungan Hidup dan Kehutanan Republik Indonesia Nomor P.92/MENLHK/SETJEN/KUM.1/8/2018 tentang Jenis Tumbuhan dan Satwa yang Dilindungi.

Peterson, G.D., G.S. Cumming, and S.R. Carpenter. 2003. Scenario planning: a tool for conservation in an uncertain world. *Conservation Biology* 17(2): 358-366.

Purnomo, D.,M. Magandhi, F. Kuswantoro, R.A. Risna and J.R. Witono. 2015. Developing plant collections on the regional botanic gardens in framework of plant conservation strategy in Indonesia. *Buletin Kebun Raya* 18(2): 111-124. [Indonesian]

Richards, C.M., M.F. Antolin, A. Reilley, J. Poole, and C. Walters. 2007. Capturing genetic diversity of wild populations for ex-situ conservation: Texas wild rice (*Zizania texana*) as a model. *Genetic Resource and Crop Evolution* 54(4): 837–848.

Registration Unit-CBG. 2017a. *Monthly reports of plants collection richness of CBG-March 2017*. CBG, Cianjur. [Indonesian] [Unpublished]

Registration Unit-CBG. 2017b. *Annual reports of microclimates of CBG from 2012 to 2016: Form D10*. CBG, Cianjur. [Indonesian] [Unpublished]

Rodriguez, V. 2005. Material transfer agreements: open science vs. proprietary claims. *Nature Biotechnology* 23: 489–491.

Shapiro, C., H.R. Varian and W.E. Becker. 1999. Information rules: a strategic guide to the network economy. *Journal of Economic Education* 30: 189-190.

Streitz, W.D. and A.B. Bennett. 2003. Material transfer agreements: A University perspective. *Plant Physiology* 133(1): 10–13.

[UN] United Nations.1992. *Convention on Biological Diversity*. United Nations, New York.

Vitt, P., K. Havens, A.T. Kramer, D. Sollenberger, and E. Yates. 2010. Assisted migration of plants: changes in latitudes, changes in attitudes. *Biological Conservation* 143(1): 18–27.

Widyatmoko, D., N. Suryana, A. Suhatman, and Rustandi (Eds.). 2010. *List of Living Plants Collection Cultivated in Cibodas Botanic Gardens.* CBG-LIPI, Cianjur.

Establishment of Kebun Raya Universitas Halu Oleo

Julisasi Tri Hadiah
Pusat Konservasi Tumbuhan Kebun Raya – LIPI
Jl. Ir. H. Juanda 13, Bogor
e-mail: jhadiah@yahoo.com

ABSTRACT

Despite its high endemicity, Sulawesi is facing serious threats due to habitat loss, deforestation, plantation development and farming. A new botanic garden, namely Kebun Raya Universitas Halu Oleo (KR UHO), is established in the city of Kendari, Sulawesi Tenggara Province, to conserve the endemic plants of Sulawesi. KR UHO became the first university garden established in Indonesia; a botanic garden managed by a university. This paper describes the establishment of KR UHO and its more recent development. Since a Memorandum of Understanding on the establishment of KR UHO was signed in 2015, between the Universitas Halu Oleo and the Center for Plant Conservation Botanic Gardens LIPI, the garden has carried out several collecting expeditions in Sulawesi Tenggara, as well as establishing some basic infrastructure. To date, the garden houses approximately 104 collection numbers of existing plants (representing 29 families of flowering plants, excluding Orchidaceae, 48 genera, 40 species, 31 unidentified), and 71 collection numbers of orchids comprising 42 genera, 44 species and 27 unidentified.

Key words: conservation, establishment, first university garden, Kebun Raya Universitas Halu Oleo, Sulawesi endemic plants

INTRODUCTION

Indonesia as a country with mega diversity has committed to international conventions such as the CBD (1993) and GSPC (2002) to play roles on conservation of its biodiversity. One part of this commitment, started in the mid 1990s, has been to establish new botanic gardens throughout the country as *ex situ* conservation sites. The establishment of new botanic gardens also benefit the local community and represent national and international needs (Sutrisno, 2010).

Kebun Raya Bogor (KRB), established two centuries ago (18 May 1817), together with its four satellite (newer) gardens, namely Kebun Raya Cibodas, Kebun Raya Purwodadi, Kebun Raya Eka Karya Bali, Cibinong Science Centre and Botanic Garden, are administratively under the management of Lembaga Ilmu Pengetahuan Indonesia (LIPI/Indonesian Institute of Sciences) and is named Pusat Konservasi Tumbuhan Kebun Raya LIPI (Center for Plant Conservation Botanic Gardens LIPI). However, these five botanic gardens only manage to conserve c. 30–40% of Indonesian plants (Anonymous, 2016). Therefore, KRB aims to develop at least 47 botanic gardens throughout the archipelago based on the 47 eco-regions existing in Indonesia (Olson *et al*., 2001). Currently, there are 32 botanic gardens in Indonesia representing different eco-regions, including the four older botanic gardens under LIPI (see Fig. 1 and Table 1).

Figure 1. Distribution of new botanic gardens in Indonesia (after Anonymous, 2016)

- Botanic gardens of LIPI
- Botanic gardens of local authorities
- Botanic gardens of university

Table 1. Current list of Indonesian botanic gardens (Anonymous, 2016). Note: KR = Kebun Raya

No.	Botanic Garden's Name	Location (Province)	Size (ha)	Theme of the garden
1.	KR Bogor	Jawa Barat	87	Wet lowland plants
2.	KR Cibodas	Jawa Barat	85	Highland plants of western Indonesia
3.	KR Purwodadi	Jawa Timur	85	Dry lowland plants
4.	KR Eka Karya Bali	Bali	157.5	Highland plants of eastern Indonesia
5.	Cibinong Science Center & Botanic Gardens	Jawa Barat	189	Plants represented Indonesian Bioregions
6.	KR Balikpapan	Kalimantan Timur	309	Indonesian timber trees
7.	KR Banua	Kalimantan Selatan	100	Medicinal plants of Kalimantan
8.	KR Batam	Kepulauan Riau	86	Plants of small islands
9.	KR Baturraden	Jawa Tengah	142	Montane plants of Jawa
10.	KR Bukit Sari	Jambi	425	Lowland plants of Sumatera
11.	KR Danau Lait	Kalimantan Barat	328	Equator plants
12.	KR Gianyar	Bali	9.7	Native plants to Gianyar
13.	KR Indrokilo Boyolali	Jawa Tengah	8	Lowland rainforest plants from eastern Jawa
14.	KR Jagatnatha Jembrana	Bali	5.8	Usada and Balinese ceremonial plants
15.	KR Jompie Parepare	Sulawesi Selatan	13,5	Plants of Wallacean coastal areas
16.	KR Katingan	Kalimantan Tengah	102.47	Indonesian fruit plants
17.	KR Kendari	Sulawesi Tenggara	113	Ultra basic plants
18.	KR Kuningan	Jawa Barat	172	Plants from rocky areas and Mount Ciremai
19.	KR Liwa	Lampung	86.7	Indonesian ornamental plants
20.	KR Lombok	Nusa Tenggara Barat	130	Plants of Nusa Tenggara
21.	KR Massenrempulu Enrekang	Sulawesi Selatan	300	Wallacean plants
22.	KR Megawati Soekarnoputri	Sulawesi Utara	221	Wallacean lowland rainforest plants
23.	KR Minahasa	Sulawesi Utara	186	Plants of Wallacean highlands
24.	KR Pelalawan	Riau	100	Peat swamp plants of Sumatera

No.	Botanic Garden's Name	Location (Province)	Size (ha)	Theme of the garden
25.	KR Pucak	Sulawesi Selatan	120	Plants with economic values
26.	KR Sambas	Kalimantan Barat	300	Riparian plants of Kalimantan
27.	KR Samosir	Sumatera Utara	100	Highland plants of Sumatera Utara
28.	KR Sampit	Kalimantan Tengah	600	Heath forest plants
29.	KR Solok	Sumatera Barat	112.6	Herbs of Indonesia
30.	KR Sriwijaya	Sumatera Selatan	100	Medicinal and wetland plants of Sumatera
31.	KR Universitas Halu Oleo	Sulawesi Tenggara	22.88	Endemic plants to Sulawesi
32.	KR Wamena	Papua (Indonesia)	160	Montane plants of middle Papua

This paper presents the establishment (in 2015) and the subsequent development of a new botanic garden Kebun Raya Universitas Halu Oleo, Sulawesi Tenggara.

MATERIAL AND METHODS

This study used a direct observation method, where data directly observed and collected from the garden site. The garden is located within the new campus of Universitas Halu Oleo in Anduonohu area of the city of Kendari, Sulawesi Tenggara. Identification and validity of plant names were checked against specimens at Herbarium Bogoriense (BO), by personal communication with relevant experts and using the Plant List (2013).

RESULTS AND DISCUSSION

Establishment of Kebun Raya Universitas Halu Oleo

Sulawesi is the largest island in the Wallacea region, a biodiversity hotspot situated in between Sunda Shelf in the west and Sahul Shelf in the east. It covers about 53% of the area, with c. 5000 species of plants, c. 500 of which are endemic. Vegetation types in the island are mostly lowland rainforest (42%) and montane rainforest (11.9%). No particular families dominate the lowland rainforest, but *Diospyros* spp. and palms are common. Although many new species have been discovered from the island, the island's plant diversity is still poorly known as the number of specimens collected per 100 km^2 is still quite low (23 specimens/100 km^2). Ideally, an adequate representative of collections would be 100 specimens per 100 km^2 (Whitten et al. 2002).

Sulawesi's plant diversity is facing serious threats, similar to that observed in many parts of Indonesia, due to habitat loss, deforestation, plantation development and farming. The establishment of a new botanic in the city of Kendari, Sulawesi Tenggara, is therefore timely as this plant diversity needs to be represented in *ex situ* living collections in botanic gardens.

Universitas Halu Oleo is the foremost university in Sulawesi Tenggara that is concerned with the environment and conservation of habitat. It has been declared as a green campus since 1995. The University is committed to actively be involved in the conservation of Indonesian biodiversity, particularly endemic species to Sulawesi. The then Rector of the University wrote to the Head of LIPI (correspondence No.1152a/UN29/KP/2015) proposing the establishment of a new botanic garden, Kebun Raya Universitas Halu Oleo, that specializes in conserving endemic plants of Sulawesi.

After an initial site survey (7–9 May 2015) by a Team from KRB, a Memorandum of Understanding (MoU) was signed (18 May 2015) between Center for Plant Conservation Botanic Gardens LIPI (KRB) and the Universitas Halu Oleo (UHO) on the establishment of Kebun Raya Universitas Halu Oleo (KR UHO). KR UHO is located within the new campus in Kelurahan Kambu, Kecamatan Kambu, City of Kendari, and covers a total area of 22.88 ha comprising three separate blocks: main garden (18.46 ha), arboretum (1 ha) and sago wetland (3.42 ha).

The signing of the MoU between KRB and UHO has marked a starting point of the establishment of KR UHO. A field survey was undertaken (November 2015) to observe various aspects for designing

a Master Plan for the new garden. The current vegetation, wildlife, soil types and water sources were evaluated. The socio-economic status of the local people was also evaluated. The field survey reported that the existing plants in the garden's site comprised 45 families, 72 genera and 86 species. The myrtle family (Myrtaceae) was the most abundant (nine species), followed by Apocynaceae (six species), Arecaceae (five species) and Phyllanthaceae (five species). Four endemic plant species were found growing in the garden, namely *Dillenia serrata* Thunb. (Dilleniaceae), *Kjellbergiodendron celebicum* (Koord.) Merr. (Myrtaceae), *Helicia kjellbergii* Sleumer and *Lasjia hildebrandii* (Steenis) P.H.Weston & A.R.Mast. (both Proteaceae). In addition, some additional species are listed in the IUCN Redlist of Threatened Species, namely *Cycas circinalis* L., Cycadaceae (Endangered A2acd ver 3.1), *Maranthes corymbosa* Blume, Chrysobalanaceae (Lower Risk/least concern ver 2.3), *Gnetum gnemon* L., Gnetaceae (Least Concern ver 3.1), *Cratoxylum formosum* (Jacq.) Benth. & Hook.f. ex Dyer, Hypericaceae (Lower Risk/least concern ver 2.3) and *Nymphoides indica* (L.) Kuntze, Menyanthaceae (Least Concern ver 3.1).

There are some plant of horticultural/ornamental value growing on the site, such as ruruhi (*Syzygium* sp., Myrtaceae). Ruruhi is a shrub or small tree that produces beautiful pink flowers on its trunk and branches. Its dark red fruits are edible with refreshingly sour taste (Figure 2). The occurrence of this species on the site is abundant, flowering and fruiting at the same time, thus creating beautiful pink pathways in the garden. Other plants with ornamental value include pakis aji (*Cycas circinalis* L., Cycadaceae) (Figure 3), soni (*Dillenia serrata* Thunb., Dilleniaceae), kayu cina (*Dracaena angustifolia* (Medik.) Roxb., Asparagaceae), palem *Hydriastele* sp. (Arecaceae) and soka (*Ixora lanceolata* Lam., Rubiaceae) (Figure 4).

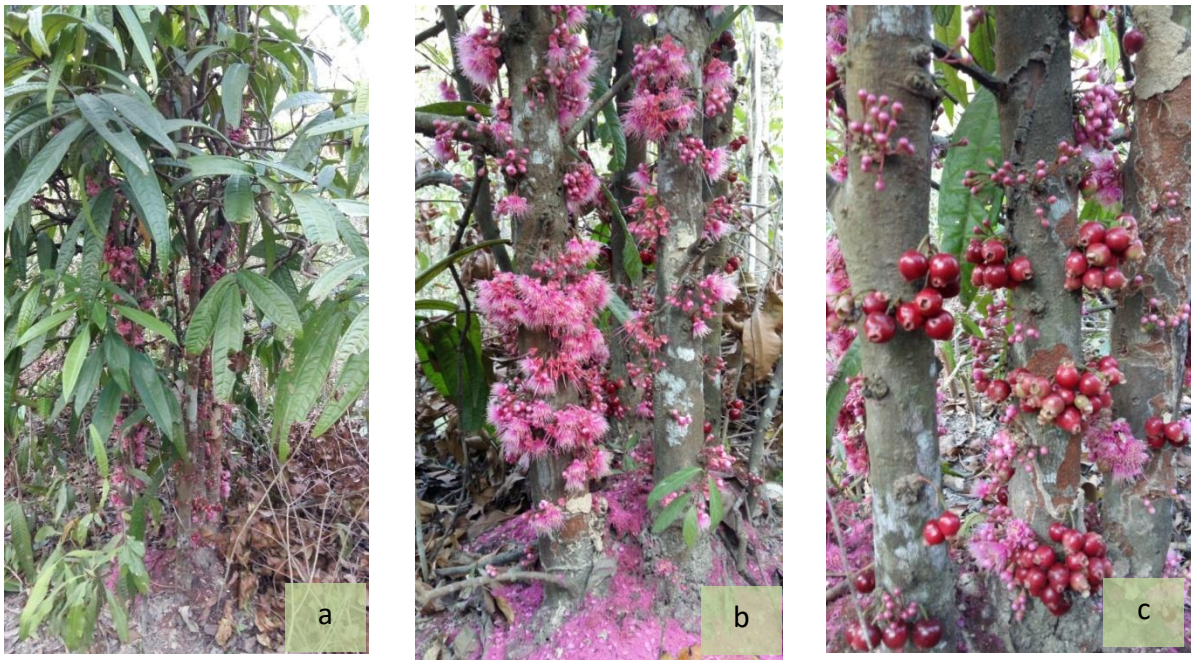


Figure 2. The blooming Ruruhi, *Syzygium* sp. (Myrtaceae) in the garden: (a) shrubby habitus, (b) flowering trunk, (c) fruits

Figure 4. Soka flower, *Ixora lanceolata* Lam. (Rubiaceae)

Figure 3. Pakis aji, *Cycas circinalis* L. (Cycadaceae), a threatened species (Endangered, A2acd) with ornamental values

The Master Plan for the KR UHO gardens was published in early 2016. It provides guidance for the construction of infrastructure, development of theme display and collection management (Figures 5 and 6). The document also states that the overall theme of the new garden is conservation of endemic plants of Sulawesi, and designates *Lasjia hildebrandii* (Steenis) P.H.Weston & A.R.Mast (Proteaceae) as the icon of the new garden; an endemic tree which has an alliance with Australian species. In the future, several themed displays and other features to be built at KR UHO, include Ebony Garden, Muna Island Plants, Myrtle Garden, Sagoo Park, Sulawesi Endemic Plants, Sulawesi Orchid House, Sulawesi Palms, Wood of Sulawesi.

Figure 5. Designs of Kebun Raya Universitas Halu Oleo (the dark green areas) within the campus (after the Master Plan; Witono *et al.*, 2016)

Figure 6. Aerial view of Kebun Raya Universitas Halu Oleo (after the Master Plan; Witono *et al.*, 2016)

Since the publication of the Master Plan, three collecting expeditions has been carried out to several sites in Sulawesi Tenggara, such as Rawa Aopa National Park, Tanjung Peropa Wildlife Sanctuary and Wawonii Island. KR UHO focuses on collecting plant species native to Sulawesi with particular interest on those endemic to Sulawesi, some examples include the orchid *Coelogyne celebensis* and the iconic species *Lasjia hildebrandii.*

Soft launching ("Pra Launching") of the garden was held on 23 March 2016, when the Rector of UHO officially launched KR UHO as a conservation site for Sulawesi endemic plants, and thus made KR UHO the first Indonesian botanic garden that is managed by a university (Figure 7). On the same occasion, the a temporary Orchid House was officially opened. It is located in front of the Auditorium building (Figure 8) and a 2 km walking trail circling inside the garden (Figure 9), including eight wooden bridges along the track (Figure 10).

Development of Kebun Raya Universitas Halu Oleo and its function

In addition to the basic infrastructure built prior to the garden's soft launching (in 2016), subsequent development of this new garden included a nursery, a compost house and an orchid house (the latter is ongoing). Three new iron bridges and walking trails have been constructed to provide access to the above three facilities. From 2018 onwards, it is expected that the Ministry of Public Works and Housing will participate in the acceleration of the garden's development by completing the garden's infrastructure and facilities.

This year the garden will continue developing its plant collections by conducting more collecting expeditions, one of which is in collaboration with Kebun Raya Bogor. To date, the garden houses as many as 104 collection numbers of existing plants (29 families, 48 genera, 40 species, 31 unidentified), and 71 collection numbers of orchids comprising 42 genera, 44 species and 27 unidentified. The orchids collections are currently housed at the temporary Orchid House located in front of the UHO Auditorium, and will be moved to the new Orchid House that is currently being built within the garden.

The current plant collections of KR UHO are listed in Tables 2 and 3, below

Table 2. Plant collections (excluding orchids) held at Kebun Raya Universitas Halu Oleo

No	Species	Families
1.	*Acronychia trifoliolata* Zoll. & Moritzi	Rutaceae
2.	*Alangium* sp.	Alangiaceae
3.	*Alstonia spectabilis* R.Br.	Apocynaceae
4.	*Antidesma montanum* Blume	Phyllanthaceae
5.	*Antidesma ghaesembilla* Gaertn.	Phyllanthaceae
6.	*Archidendron pauciflorum* (Benth.) I.C.Nielsen	Fabaceae
7.	*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg	Moraceae
8.	*Baccaurea* sp.	Phyllanthaceae
9.	*Barringtonia* sp.	Lecythidaceae
10.	*Calamus ornatus* Blume	Arecaceae
11.	*Canthium* spp. (2 numbers)	Rubiaceae
12.	*Castanopsis buruana* Miq.	Fagaceae
13.	*Cinnamomum* sp.	Lauraceae
14.	*Cleistanthus oblongifolius* (Roxb.) Müll.Arg.	Phyllanthaceae
15.	*Cratoxylum formosum* (Jacq.) Benth. & Hook.f. ex Dyer	Hypericaceae
16.	*Cratoxylum* sp.	Hypericaceae
17.	*Cycas circinalis* L.	Cycadaceae
18.	*Dillenia serrata* Thunb.	Dilleniaceae
19.	*Diospyros javanica* Bakh.	Ebenaceae
20.	*Dracaena angustifolia* (Medik.) Roxb.	Agavaceae
21.	*Dracaena* sp.	Agavaceae
22.	*Elaeocarpus serratus* L.	Elaeocarpaceae
23.	*Fagraea fragrans* Roxb.	Gentianaceae
24.	*Fagraea racemosa* Jack	Gentianaceae
25.	*Ficus crassiramea* (Miq.) Miq.	Moraceae
26.	*Ficus drupacea* Thunb.	Moraceae
27.	*Ficus oleifolia* King	Moraceae
28.	*Garcinia celebica* L.	Clusiaceae
29.	*Garcinia* sp.	Clusiaceae
30.	*Garcinia tetrandra* Pierre	Clusiaceae
31.	*Gardenia* spp. (2 numbers)	Rubiaceae
32.	*Gnetum gnemon* L.	Gnetaceae
33.	*Grevillea elbertii* Sleumer	Proteaceae
34.	*Guioa* sp.	Sapindaceae
35.	*Helicia kjellbergii* Sleumer	Proteaceae
36.	*Hydriastele* spp. (4 numbers)	Arecaceae
37.	*Ixora lanceolata* Lam.	Rubiaceae
38.	*Kibatalia* sp.	Apocynaceae

No	Species	Families
39.	*Kjellbergiodendron celebicum* (Koord.) Merr.	Myrtaceae
40.	*Lasjia hildebrandii* (Steenis) P.H.Weston & A.R.Mast	Proteaceae
41.	*Lepisanthes amoena* (Hassk.) Leenh.	Sapindaceae
42.	*Licuala rumphii* Blume	Arecaceae
43.	*Lithocarpus* spp. (4 numbers)	Fagaceae
44.	*Maranthes corymbosa* Blume	Chrysobalanaceae
45.	*Pandanus tectorius* Parkinson ex Du Roi	Pandanaceae
46.	*Peltophorum pterocarpum* (DC.) K.Heyne	Fabaceae
47.	*Polyscias kjellbergii* (Phillipson) Lowry & G.M. Plunkett	Araliaceae
48.	*Rauvolfia sumatrana* Jack	Apocynaceae
49.	*Rhodamnia cinerea* Jack	Myrtaceae
50.	*Rhodomyrtus* spp. (2 numbers)	Myrtaceae
51.	*Syzygium acuminatissimum* (Blume) DC.	Myrtaceae
52.	*Syzygium* spp. (6 numbers)	Myrtaceae
53.	*Tristaniopsis whiteana* (Griff.) Peter G.Wilson & J.T.Waterh.	Myrtaceae
54.	*Vernonia arborea* Buch.-Ham.	Asteraceae
55.	*Vitex cofassus* Reinw. ex Blume	Lamiaceae
56.	*Xanthophyllum* spp. (2 numbers)	Polygalaceae
57.	*Xanthostemon petiolatus* (Valeton) Peter G. Wilson	Myrtaceae
58.	*Xylopia* sp.	Annonaceae

Table 3. Orchid collection at Kebun Raya Universitas Halu Oleo

No	Species	No	Species
1	*Acriopsis liliifolia* (Koen.) Omerod.	34	*Grosourdya* sp.
2	*Aerides odorata* Lour.	35	*Habenaria* sp.
3	*Agrostophyllum stipulatum* Schltr.	36	*Hippeophyllum* sp.
4	*Ascocentrum miniatum* (Lindl.) Schltr.	37	*Liparis condylobulbon* Rchb.f.
5	*Bromheadia* sp.	38	*Liparis* sp.
6	*Bulbophyllum acuminatum* Rindl.	39	*Luisia* sp.
7	*Bulbophyllum macranthum* Lindl.	40	*Macropodanthus* sp.
8	*Cadetia micronephelium* Schltr.	41	*Malaxis koordersii* var. *celebensis*
9	*Calanthe triplicata* Ames.	42	*Malaxis* spp. (3 numbers)
10	*Cleisostoma simondii* Seidenf.	43	*Micropera* sp.
11	*Cleisostoma subulatum* Blume.	44	*Nervilia aragona* Gaundich.
12	*Coelogyne celebensis* J.J.Sm.	45	*Oberonia padangensis* Schltr.
13	*Coelogyne rochussenii* de Vriese.	46	*Oberonia* sp.
14	*Corymborkis veratrifolia* Blume.	47	*Oxystrophyllum cultratum* M.A.Clem.
15	*Cymbidium finlaysonianum* Lindl.	48	*Phalaenopsis amabilis* (L.) Blume.
16	*Cymbidium pubescens*	49	*Phalaenopsis amboinensis* J.J.Sm.
17	*Dendrobium anosmum*	50	*Phalaenopsis deliciosa* Rchb.f.

No	Species	No	Species
18	*Dendrobium crumenatum* Sw.	51	*Plocoglottis* sp.
19	*Dendrobium grande* Hook.f	52	*Podochilus* sp.
20	*Dendrobium litorale* Schltr.	53	*Pomatocalpa diffusum* Breda.
21	*Dendrobium reflexitepalum* J.J.Sm.	54	*Pomatocalpa spicata*
22	*Dendrobium* spp. (4 numbers)	55	*Pteroceras unguiculatum* H.A.Pedersen.
23	*Dendrobium tetrodon*	56	*Renathera* sp.
24	*Diplocaulobium* sp.	57	*Spatoglottis plicata* Blume.
25	*Eria javanica* Blume	58	*Thaneophyllum* sp.
26	*Eria ornata* Lindl.	59	*Thelasis spicrantha* J.J.Sm.
27	*Eria quadricolor* J.J.Sm.	60	*Thrixpermum* spp. (2 numbers)
28	*Eria* sp.	61	*Thrixpermum trichoglottis* Kuntze.
29	*Eria xanthocheilla*	62	*Trichoglottis geminata* (Teijsm. & Binn.) J.J.Sm.
30	*Flickingeria* sp.	63	*Vanda* sp.
31	*Goodyera* sp.	64	*Vandopsis lissochilodes* (Gaundich.) Pfitzer.
32	*Grammatophyllum scriptum* Blume.	65	*Vanilla albida* Blume.
33	*Grammatophyllum stapeliiflorum* J.J.Sm		

Figure 7. Soft launching of Kebun Raya Universitas Halu Oleo, 23 March 2016

Figure 8. The orchid collections of Kebun Raya Universitas Halu Oleo

Figure 9. Walking trails circling inside the garden area

Figure 10. One of the eight wooden bridges along the walking trails

Although KR UHO is new, and its infrastructure and supporting facilities are still very limited, the garden has already served the public and run the five functions of botanic gardens as stated in the Presidential Decree Peraturan Presiden Republik Indonesia No. 93/2011 (Anonymous, 2011), namely: conservation, research, education, environment service and recreation. The garden has been serving the five functions since its establishment in 2015, particularly as it is located within the campus of Universitas Halu Oleo. Many students from different schools of the university conducted practical classes in the garden, such as composting using the abundant forest litter from the garden and ecological studies in the forested area of the garden. The university's architectural students joined an internship project to assist in the designing and building the Orchid House and walking tracks. Some research has been carried out in the garden as well. For instance, six research studies were conducted in the garden (in 2017) by UHO students for their final year/thesis projects. The recreational aspect of the garden has resulted in an increase in the number of visitors. Most of the visitors were students and local community groups who were interested in the establishment and development of garden, wanting to enjoy the natural features and beauty of the new garden.

Lasjia hildebrandii (Steenis) P.H.Weston & A.R.Mast – the icon for Kebun Raya Universitas Halu Oleo

Each new botanic garden in Indonesia chooses an icon plant for their unique identity. *Lasjia hildebrandii* (Steenis) P.H.Weston & A.R.Mast (Proteaceae) was designated as the icon for Kebun Raya Universitas Halu Oleo as it is an endemic species to Sulawesi and naturally abundance in the new garden.

The genus *Lasjia* P.H.Weston & A.R.Mast is a member of Tribe Macadamieae (Proteaceae) consisting of five species; three of which are Australian endemic and two are endemic to Sulawesi. *Lasjia hildebrandii* (Steenis) P.H.Weston & A.R.Mast is a small tree up to 10 m high, leaves simple, 5–7 leaves are vertically or spirally arranged on the trunk/branches, with margin entire; inflorescence terminal or lateral; fruits globose, bitter (Figure 11).

The plant is also known as pohon tahan api (Sulawesi Barat) or maladewata (Indonesia), and is related to the more well-known macadamia nut from Australia. It is distibuted in lowland rainforests of Sulawesi at an elevation up to 450 m above sea level. The fruits are not edible, but are used as forage for pigs and poultry with 6.05% protein content, 48.44% carbohydrate, and 8.42% tannin (Tandi, 1989). The species is rare and may be endangered in the wild.

Figure 11. The icon plant of Kebun Raya Universitas Halu Oleo, *Lasjia hildebrandii* (Steenis) P.H.Weston & A.R.Mast; (a) habitus, (b) inflorescence, (c) fruits

CONCLUSION

Establishment of new botanic gardens in Indonesia is still required as the country is facing serious threats to its biodiversity, such as forest and other natural habitat degradation. Also, the 47 ecoregions in Indonesia are not yet all represented in order to conserve the ecoregions and its biodiversity. Kebun

Raya Universitas Halu Oleo is one of the new botanic gardens in Indonesia that answers the challenges to conserve Indonesia biodiversity particularly endemic plants to Sulawesi.

REFERENCES

Anonymous. 1997. *Agenda 21 – Indonesia: A national strategy for sustainable development.* State Ministry for Environment, Republic of Indonesia: United Nations Development Programme, Jakarta.

Anonymous. 2011. Peraturan Presiden Republik Indonesia Nomor 93 Tahun 2011 Tentang Kebun Raya.

Anonymous. 2014. Bidang Pengembangan Kawasan Konservasi Tumbuhan Ex Situ. 2014. *Roadmap Pembangunan Kebun Raya Sebagai Ruang Terbuka Hijau Pada Kawasan Perkotaan di Indonesia tahun 2015-2019*. Pusat Konservasi Tumbuhan Kebun Raya LIPI & Kementerian Pekerjaan Umum dan Perumahan Rakyat, Bogor.

Anonymous. 2016. Bidang Pengembangan Kawasan Konservasi Tumbuhan Ex Situ. 2016. *Perkembangan Pembangunan Kebun Raya Daerah di Indonesia Desember 2016*. Pusat Konservasi Tumbuhan Kebun Raya LIPI, Kementerian Pekerjaan Umum dan Perumahan Rakyat & Kementerian Dalam Negeri, Bogor.

CBD. 1993. Convention on Biological Diversity. Article 9 Ex Situ Conservation. http://www.cbd.int/convention/articles.shtml?a=cbd-09 (accessed 4 Sep 2010).

GSPC. 2002. *Global Strategy for Plant Conservation*. Convention on Biological Diversity & Botanic Gardens Conservation International. http://www.cbd.int/gspc/ (accessed 2 May 2017).

IUCN. 2015. *The IUCN Red List of Threatened Species. Version 2015-4.* http://www.iucnredlist.org. (downloaded on 19 November 2015).

Mast, A.R., C.L. Willis, E.H. Jones, K.M. Downs and P.H. Weston. 2008. A smaller *Macadamia* from a more vagile tribe: inference of phylogenetic relationships, divergence times, and diaspore evolution in *Macadamia* and relatives (tribe Macadamieae; Proteaceae). *American Journal of Botany* **95** (7): 843–870. doi: 10.3732/ ajb.0700006.

Olson, D.M., E. Dinerstein, E.D. Wikramanayake, N.D. Burgess, G.V.N. Powell, E.C. Underwood, J.A. D'Amico, I. Itoua, H.E. Strand, J.C. Morrison, C.J. Loucks, T.F. Allnut, T.H. Ricketts, Y. Kura, J.F. Lamoreux, W.W. Wettengel, P. Hedao and K.R. Kassem. 2001. Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* 51(11): 933–938.

Sutrisno. 2010. Membangun Kebun Raya Baru di Indonesia. *Warta Kebun Raya* 10 (1): 3–12

Tandi, E.J. 1989. *Penggunaan Biji Pohon Tahan Api (Macadamia hildebrandii) dalam Ransum Ternak Babi Ditinjau dari Kandungan Taninnya*. Doctorate dissertation, Program Pasca Sarjana Universitas Hasanudin, Makasar.

The Plant List. 2013. Version 1.1. Published on the Internet. http://www.theplantlist.org/ (accessed 1 January 2017).

Whitten, T., G.S. Henderson and M. Mustafa. 2002. *The Ecology of Indonesia*. *Vol. IV. The Ecology of Sulawesi*. Periplus Editions (HK) Ltd., Singapore.

Witono, J.R., D.W. Purnomo, D. Safarinanugraha, J.T. Hadiah, R.F. Lubis, H. Bakti, I.R.D. Ari, E.B. Walujo, D.M. Puspitaningtyas, D. Narko, D. Guswandi, W. Hendratmo, Suyanto, M.T. Hemun, Zulfikar & Jufri. 2016. *Master Plan Kebun Raya Universitas Halu Oleo*. Pusat Konservasi Tumbuhan Kebun Raya LIPI & Universitas Halu Oleo, Bogor.

Plants collection enrichment of Cibodas Botanic Garden as *ex-situ* conservation efforts

[1*]Imawan W. Hidayat, [1]Neneng I. Kurnita, and [1]Dimas Ardiyanto
[1]Cibodas Botanic Garden - Indonesian Institute of Sciences (LIPI).
Kebun Raya Cibodas Street, P.O. Box 19 SDL, Sindanglaya, Cianjur 43253 West Java, Indonesia. Tel. /Fax: +62-263-512233.
*Email: imawan.wahyu.hidayat@lipi.go.id

ABSTRACT

Cibodas Botanic Garden (CBG) is a government institution which has a main assignment and function on *ex-situ* plant conservation efforts, especially native plants of Indonesian tropical highland. During the last five years, CBG has conducted exploration and plants collection from Sumatran highland. Collected plants were those species which threatened, has no collection in the garden or has a potential usefulness. Plants which collected from their natural habitat, maintained, and planted in the garden will enrich plants collection of CBG. Moreover, plants enrichment of CBG was also achieved through donation and propagation. This study was to analyze the increasing of plants collection of CBG from 2012 to 2016. The inventory was conducted since plants acceptance, which achieved through exploration and plants collection of CBG, donation, and propagation until these to be planted and become plants collection. At the end of 2016, the increasing of plants collection which planted in the garden was 734 specimens. These collections were originated from exploration and plants collection 380 specimens, donation 235 specimens and propagation 119 specimens. The average of enrichment was 147 specimens per-year. The most planted were Lauraceae with 71 specimens, Arecaceae with 47 specimens, and Fagaceae 41 specimens.

Keywords: Cibodas Botanic Garden; *ex-situ* plants conservation; plants collection enrichment

INTRODUCTION

The conservation of plant diversity has received considerably less attention than the conservation of animals, perhaps because plants lack the popular appeal of many animal groups (Goettsch *et al*., 2015). As a result, plant conservation is greatly under-resourced in comparison with animal conservation (Havens *et al*., 2014). Yet plants are much more important to us. Plants provide food for us and our livestock, as well as a huge diversity of other products and services, from timber and fibers to clean water and erosion control. Although most commercial plant products come from a very narrow range of plant species, a life based on only these species would be both unhealthy and dull: even urban dwellers use a wide range of other plant species for various purposes and rural people tend to use many more. For example, Sumatran tropical rain forest in 2007 has only 29% of forests were protected by conservation areas, only nine of the 38 eco-floristic sectors had more than 50% of their remaining forest cover protected. Thirty-eight percent of remaining forest was "critically endangered", "endangered" or "vulnerable" eco-floristic sectors (5 million ha) but only 1 million ha (20%) were protected (Laumonier *et al*., 2010). It has also occurred in mountains forest, since 1985-2007, in totally, 41% forest loss has occurred in South, Central and North Sumatra. Specifically of submontane, montane and upper montane in Central Sumatra has been occurred forest lost as much as 6% and 4% in its years' range (Laumonier *et al*., 2010). Within extraordinary pressures to the existence of conservation areas, therefore it should be carried out immediately noticeable efforts in conservation sectors, one of which was *ex situ* conservation.

An *ex situ* conservation should be placed an important part of conservation efforts, because the extant habitats of a growing number of species are becoming less viable for their survival for a variety of reasons, including global warming, disease, drought, fragmentation, and deforestation (Braverman, 2014). To maintain many species, an alternative habitat (or the existing one) might be (re)constructed and managed (Braverman, 2014). Moreover, *ex situ* plant collections would help conserve threatened species (Richards *et al*., 2006), function as a source of traits for agricultural improvement (Tanksley & Mccouch, 1997; Brummer *et al*., 2011; Neale & Kremer, 2011), or has been used to propagate large numbers of individual plants for ecosystem restoration or assisted migration (Broadhurst *et al*., 2008; Vitt *et al*., 2010; Aitken & Whitlock, 2013).

Cibodas Botanic Garden (CBG) at the beginning of the establishment as an acclimatization area of highland plants which imported from foreign. This was led to the introduction of plant collections

more dominant than native plants from Indonesia. Based on the Decree of the Chairman of Indonesian Institute of Sciences (*LIPI*) January 17, 1987, *No.25/KEP/ D.5./87*, CBG has the task to conduct exploration and plant conservation of tropical wet highlands. CBG is an Indonesian government institution which has a main assignment and function on *ex-situ* plant conservation efforts, especially native plants of Indonesian tropical highland. Based on the duties and functions as an institution of *ex-situ* conservation of plants, CBG has an important role to conducted exploration and research activities of wet highlands plants. Recent years, the areas that became the focus of exploration activities were mountains zone in western Indonesia including Sumatra.

Since 2012, CBG has conducted of exploration and plants collection at Sumatran highlands. These collected plants were those species which threatened, has no collection in the garden or has a potential usefulness. Plants which collected from their natural habitat will be maintained and planted in the garden which will enrich plants collection of CBG. Moreover, plants enrichment of CBG was also achieved through donation and self-propagation effort of CBG staff. Before a plant becomes a collection of CBG, it must go through a series of data processing in order to inventory good and right plant data. The inventory was conducted since plants acceptance, which achieved from exploration and plants collection of CBG, donation, and propagation, until these to be planted and become plants collection, these process called "registration". This registration is ensuring of data validity of a plant which planted and displayed in the garden, so it has scientific integrity and a high conservation value. This enrichment is a part of CBG *ex situ* conservation of plants, and contribution in research and education.

The objectives of the study were to assess the increase of plants collection of CBG which planted in the garden for several years and to identify the background data of plants, based on registration inventory. The results were expected to give an overview of CBG contribution efforts in *ex situ* conservation of plants.

METHODS

Study site

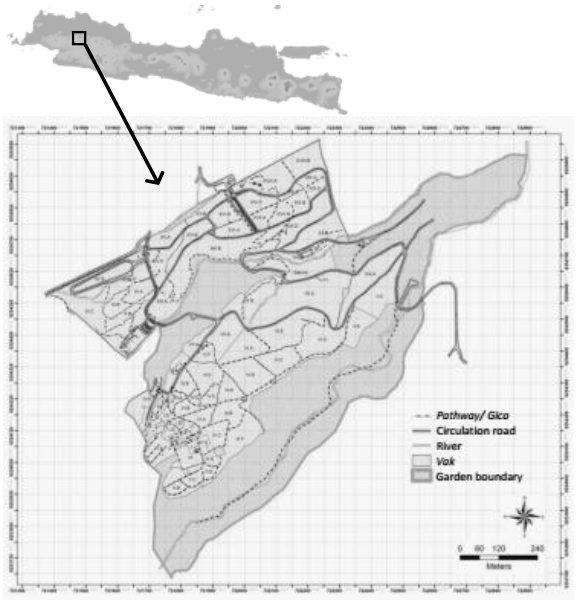


Figure 1. Study area at Cibodas Botanic Garden, Cipanas-Cianjur, West Java. (*Courtesy of* Agus Darmawan).

The study was conducted at CBG, which administratively located in Cipanas District, Cianjur Regency, West Java Province (Figure 1). CBG is located at mountainside of Mount Gede and Mount Pangrango at an altitude of approximately 1,300-1,425 meters above sea level, with an area of 84.99 hectares. The average temperature is 20 °C, humidity of 80.82% and an average rainfall of 2,950 mm per year. CBG is a comfortable place to rest while enjoying the beauty of the various types of plants that originated from Indonesia, especially from tropical wet highland, and other foreign countries. CBG is ± 100 Km from Jakarta and ± 80 Km from Bandung.

Study framework

In this study, plants collection enrichment was only assessed from three sources of plants acceptance. There were plants collection resulted from exploration, donation, and self-propagation effort of CBG staff. These sources were routinely accepted by CBG every year. Plants collection resulted from exploration were registered in "A1" form, donation registered in "A2" form, self-propagation effort registered in "A4" form. The "A" code is a unique code for plants acceptance in plants registration system of CBG. A plant can be referred to as enrichment of garden collection if it has been through a series of the registration process and finally determined as the addition of garden collection. The number of plants enrichment was assessed from acceptance until planted in the garden in the same year. Planted plants with over past periods of acceptance or before 2012, will not be counted in the data. This was to facilitate an understanding of the enrichment of CBG collections which conducted in a year, along with a series of processes. Although in fact, the cumulative number of planting was exceeded the data would be presented.

Procedures

The subject of the study was the number of planted plants which established as the garden collection. The sources of plants collection of CBG come from three sources, which are the exploration and plants collection derived from natural habitats, plant donations or contributions from outside, and the propagation and reproduction which conducted by CBG staffs. Plants which collected from their natural habitat were recorded in "A1" form, plants donation recorded in "A2", and propagation recorded in "A4" form. These forms are part of plants registration system of CBG as a form of "plants acceptance". The flowing chart of the CBG registration system from plants acceptance (A1, A2 dan A4) those plant become plant collection of CBG is explained by this diagram (Figure 2).

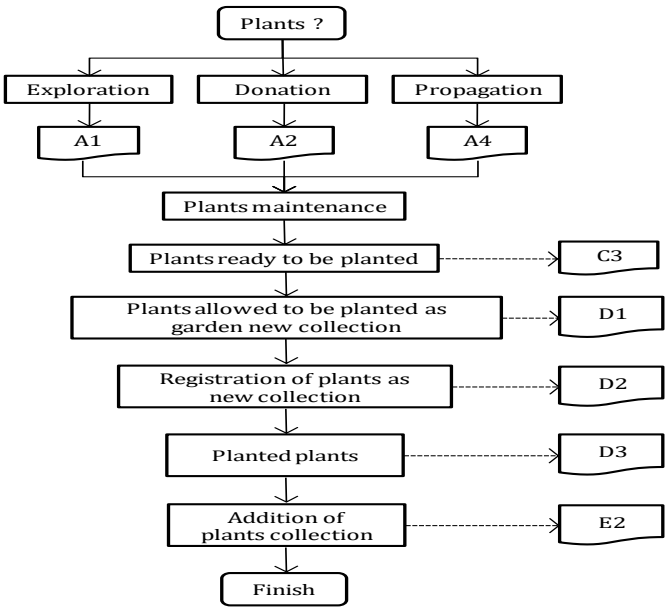


Figure 2. Flowing chart of plants collection enrichment at CBG registration system record, since acceptance until established as a garden new collection. (*Note*: this chart regardless of the dynamics of plants condition, such as death, species name alteration etc. The dynamics are also recorded in the registration system, but not to be further explained)

Data analysis

In this study, plants collection enrichment of CBG were recorded from 2012 to 2016. In each year, will be analyzed the number of enrichment, the sources of acceptance and plants species were dominated planted in the garden. Then statistically will be analyzed the addition of each year. Moreover, it will be explained plants species which have a high conservation value, such as a threatened or

vulnerable etc., and/or has no collection in the garden. This is expected to give an explanation of CBG contribution efforts in *ex situ* conservation of plants, and so for further research and education.

RESULTS AND DISCUSSION

Based on 2012 to 2016 data (Table 1), plants collection enrichment of CBG was experienced a significantly increased. Based on three sources of plants acceptance, plants collection resulted from exploration were dominated by these achievements. Since 2010 to 2016, CBG has routinely conducted exploration activities and plants collection, as part of *ex situ* conservation, with a focus on the mountains of Sumatra. The activities were conducted once or twice a year depends on institution budget. These efforts were expected to help conserve threatened species in their natural habitat and as a part of *ex situ* conservation (Richards *et al*., 2006).

Plants collection was an act of taking plant materials both seedlings, cuttings or seeds of plants, from the original habitat, which would later be re-planted and breed at CBG. List of priority plants that would be collected from the field, previously conducted through literature study, inventory and recommendations from the nursery and registration unit of CBG, consultation with the Research Center of Biology-LIPI, and the recommendations of the Ministry of Forestry and Environment. Nevertheless, it was possible to be done a plants collection outer of the list, given that the plant was found and would be collected meets the requirements of priority plants to be collected in CBG (e.g. unique, endangered, and had a high potential value of economic, ethnobotany, ornamental, development of science, or other value). Then, the results were recorded in "A1" form of registration system of CBG.

Table 1. CBG's plants collection enrichment from 2012 to 2016[*)].

Year	Sources of plants acceptance			Total (*N* specimens)
	Exploration (*n* specimens)	Donation (*n* specimens)	Propagation (*n* specimens)	
2012	118	106	47	271
2013	163	44	19	226
2014	98	66	21	185
2015	1	16	32	49
2016	-	3	-	3
Σ	380	235	119	734

[*)] *Note*: the number of plants collection was established based on the similarity period between "the access number" code of plants and related year.
The *access number* is a series unique number which established for a plant acceptance. The code consists eleven digits, the first digit is "C" code for Cibodas, four digits afterwards are code for the year, next two digit is a month in the year which plant accepted, and last four digit is the order of plant registered in that month. For example, access number C2016020016, this means the plants accepted to Cibodas in February 2016, with registered as the sixteenth order.

Table 1 described the enrichment of plants collection of CBG since plants acceptance until the plants were established as plants collection of the garden during the same year. The presentation of data was deliberately organized and restricted as such to provide a common perception of *ex situ* conservation efforts in plants collection which conducted by CBG in each year. Nevertheless, in fact at the garden, the number of additional collection plants performed each year is greater than the data presented, but the plants were accepted in the past, like two or three years ago and recently planted. The process of plants maintenance until ready to be planted is often required a long period, and the order of which plants urgently needed for planted is adapted to the needs of space of the garden, and the important value of conservation. Because of the potential for misperception and understanding of the study framework, and then the data were presented as above.

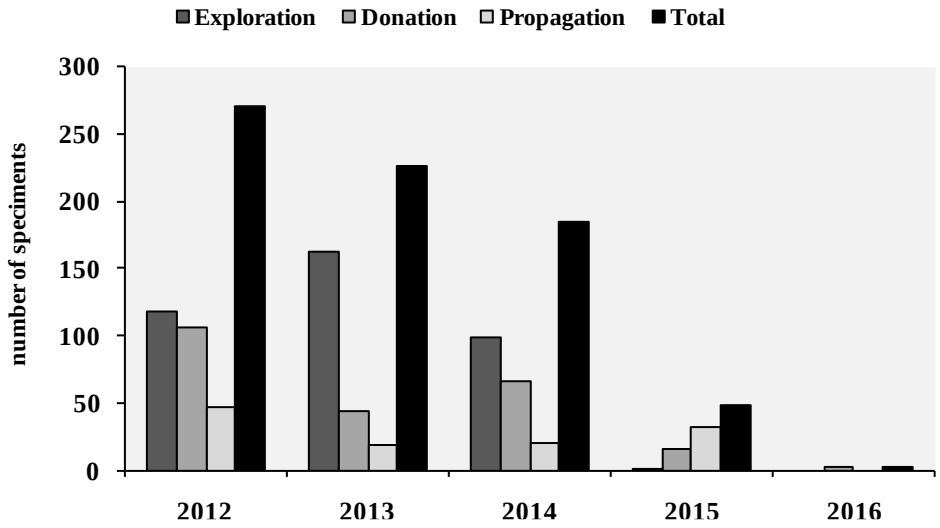


Figure 3. The chart of CBG's plants collection enrichment from 2012 to 2016, based on plants acceptance.

Table 1 also described that the average of enrichment was about 147 specimens' per-year. Plants accepted from exploration efforts were dominated contribute to garden enrichment, except in 2015 and 2016 (Table 1). And totally, more than 50% of enrichment in last five years came from exploration effort. In each exploration trip, they can collect average 300 to 400 of live plant specimens in the form of seedlings, seeds or cuttings. After maintenance and with an average survival rate above 60%, each year CBG has sufficient plant stock in the framework of the collection enrichment, beside from other sources. Nevertheless, since 2014 the contribution of exploration result has decreased in enriching the garden collection (Figure 3). This was due to the plants from the exploration from 2014 to 2016 still many in the maintenance phase, and not ready to be set as ready for planting.

The maintenance phase plays a very important role, as it provides ready-to-plant plant stock. In the maintenance phase, each plants specimen was grown in different medium to adapt their natural habitat in the wild. Growth medium has previously given *Furadan* at a dose of 7.5 to 10 gr. per sack sized 40-50 kg, in order to prevent disruption of worms and termites. Materials of growth medium were inserted into polybag of 25 cm size of each specimen which has been equipped by plant identity (i.e. species name, family, collector, date of collections and elevation) and planted in polybag per individual.

Plants seedling and terrestrial orchids were grown in forest compost medium and paddy husks with a ratio of 1:1. Epiphyte orchids were grown by using growth medium in the form of chopped ferns, and Rhododendrons were grown in the growth medium of a mashed root of *Kadaka*. Growth medium as maturate place of roots organ is an environmental factor which influences the seedling growth of plants. Based on Bradi & Well (2014), a good growth medium has four main functions, there are nutrients suppliers and roots medium, provides water and water catchment, provides air circulation for roots respiration, and for the medium of plants development. Hartmann *et al.* (2010), a good medium has several requirements, which are the ability to preserve humidity, good aeration and drainage, low salinity and clear from pests and diseases.

Based on data, species which planted as collection were dominated by the family of Lauraceae 71 specimens, Arecaceae 47 specimens, Fagaceae 41 specimens, Araliaceae 27 specimens, and Cupressaceae and Myrtaceae of each was 26 specimens (Table 2). The most planted plants were *Pinanga malaiana* (Arec.) with 18 specimens. Another species were planted such as *Callitris rhomboidea* (Cupress.) with 15 specimens, *Schefflera* sp. (Aral.) with 13 specimens, *Castanopsis argentea* (Fag.) 12 specimens, and *Archontophoenix alexandrae* (Arec.), *Cupressus cashmeriana* (Cupress.), *Castanopsis tungurrut* (Fag.), *Syzygium* sp. (Myrt.) with each of 10 specimens.

Table 2. The most planted family specimens as the collection of CBG from 2012 to 2016 (> 25 specimens).

Family	Genus	Species	*n*-specimens	*N*-specimens	IUCN/ garden status
Araliaceae	*Aralia*	*montana*	2	27	Limited/ has no
	Harmsiopanax	sp.	1		Limited/ has no
	Macropanax	sp.	2		Limited/ has no
	Polyscias	*diversifolia*	1		Limited/ has no
	Schefflera	*scandens*	1		Limited/ has no
	Schefflera	sp.	13		VU
	Trevesia	*palmata*	5		Limited/ has no
	Trevesia	*sundaica*	2		Limited/ has no
Arecaceae	*Archontophoenix*	*alexandrae*	10	47	Limited/ has no
	Areca	sp.	4		Limited/ has no
	Areca	*vestiaria*	1		Limited/ has no
	Caryota	sp.	4		Limited/ has no
	Caryota	*mitis*	1		Limited/ has no
	Licuala	*celebica*	1		Limited/ has no
	Oncosperma	*horridum*	1		Limited/ has no
	Pinanga	sp.	3		Limited/ has no
	Pinanga	*malaiana*	18		Limited/ has no
	Pinanga	*densiflora*	2		Limited/ has no
	Pinanga	*coronata*	2		Limited/ has no
Cupressaceae	*Callitris*	*rhomboidea*	15	26	LC
	Cupressus	*cashmeriana*	10		NT
	Widdringtonia	*whytei*	1		CR
Fagaceae	*Castanopsis*	*argentea*	12	41	EN
	Castanopsis	*tungurrut*	10		EN
	Castanopsis	sp.	2		Limited/ has no
	Lithocarpus	*hystrix*	1		Limited/ has no
	Lithocarpus	*blumeanus*	2		Limited/ has no
	Lithocarpus	sp.	4		Limited/ has no
	Nothofagus	sp.	1		Limited/ has no
	Quercus	sp.	8		Limited/ has no
	Quercus	*lineata*	1		Limited/ has no
Lauraceae	*Actinodaphne*	sp.	5	71	Limited/ has no
	Actinodaphne	*sesquipedalis*	5		Limited/ has no
	Actinodaphne	*procera*	2		Limited/ has no
	Alseodaphne	sp.	1		Limited/ has no
	Cinnamomum	*cassia*	3		Limited/ has no
	Cinnamomum	*heyneanum*	1		DD
	Cinnamomum	*iners*	2		Limited/ has no
	Cinnamomum	sp.	6		Limited/ has no
	Cinnamomum	*javanicum*	1		Limited/ has no
	Cinnamomum	*porrectum*	1		Limited/ has no
	Cryptocarya	*ferrea*	5		Limited/ has no
	Cryptocarya	*vulgaris*	1		Limited/ has no
	Cryptocarya	sp.	1		Limited/ has no
	Cryptocarya	*lanceolata*	1		Limited/ has no
	Cryptocarya	*affinis*	4		Limited/ has no
	Dehaasia	sp.	1		Limited/ has no
	Endiandra	*rubescens*	4		Limited/ has no
	Endiandra	sp.	1		Limited/ has no
	Litsea	*cubeba*	3		Limited/ has no
	Litsea	*garciae*	6		Limited/ has no
	Litsea	*leefeana*	1		Limited/ has no
	Litsea	*javanica*	1		Limited/ has no
	Litsea	*firma*	1		Limited/ has no
	Litsea	*ferruginea*	4		Limited/ has no
	Litsea	sp.	3		Limited/ has no
	Litsea	*oppositifolia*	1		Limited/ has no
	Neolitsea	sp.	4		Limited/ has no
	Persea	*excelsa*	1		Limited/ has no
	Phoebe	*grandis*	1		Limited/ has no

Family	Genus	Species	*n*-specimens	*N*-specimens	IUCN/ garden status
Myrtaceae	*Corymbia*	*citriodora*	4	26	Limited/ has no
	Eucalyptus	*saligna*	2		Limited/ has no
	Eucalyptus	sp.	1		Limited/ has no
	Plinia	*cauliflora*	5		Limited/ has no
	Rhodamnia	*cinerea*	1		Limited/ has no
	Syzygium	sp.	10		Limited/ has no
	Syzygium	*nigricans*	1		Limited/ has no
	Syzygium	*lineatum*	1		Limited/ has no
	Syzygium	*aqueum*	1		Limited/ has no collection

Note: IUCN status, LC: Least Concern; DD: Data Deficient; NT: Near Threatened; VU: Vulnerable; EN: Endangered; CR: Critically Endangered. *Limited/ has no collection* means that the plant has limited stock in the nursery of CBG (less than 5 specimens), or CBG has not has the collection of this species before.

Van Steenis (1972) was claimed that some families such as Arecaceae, Araucariaceae, Clethraceae, Cunoniaceae, Ericaceae, Fagaceae, Lauraceae, Myrtaceae, Pentaphylaceae, Podocarpaceae, Symplocaceae and Theaceae were a better presence in medium and high elevation than in the lowlands. Lauraceae as the highest planted plants has 29 different species which enriching plants collection of CBG. *Litsea garciae* and *Cinnamomum* sp. were most planted with every 6 specimens (Table 2). Each plant has an adaptation mechanism which enables to life adjacent to the surrounding environment. Environment parameters are determining ecological habitat in most of the plants. The interacted factors with a physiological mechanism of plants are temperature, lighting duration, wind and humidity. CBG with the altitude of approximately 1,300-1,425 meters above sea level is a suitable location for breeding of typical of tropical highland plants.

Based on the enrichment of plant collections in CBG it was expected to provide added value to the efforts of conservation *ex situ* of plants and play a role in the development of research and education. Collection enrichment is only a small part of the conservation efforts which conducted by CBG. Existing conservation efforts are not only limited to enrichment but also how CBGs can disseminate them to the wider community. It is intended that the efforts are more leverage and ability to arouse public awareness, especially in the plants conservation.

CONCLUSION

CBG has actually conducting ex situ plants conservation efforts through enrichment of plants collection, especially for wet-highland plants species. Last five years, from 2012 to 2016, the total addition collection was 734 specimens (regardless of the risk of mortality). The most contribution of the collection comes from plants exploration and collection, which routinely conducted by the staff of CBG, especially from Sumatran mountains. These efforts were expected to not only be able to provide added value to plants conservation, but also to foster the public awareness of its importance in rescuing and preserving natural resources. And, these collections are not only displayed for exhibition but also living material which can be further explored and researched, so that someday can be described the benefits for public and environment needs.

ACKNOWLEDGEMENTS

We are grateful towards all registration unit staffs of CBG, Mr. Didi Rasidi, Mr. Habibulloh, Puji Soepriati, Yudi Suhendri, and especially to Agus Darmawan for cooperation in data compilation. This paper was expected to be our small contribution in disseminating information and knowledge about CBG plants property.

REFERENCES

Bradi, N.C. and R.Well. 2014. *Nature and Properties of Soils* (14th Edition). Pearson India, New Delhi.

Braverman, I. 2014. Conservation without nature: the trouble with *in situ* versus *ex situ* conservation. *Geoforum* 51: 47-57.

Broadhurst, L.M., A. Lowe, D.J. Coates, S.A. Cunningham, M. McDonald, P.A. Vesk and C. Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* 1(4): 587–597.

Brummer, E.C., W.T. Barber, S.M. Collier, T.S. Cox, R. Johnson, S.C. Murray, R.T. Olsen, R.C. Pratt and A.M. Thro. 2011. Plant breeding for harmony between agriculture and the environment. *Frontiers in Ecology and the Environment* 9(10): 561-568.

Goettsch, B., C. Hilton-Taylor, G. Cruz-Piñón, *et al.* 2015. High proportion of cactus species threatened with extinction. *Nature Plants* 1(2015): 15142.

Hartmann, H.T., D.E. Kester, F.T. Davies Jr. and R.L. Geneve. 2010. *Plant Propagation: Principles and Practices* (8th Edition). Pearson, New Jersey.

Havens, K., A.T. Kramer and E.O. Guerrant Jr. 2014. Getting plant conservation right (or not): the case of the United States. *International Journal of Plant Sciences* 175: 3-10.

Laumonier, Y., Y. Uryu, M. Stuwe, A. Budiman, B. Setiabudi and O. Hadian. 2010. Eco-floristic sectors and deforestation threats in Sumatra: identifying new conservation area network priorities for ecosystem-based land use planning. *Biodiversity and Conservation* 19(4): 1153–1174.

Neale, D.B. and A. Kremer. 2011. Forest tree genomics: growing resources and applications. *Nature Reviews Genetics* 12(2): 111–122.

Richards, C.M., M.F. Antolin, A. Reilley, J. Poole and C. Walters. 2007. Capturing genetic diversity of wild populations for *ex-situ* conservation: Texas wild rice (*Zizania texana*) as a model. *Genetic Resource and Crop Evolution* 54(4): 837–848.

Surat Keputusan Ketua LIPI Nomor 23/Kep/D.5/87, tanggal 17 Januari 1987, tentang Organisasi dan Tata Kerja Lembaga Ilmu Pengetahuan Indonesia. [Indonesian]

Tanksley, S.D. and S.R. Mccouch. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. *Science* 277(5329): 1063–1066.

van Steenis, C.G.G.J. 1972. *The Mountain Flora of Java*. E.J. Brill, Leiden.

Vitt, P., K. Havens, A.T. Kramer, D. Sollenberger and E. Yates. 2010. Assisted migration of plants: changes in latitudes, changes in attitudes. *Biological Conservation* 143(1): 18–27.

Conservation Strategy of Hoya Species in Kutai National Park, Indonesia

Sri Rahayu[1)]* and Erly Sukrismanto[2]
1) Center for Plant Conservation Bogor Botanic Garden
Jl. Ir. H. Juanda 13 Bogor – Indonesia
Email: srirahayukrb@yahoo.com
2) Balai Taman Nasional Kutai Jl Awang Long Tromol Pos 1 Bontang 75311. - Indonesia

ABSTRACT

Hoyas (Apocynaceae, Asclepiadoideae) has high economic important as ornamental plant and medicinal source. Indonesia has high species diversity of Hoya especially in Kalimantan, which now facing problem in habitat destruction, while the species number is still not known clearly as well as its spatial distribution. An integrated conservation strategy and inventory is needed, and may be started by the small scale. This research aimed to inventory and develop conservation strategy of Hoya species in Kutai National Park, East Kalimantan, Indonesia. A survey on the occurrence of Hoya species in Kutai National Park, Indonesia, has been conducted by doing examination at Herbarium Bogoriense, Bogor Botanic gardens Collections and field observation at Kutai National Park in 2015. Fifteen species of Hoya, all containing ornamental values has been identified from the area, i.e. eleven species found form the field observationand six species from herbarium observation. Conservation strategy of These Hoya species was proposed via both in situ and ex situ collection in the area of Kutai National Park and outside, such as involvement of the local communities. These Hoya species should be promoted also as ornamental plat. The propagation technique of these species was described.

Keywords: *Hoya (Asclepiadaceae), ex-situ conservation, East Kalimantan, in-situ conservation*

INTRODUCTION

The genus *Hoya* (Asclepiadaceae) is one of the wild Indonesian Floras, which have high economic potential as ornamental plant. It has unique star waxy beautiful flowers. Most of the species also have decorative vein on leaves, succulent, and nice arrangement. The appreciation on this plant as ornamental is increasing particularly among the European, American and Australian. Several association dedicated on the Hoya cultivation have been founded following the increase of the popularity (Hodgkiss, 1997). Unfortunately, the hunting of the species by some collectors to the native habitat also increased. It becomes the threat to the existention of some species. Instead, the habitat conversion is also increased day by day. Several species become rare and toward extinction in the wild.

Indonesia was supposed as a center for Hoya species diversity (Kleijn & van Donkelaar, 2001). The geographical distribution of the Genus start from Japan to Samoa and Fiji Islands, Some part of New Zealand, Tropical part of Australia, Madagascar, India, Indochina, and South of China. The greatest diversity is in the Malesian region (Goyder, 1990). But, there is still less attention to these plants. Borneo is the Island which has been predicted to have high diversity of Hoya species. The widest area of Borneo is in Indonesia (Kalimantan). The species of Hoya in Borneo has been compiled by Lamb and Rodda (2016), which listed more than 90 species. Another new species then has been published from Kalimantan (Rahayu & Rodda 2017). The inventory of Hoya in Kalimantan is still promising new result and discovery of new species possibilities. Kalimantan is the area with high rate on habitat conversion besides of its conservation areas. Several National Parks have been established in Kalimantan with the aims to protect the biodiversity from extinction. There large one of National Park in Kalimantan is Kutai National Park, which is located in East Kalimantan. There are several habitat types which includes Hoya species.

The aims of this study is to do the inventory and to know how many Hoya species occurs in Kutai National Park as well as its distribution inside the National Park. The number of species and its

distribution will be used to develop the conservation strategy of these Hoya species in Kutai National Park.

METHODS

Conservation strategy of Hoya species in Kutai National Park was proposed after doing some inventory of oya species in Kutai National Park, Habitat assessment and potencial uses.

The inventory of the Kutai National Parks' Hoya species was done based on the literature, herbarium sheets at Herbarium Bogoriense (BO), living collection at Bogor Botanic gardens (BBG) as well as the data base, and by doing field study in the sampling areas of National Park. The inventory of Hoya species from Kutai National Parks have been done several times in the past hence the living collection at BBG obtained particularly as result from the Flora Exploration Program since 1995. The program is still continued, recently with the program of enrichment of living plant collection for local botanic gardens in Indonesia. The filed study also done recently in 2015 by the authors.

Identification of living collection and herbarium sheets was refer to Rintz (1978), Backer and Bakhuizen Jr. (1965), Hooker (1885), Merrill (1923), Schlechter (1914), Lamb and Rodda (2016).

A comparative study to the herbarium collection also did at University Pertanian Malaysia Herbarium (Selangor, Malaysia) and Forest Research Institute Malaysia Herbarium (Kepong, Malaysia), Singapore Herbarium, Leiden Herbarium, Paris Herbarium, Kew, and Edinburgh.

The assessmnet of habitat quality was done using maps of Kuati National Park i.e land cover map, supportin area map, important value map, work area map, zonation map and open area map, and ground visit. The situation map of Kutai National Park is presented in Figure. 1. The analyses was done with the recoginition to the strategic plan and action priority of Kutai National Park.

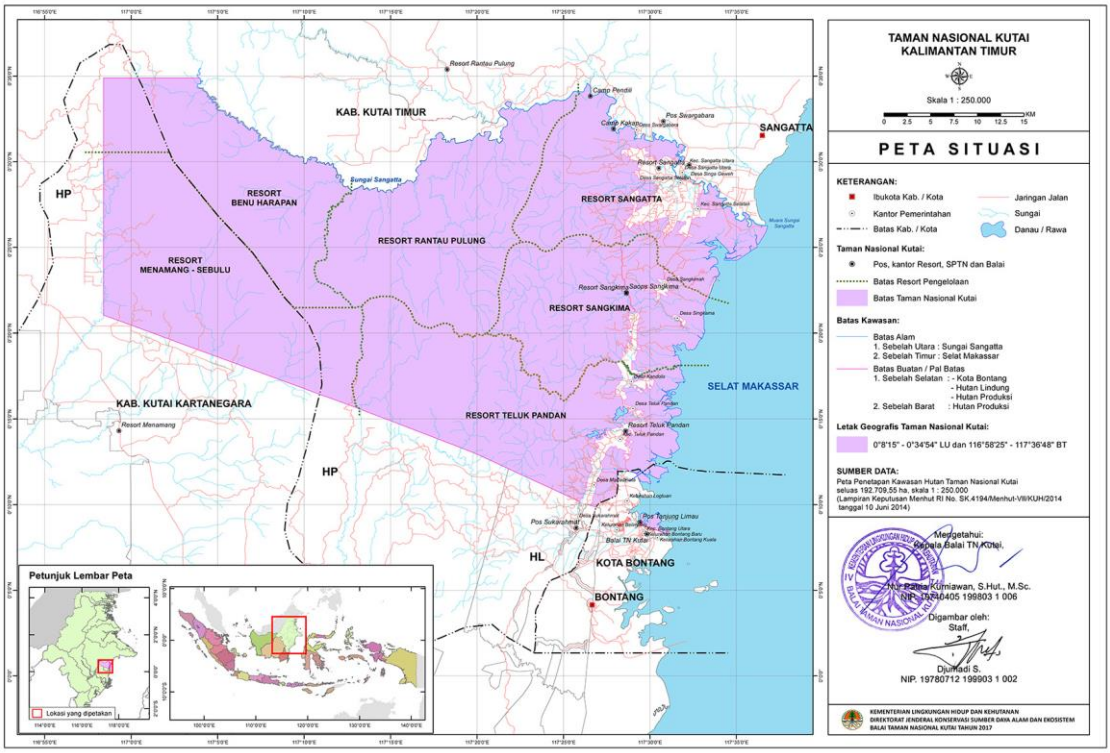


Figure 1. Situation map of Kutai National Park (Source. Kutai National Park)

RESULT AND DISCUSSION

Inventory of the Hoya Species in Kutai National Park

Any difficulties were found on each determination of species on the herbarium sheets. The herbarium sheets are very different from the living plant, particularly on the succulent species. Observation on living collection showed high plasticity on leaf morphology. The observation must do several times both on the herbarium sheets and the living collection to conclude the exact identification and determination.

There are fifteen (15) *Hoya* species as listed in Table 1 found in Kutai National Park.. These species has high potency as ornamental plant as in this time, the popularity of Hoya species as ornamental plant is increasing. The number of species is very limited from the data collected from herbarium and botanic gardens collection and several visit to the location. As the inventory were done in several representative area, but not all area was covered yet, the probality to find new species or increasing the species number is still possible. Kalimantan is the area with the highest Hoya species diversity. Lamb and Rodda (2016) listed more than 72 Hoya species were found in Borneo, which still new Hoya species from Borneo or Kalimantan is still on going published. After the publication of Lamb and Rodda (2016), a new species was published from west Kalimantan namely *Hoya narcissiflora* (Rahayu & Rodda, 2017), and very recently a new sub species of Hoya also published from Borneo and Sumatra (Rodda et all 2018). A more intensive inventory is proposed to find more species in Kutai National Park.

Table 1. Alphabetical list of Hoya Species in Kutai National Park

No	Species Name		Sumatra.	Java	Kalim.	Sulaw.	Moluc.	L.Sunda	Papua
1	*H. coriacea* Blume	* H 1			+				
2	*H. coronaria* Blume	*H 1	+		+				
3	*H. elliptica* Hook.f.	* H	+		+				
4	*H. finlaysonii* Wight	* H	+		+				
5	*H. ignorata*								
6	*H. imperialis* Lindl.	* H	+		+				
7	*H. lasiantha* Korth.	* H 1	+	+	+				
8	*H. mitrata* Kerr.	* H	+		+				
9	*H. multiflora* Blume	* H	+	+	+	+			
10	*H. nyhussiae*								
11	*H. pusilla* Rintz	*			+				
12	*H. revoluta* Wight	* H	+		+				
13	*H. scortechinii* K. & G.	*H	+						
14	*H. spartioides*								
15	*H. waymaniae*								

Notes: *collected during Flora exploration 1995 – 2002,

The Potency of In situ Conservation

The area of Kutai National Park was mostly covered by the forest, and categorized as protected area. With the habitat and forest type occured in Kutai National Park, Hoya species will be save in situ if there is no habitat changes. Kutai National Park comprised of both primary forest and secondary forest (Figure 2), which were both are good for Hoya species. The vegetation type was dominated by Dipterocarps forest, Mixed Dipterocarp forest and ironwood forest (Eusyderoxylon swagery). Kutai National Park also has a wide area at coastal area covered with the mangrove forest (Figure 3).

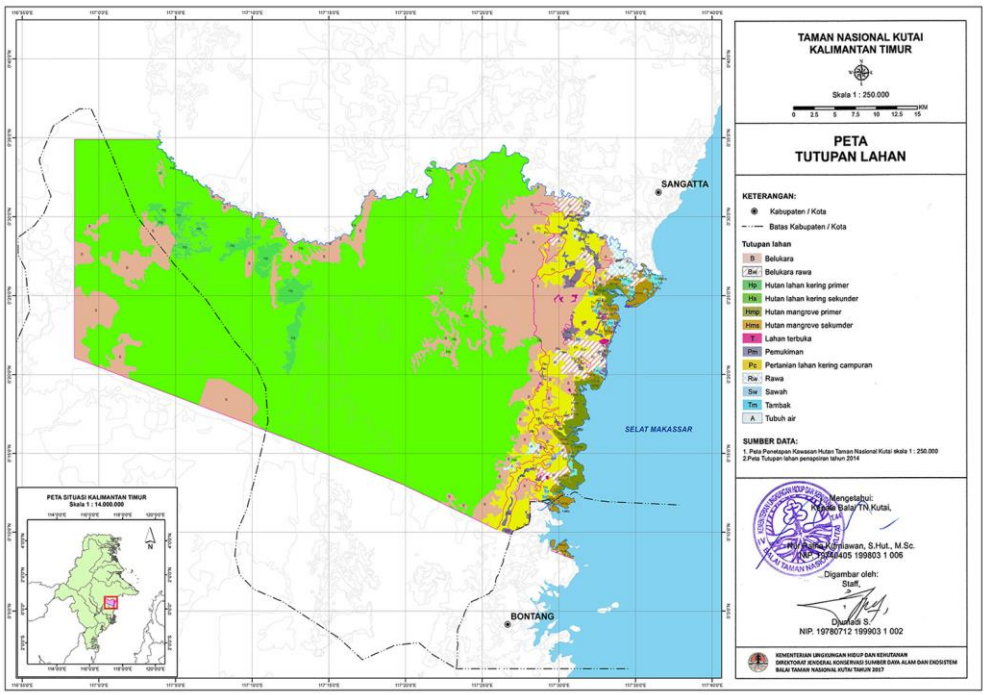


Figure 2. Land cover maps of Kuati National Park

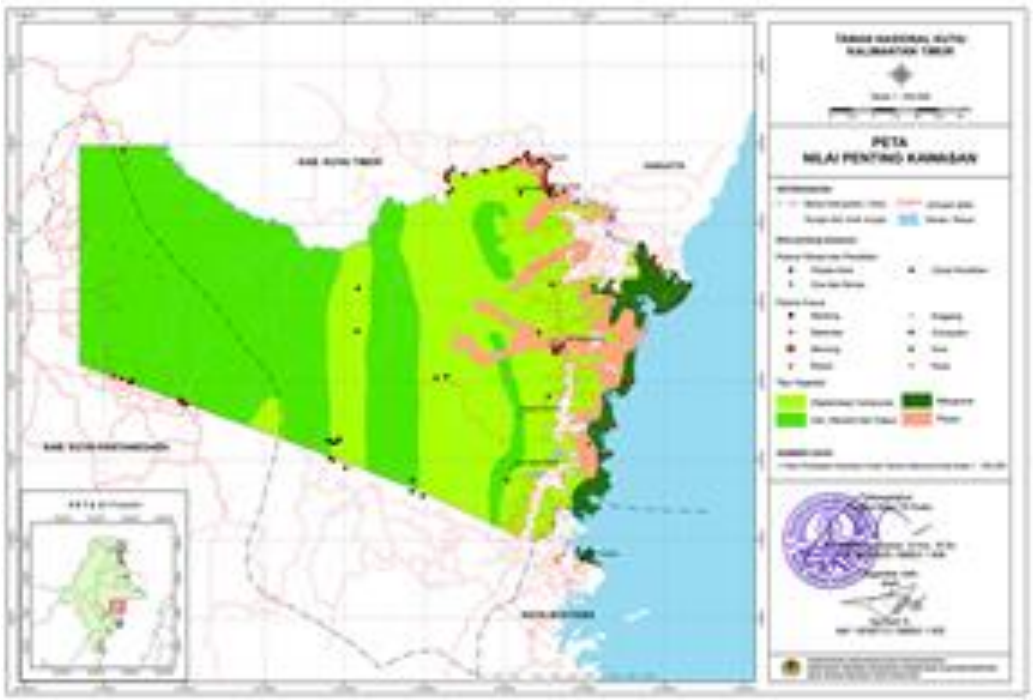


Figure 3. Important value map of Kutai National Park

The potency of *in-situ* conservation is open for all habitat and forst type in Kutai National Park. Hoya species can be found from the coastal area at altitude 0 m above sea level until 1500-200 m above sea level (Rintz 1978, Rintz 1980, Rahayu 2012 and Rodda and Lamb 2016). Hoya species tend to prefer the habitat close to the the water body such as at the riverside, beaches, and lakeside. As epiphyte, Hoya loves the humid area (Rintz 1980).

As protected area, the forest and habitat in Kutai National Park should not disturbed, except from hurricane etc. The species exploitation and species hunting are also forbiden from the conservation area, and the population hopefully develop naturally and freely. The distribution of Hoya species depend on the seed dispersal mode, which is in Hoya- seed dispersal is assisted by wind and ants (Rahayu et all, 2010). The life sycle of Hoya species in the natural habitat is depend also on the presence of pollinator. The reproductive structur of Hoya is unique and has lots of modification (Liede, 1996). Pollen and Stigma is compacted in the gynostegium, and the pollen has been evoluted to the compact form as pollinia (Endress et all, 2000). The pollinia will not moved by wind as well as by water, but it need a lifing creature to move it from the corpusculum to the receptive area of stigma. The pollinator is commonly done by insect (Rintz, 1980), which has attracted by color and odor and the nectar producing at the base of corona. Forster (1992) reported *Ochibadistes wlakeri* as pollinator of *Hoya uastralis*.

The Potency of *Ex situ* Conservation and Sustainable Utilization

Several activities on observation/characterization on the morphology of Hoya species have been done (Rahayu, 2001b). Most of Hoya species was recommended as pot plant. Hoya species of Kutai National Park has high potency to be promoted as ornamental plants. Beside, there were several uses of Hoya species as traditional medicine source by the local people. For exapmple, Hoya lasinatha and Hoya coriaceae was used in East Kalimantan to treat toothache. Another species, Hoya waymaniae used to treat stomach ache.

Among the species, *Hoya imperialis* has the most interristing floer with the biggest size and red corolla color. The utilization can be develop by implementing the Strategic plan and priority of Kutai National Park by involving the local people who living in the rural areas arround Kutai National Park. This strategy also answer the issues of traditional conflic between conservation and utilization.

The ex situ conservation strategy can be develop by establisheing "collection center garden" including Hoya species at the buffer zone. Ex situ collection can be maintain and arranging as a specific or thematic gardens which in turn will attract people to visit and learn more about plant / Hoya diversity in Kutai National Park without enter or damage the real population in the wild. The garden can be located at the atrraction point such as at

The involvement of local people will be very good. There were Bugis settlement in Kutai National Park (Vayda and Sahur, 1996). The involvement of local people can be develop by building cappacity on the simple biodiversity management; in this case the Hoya culture, propagation and maintanace. The propagataion result by the local people can be put on the show unit at the attraction area.

EPILOGUE

These activities are still continued involving a line research on the biology and cultivation of this genus. The inventory of the species is also continued. We are still waiting for a complete taxonomical revision. The living collection is hopefully increased in number of species through annually exploration program. Several programs in cultivation would be the priority instead improvement on determination and identification of living and herbarium collection.

The promotion program as ornamental should be accompanied with plant sale and publication program. At the moment we still develop the clonal propagation techniques as a tool for providing "sold plant" stock. Establishing the Hoya Garden is an effort to increase the awareness of the people appreciation on this plant.

REFFERENCES

Backer CA and Bakhuizen Jr. 1965. Flora of Java. Vol. II. Groningen, Noordrof.

Endress, M.E. & Bruyns, P.V. 2000. A revised classification of the Apocynaceae s.l. The Botanical Review 66: 1–56.

Endress, M.E., Liede- Schumann, S. and Meve, U. 2014. An updated classification for Apocynaceae. Phytotaxa 159: 175–194.

Forster PI and DJ. Liddle. 1993. Taxonomic studies on the genus *Hoya* R.Br. (Asclepiadaceae: Marsdenieae) in Papuasia, 6. Austrobaileya 4(1): 51-55

Goyder D. 1990. *Hoya multiflora* Blume. Kew Magazine 7:3-6

Hodgkiss J. 1997. The Hoya Society International. http://www.graylab.ac.uk/usr/hodgkiss/hoya1.html

Hooker, JD. 1885. The Flora of British India. Vol IV. London: L. Reeve & Co., LTD.

Kleijn D. and R van Donkelaar. 2001. Notes on the Taxonomy and ecology of the genus Hoya (Asclepiadaceae) in Central Sulawesi. Blumea 46: 457 – 483

Koorders, SH. 1898. Flora van N.O. Celebes. Batavia:s'Gravenhage G.Kolff & Co.

Lamb, A.L. & Rodda, M. 2016. A Guide to Hoyas of Borneo. Natural History Publications Borneo, Kota Kinabalu, 204 pp.

Liede S. 1996. Anther differentiation in the Asclepiadaceae-Asclepiadeae: form and function. In: D'Arcy WG, Keating RC (Eds.). The Anther: form, function and phylogeny. Cambridge: Cambridge Univ. Pess. Pp.221-235.

Merrill ED. 1923. An Enumeration of Philipine Flowering Plants. Vol. III. Manila: Bureau of Printing

Miquel J. 1856. Flora van Netherlandsch Indie. Tweede deel. Liepzig: Fried. Fleischer

Rahayu S, Rodda, M. 2015. Hoya undulata a new myrmecophytic species from Borneo and the typification of Hoya darwinii. Garden Bull Singapore 16 (2): 12-25 (2015)

Rahayu, S, Rodda, M. 2017. Hoya narcissiflora, a new species from Borneo. Reinwardtia 16 (1): 16-28 (2017)

Rahayu S, Kusmana C, Abdulhadi R, Jusuf M, Suharsono. 2010. Distribution of Hoya multiflora Blume at the Gunung Gede Pangrango National Park, Indonesia. J For Res 7 (1): 42-52.

Rahayu S. 2012. Hoya (Apocynaceae: Asclepiadoideae) diversity in Gunung Gede Pangrango National Park, West Java, Indonesia. Reinwardtia 13 (4): 331-339

Rahayu, S. 2001a. Keanekaragaman Genetik Hoya Sumatera. S2 Thesis. Program Pascasarjana IPB, Bogor. (In Bahasa, not published).

Rahayu, S. 2001b. Menjadikan Hoya asal Sumatra sebagai tanaman hias exotik asal Indonesia. Proceed. Seminar Hortikultura: 301 - 310 (In Bahasa with English abstract)

Rodda, M., A.L. Lamb, L. Gokusing, S. Rahayu. 2018. Hoya fauziana ssp angulata (Apocynaceae, Asclepiadoideae) a new subspecies from Borneo and Sumatra. Blumea Blumea 63: 144 –146.

Rintz, RE. 1978. The Peninsular Malaysian species of Hoya (Asclepiadaceae). Malay. Nat. J. 30 (3/4):467-522.

Rintz RE. 1980. The biology and cultivation of Hoyas. Asclepiadaceae 19: 9-17.

Schlechter FRR. 1914. Die Asclepiadaceen von Deutsch Neu Guinea. Engl. Bot. Jahrb.

Vayda A. P and Sahur A. 1996. Bugis Settlers in East Kalimantan's Kutai National Park. Their Past and Present and Some Possibilities for Their Future. Cifor Special Publication. Center for International Forestry Research. Bogor. 59 p.

Ecological Studies of Suweg (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) at Local Farms in Kuningan Regency, West Jawa

Syamsul Hidayat, Yuzammi, Tri Handayani
Center for Plant Conservation Botanic Gardens
Indonesian Institute of Sciences
Jl. Ir. H.Juanda No.13 Bogor 16122
Email: hidayatkbri@yahoo.com

ABSTRACT

Suweg (*Amorphophallus paeoniifolius* (Dennst.) Nicolsan), belongs to the Araceae family, is one of the plants that can be used as alternatives food. Kuningan Regency, is one of the areas within West Jawa, has known consuming suweg tuber even now. However, this practice is fading as the development and westernization in the food sector. Besides, information of ecological factors which influence in cultivation of suweg at local farms are very limited. The study aimed to determine ecological condition and distribution of local farms for recent suweg cultivation. The survey was conducted randomly to various villages in Kuningan. The results showed that there were six villages in Kuningan Regency have provided their lands to cultivate suweg and intercropping it with other commodities. The suweg intercropped mostly together with gingers, bananas and taro yarns. In general, the content of C/N ratio on the arable lands are 9.47–45.74 while the highest P and K are 418.74 ppm and 148.58 ppm, respectively. Based on the standard colour chart of the soil varies between 10 YR2/2 to 10 YR 4/5 with sandy dust texture and acidity of the soil ranging from 4.8 to 6.8. Micro-climate during the day is temperature ranging from 29-35^0 C and air humidity ranging from 58–90%. The land available for cultivation of suweg is usually located on flat and semi-open to open area. For such land condition therefore it needs to increase public awareness in terms of processing suweg in the future by optimizing the marginal land into cultivation land.

Keywords: suweg, local farms, conservation, Kuningan regency

INTRODUCTION

Amorphophallus paeoniifolius or Suweg (Javanese local name) is also known as elephant foot yam. This species is highly potential tropical tuber crop which belongs to the Araceae family. It has long been used as staple food in many countries such as in the Philippines, Indonesia (Jawa, Sumatera), Malaysia, Bangladesh, India, China and southeastern Asian countries (Chandra, 1984; Sugiyama & Santosa, 2008). The distribution of the species ranges from Madagascar through India, Malesia, Thailand, Indochina, southern China, Polynesia and northern Australia (Hetterscheid & Peng, 1995; Hetterscheid & Ittanbach, 1996; Yuzammi *et al.*, 2017). The widespread distribution of the species presumably is caused by human activities who cultivated the tuber as important tuber crop in many centuries. According to Yuzammi *et al.* (2017) suweg has a long history of cultivation. It has probably escaped from the gardens and became weedy in many areas.

This species has been cited on both traditional and ethnomedicinal literatures that is very effective and not harmful for medicinal purposes. Discovering of natural drug product could be searched from various plants species by using pharmatology approched. This method has been proved to investigate drugs that can cure various chronic diseases such as liver, cancer, arthritis and other inflammatory diseases (Dey *et al*. 2012).

There are two kinds cultivar of *A. paeoniifolius* found in Jawa, namely 'suweg' and 'walur'. Both cultivars can be differentiated by looking and rubbing the petiole. The former has slightly rough petiole, sometimes sandy-like and the tuber is edible after following several treatments. The latter has rough to very rough petiole and the tuber is not edible. However, suweg tuber has been known since Dutch Era as a staple food particularly in East and Central Jawa from 1941 to 1950. Recently, some javanese are still consuming the tuber of suweg, not as a staple food but rather than as a kinds of snack. On the other hand, Sugiyama *et al.*, (2010) mentioned that in Aceh Province and Banten Province (notably in western part) the species is considered as a weed therefore the villagers usually

removed it when it grows in their gardens. Futhermore, cultivated and escaped plants were common in Madura, Central and East Jawa, and some parts of West Jawa. Cultivation was also evident in the outskirts of larger cities, such as Medan, Padang and Lampung in Sumatera, Banjarmasin in Kalimantan, Makassar in Sulawesi, Bali and Nusa Tenggara Islands. Very limited information of suweg that is occurred in West Jawa, notably in Kuningan Regency. Therefore, this study aims to determine the ecological condition and recent distribution of local farms for suweg cultivation. The survey was conducted randomly to various villages in Kuningan Regency.

MATERIAL AND METHODS

Study site

Ecological study of *A. paeoniifolius* has been conducted on May 2016 in Kuningan Regency. In order to determine ecological condition as well as distribution of local farms for suweg cultivation, therefore the survey was conducted randomly at six villages in Kuningan Regency, namely Longkewang, Gulah Herang, Lebak Wangi, Singkup, Maleber and Cikubangsari (Fig. 1).

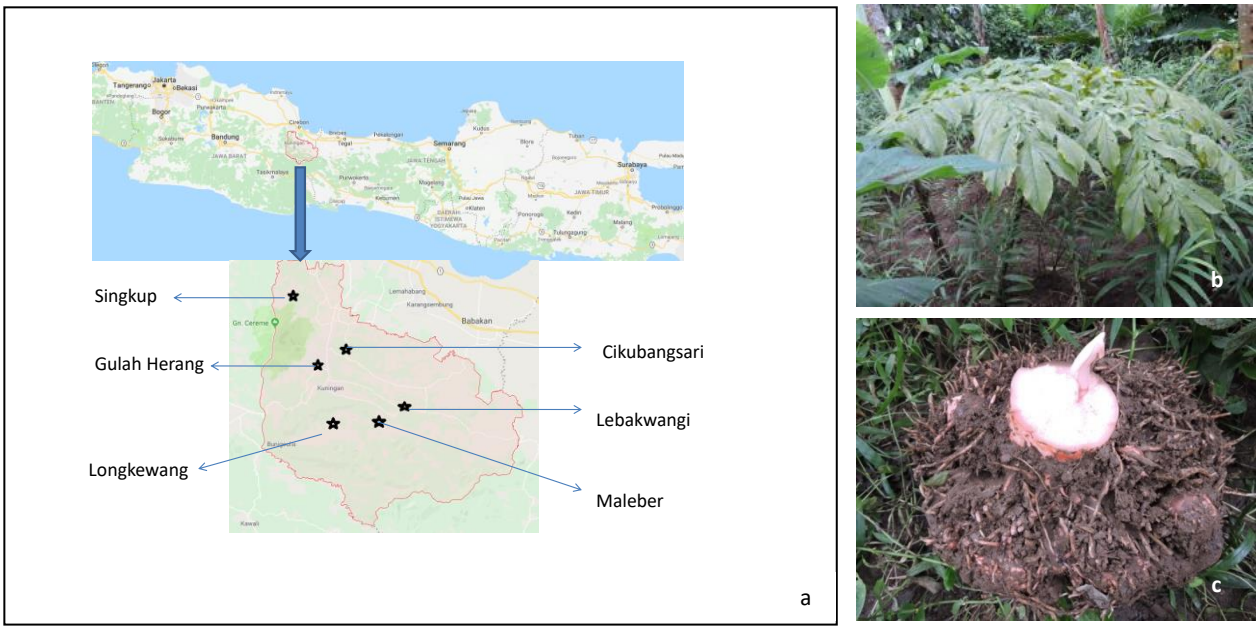


Figure 1. a) The study sites of suweg at six villages in Kuningan regency; b) vegetative phase of suweg; c) the tuber of suweg

The data of villages in Kuningan Regency, in which suwegs were cultivated at local farms, were obtained from distric offices prior to field study. Direct observation was used to collect the data from the local farms. Each local farms where planted suwegs were observed and all wild plants grew together with suweg were recorded, as well as local micro climate and soil nutrient. Several equipments were used to support local micro climate data such as thermohygrometer (temperature and humidity), pH tester (soil acidity and soil humidity), Shunto Clinometer (topography) and GPS Garmin (position and altitude). These local micro climate data then tabulated in tables and the values were made on average. Soil colour was analyzed with Munsell standard soil colour charts while nutrient content and soil texture were analyzed in a soil laboratory at Indonesian center for Biodiversity and Biotechnology in Bogor.

RESULTS AND DISCUSSION

The result showed that there are six villages, (Longkewang village, Gulah Herang village, Lebak Wangi village, Singkup village, Maleber village and Cikubangsari village of which Cipari, Winduhaji, Cibinuang as subvillage of Cikubangsari), have cultivated suweg. Mostly, other commodities (ginger, turmeric, banana and taro) or crops (cloves, cassavas, manggo, rambutan and gnetums) plantations intercropped with suweg. Ravi *et al.* (2011) mentioned that In India, suwegs are also intercropped with coconuts and bananas plantations. Furthermore, Nurlaila *et al.* (2018), mentioned that limited number of local farms cultivated suweg in Kuningan Regency. In spite of this limitation number of local farms, suweg's tubers are still well known as staple food during a shortage of rice in this regency. Commonly, suwegs could tolerate in deep shady areas though semi open areas are more preferable. Therefore, it can be intercropped between young trees (Mulyati *et al*., 2017).

Figure 2. One of dominant weed, *Nepeta cataria*, found in suweg cultivation.

During field observation, we also found many weeds growing adjacent to and were abundant among the suweg. A few of them are dominant namely *Nepeta cataria* L. (Fig. 2), *Oxalis barrelieri* L., *Biophytum umbraculum* Welw. (Fig. 3), *Phyllanthus niruri* L., *Sida rhombifolia* L. and others belong to the member of Compositae weeds such as *Ageratum conyzoides* L., *Synedrella nodiflora* (L.) Gaertn., *Mikania cordifolia* (L.f.) Willd. and *Chromolaena odorata* (L.) R.M. King & H. Rob. Mulyati *et al*. (2017) stated that the dominance of weeds in suweg cultivation is *C. odorata* which belongs to the Asteraceae family. This because *C. odorata* is able to adapt and compatitiveness compare to others. Moreover, this species does not require such certain environment to grow, propagate naturally and fast growing as well. Jintan *et al*. (2015) mentioned that understorey surrounding suweg cultivations, in Pantolan Boya village Nort Sulawesi district, were dominated by *C. odorata*, *Annona squamosa*, *Breynia sp.*, *Coccinea sp.*, *Croton sp.*, *P. niruri* and *S. rhombifolia*. Furthermore, Kunarso & Azwar (2013) explained that the diversity of understorey and the soil parameters did not showed correlation significantly. However, the abundance of *C. odorata* can be used as indicator of C-Organic and N-Total.

Figure 3. The dominant weeds from Oxalidaceae family

Suweg grows well on variety of soil types (Kumar *et al.* 2015), from medium to light soils (coarse-textured sandy soils) with adequate amounts of organic matter. This because suweg prefers well-aerated soils. The soil colour in study sited varies between 10 YR 2/2 to 10 YR 4/5 with sandy loam texture and acidity of the soil of 4.8 to 6.8, based on the standard soil colour chart. This soil acidity composed of acidic to neutral soil types. Singh & Wardhow (2014) mentioned that only dark black soil is suitable and ideal for suweg propagation. On the other hand, this research found suweg grew well on very dark brown soil and dark yellowish brown soil. The individuals of suweg can reach over 2 m high and ± 3 m in diameter of leaves and the tubers weight are over 5 kg. It can be said that the villlages in this regency have an arable lands. The C / N ratio contents are between 9.47– 45.74, the highest P content is 418.74 ppm and the highest K content is 148.58 ppm. Santosa *et al.* (2015) said that N and K are important nutrients to increase tuber yield of *Amorphophallus* species. The result of their study showed that duration growth and yield of *A. paeoniifolius* has increased with addition of application NPK 20 ton ha $^{-1}$ manure.

Suweg grows well under tropical, warm and humid condition (Ravi *et al.*, 2011), with optimum temperature 20–30°C (in Indonesia) (Sugiyama & Santoso, 2008). However, in India this species grows well on temperature 30–35°C (Sunitha *et al.*, 2013). The study in Kuningan Regency recorded the range of temperature for suweg cultivation was 29–35°C. In spite of this temperature highest than what was Sugiyama & Santoso (2008) mentioned above, only in Cikubangsari and Longkewang villages were found suweg growing well with temperature ranging from 29–30°C (Tabel 1). Moreover, air humidity is also influence on growth and development of suweg, notably at early growing stage. It can be seen at Maleber Village, in which the maximum temperature during the day is 31°C and air humidity is 90% (the highest), could generate quite well suweg growth. Besides, level of shading is also effect on growth and development of suweg. According to Santoso *et al.* (2006), suweg grows well under 75% of shade. In Kuningan Regency, in some villages showed that the suwegs were still grown well in shading area, even under 90% of shade.

Table 1. Local micro-climate at six villages in Kuningan Regency on May 2016

No	Research Sites (villages)	Altitute (m asl)	Temperature (°C)	Air Humidity (%)	pH Soil	Soil Humidity (%)	Shade level (%)
1.	Singkup	411	31–33°	59%	6.5–6.8	70–80%	70–80%
2.	Cikubangsari	700	29–30°	74%	5.9–6.2	60–80%	50–80%
3.	Gulah Herang	150	31–32°	72%	6.5–6.7	70–80%	70–90%
4.	Lebakwangi	521	34–35°	58%	6.2–6.5	60–80%	50–60%
5.	Longkewang	681	29–30°	72%	4.8–6.8	25–70%	60–90%
6.	Maleber	158	30–31°	90%	6.5–6.7	60–70%	50–70%

The acidity of soil (pH soil) in those study sites ranged from 4.8–6.8 (acid to neutral). Ravi *et al*. (2011) mentioned that the optimum pH for cultivation of suweg is 6–7, whereas in this study, such as in Longkewang Village, found that most of suwegs growing well in acid soil even untill 4.8. However, it can not justified that the soil condition is only indicated by pH but environment factors also play important role, such as mineral contents, water reserve and soil humidity. The soil humidity in which suweg growth in several villages, in Kuningan Regency, tended to high up to 80%. The wide range of diameter of lamina is influenced by soil humidity. The increasing weight of tuber corresponds to diameter of lamina (Sunitha *et al.*, 2013).

Most of suwegs in Kuningan Regency are cultivated as secondary crops, growing among other major ones. Mostly, suweg grew wild without any treatment. In some cultivation farms, suweg is considered as an unavailing plant though it is functional food by some others. Under such condition, it is needed to increase public awareness for optimizing their marginal land and is converted into cultivation land, such as suweg cultivation.

CONCLUSION

Kuningan is one of the Regency in West Java which still grows suweg for local consumpsion even now. Six villages in this regency have been choosen as an example sites of the study viz. Cikubangsari, Gulah Herang, Lebakwangi, Longkewang, Maleber and Singkup. Those villages are suitable for suweg cultivation based on ecological conditions and are planted as intercropping with bananas, taros, turmerics and gingers. Micro climate was recorded during the day as followed: temperature ranging from 29–35^0 C, air humidity ranging from 58–90% and shade level ranging from 50–90%. The acidity of soil varied from 4.8 to 6.8. The topography all of these villages mostly found flat, somewhat undulate to slightly hill, from slighly open to open areas.

ACKNOWLEDGEMENTS

This research was funded by thematic DIPA programme. We would like to thank Director of Center for Plant Conservation Botanical Gardens, Indonesian Institute of Sciences for allowing us to conduct field work. We also thank to Kuningan Botanic Garden staffs, especially to Mr. H. Maryoto, for assistance during the field work. We also express our gratitude to the villages administration staffs in the Kuningan Regency and the farmers who have supported this research activities.

REFERENCES

AICRP (All India Coordinated Research Project on tuber crops). 2008. *Elephant foot yam*. All India Coordinated Research Project on tuber crops Annual Report 2007-2008. pp. 93–106.

Dey, Y.N., S. Ota, N. Srikanth, M. Jamal and M. Wanjari. 2012. A phytopharmacological review on an important medicinal plant - *Amorphophallus paeoniifolius*. *Ayurvedic Journal* 33(1): 27–32.

Hetterscheid, W.L.A and C. Peng. 1995. Notes on the genus *Amorphophallus* (Araceae) IV. Revision of the species in Taiwan. *Botanical Bulletin Academia Sinica* 36: 101–112

Jintan, Yuzammi, I.N. Suwastika and R. Pitopang. 2015. Botany of *Amorphophallus paeoniifolius* Dennst. Nicolson (Araceae) in Palu Valley. *Online Jurnal of Natural Science* Vol 4(1): 17–31.

Kumar, R.P.K., S.K. Kolli, J. Suneetha and G. Hemanth. 2015. Cultivation of *Amorphophallus paeoniifolius* (Dennst.) Nicolson (Elephant Foot Yam) in Kovvur Mandal of west Godavari District, Andhrapradesh India. *International Journal of Current Research* 7(05): 15549–15553.

Kunarso, A and F. Azwar. 2013. Keragaman jenis tumbuhan bawah pada berbagai tegakan hutan tanaman di Benakat, Sumatera Selatan. *Jurnal Penelitian Hutan Tanaman* 10 (2): 85–98.

Kurniawan, A., I.P.A. Wibawa and B. Adjie. 2011. Species diversity of *Amorphophallus* (Araceae) in Bali and Lombok with attention to genetic study in *A. paeoniifolius* (Dennst.) Nicolson. Biodiversitas 12(81): 7–11.

Mulyati, Djufri and Supriatno. 2017. Analysis vegetation of shade corpse flower (*Amorphophallus peoniifolius* (Dennst.) Nicolson) in Padang Tiji sub-district of Pidie District. *Jurnal Ilmiah Mahasiswa Fakultas Keguruan dan Ilmu Pendidikan Unsyiah* 2(1): 98–105.

Nurlaila, A., I. Karyaningsih and N. Herlina. 2018. Diversification of forest food plants on agroforestry system in Kuningan District. *Journal of Forestry and Environment* 01: 12–15.

Ravi, V., C.S. Ravindran, G. Suja, J. George, M. Nedunchezhiyan, G. Byju and S.K. Naskar. 2011. Crop physiology of elephant foot yam [*Amorphophallus paeoniifolius* (Dennst. Nicolson)]. *Advances in Horticultural Sciences* 25(1): 51–63.

Santosa, E., N. Sugiyama, M. Nakata and O.N. Lee. 2006. Growth and corm production of *Amorphophallus* at defferent shadinglevel in Indonesia. *Japan Journal of Tropical Agriculture* 50(2): 87–91.

Santosa, E., A.D. Susila, A.P. Lontoh, A, Noguchi, K. Takahat and N. Sugiyama. 2015. NPK fertilizers for Elephant Foot Yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) intercropped with coffee trees. *Journal Agronomy of Indonesia* 43(3): 257–263.

Singh, A and N. Wadhwa. 2014. A review on multiple potential of aroid: *Amorphophallus paeoniifolius. International Journal of Pharmaceutical Sciences Review and Research* 24(1): 55–60.

Sugiyama, N. and E. Santosa. 2008. *Edible Amorphophallus in Indonesia. potential crops in agroforestry*. Gadjah Mada University press, Bulaksumur, Yogyakarta.

Sugiyama, N., E. Santosa and M. Nakata. 2010. Distribution of elephant foot yam (*Amorphophallus paeoniifolius*) in Indonesia. *Tropical Agriculture and Development* 54(1): 33–34.

Sunitha, S., V. Ravi, J. George and G. Suja. 2013. Aroids and water relations: An overview. Journal of Root Crops 39(1): 10–21.

Yuzammi, A. Kurniawan, N.P.S. Asih, I. Erlinawati and W. Hetterscheld. 2017. *The Amorphophallus of Indonesia*. Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences.

Ecosystem Services on Three Land Use System around Janitri Water Springs, Sumber Brantas Village, Batu

Titut Yulistyarini and Abban Putri Fiqa
Purwodadi Botanic Garden-Indonesian Institute of Sciences
Jl. Raya Surabaya-Malang km. 65, Purwodadi-Pasuruan, Jawa Timur 67163,
Phone/Fax: 0343-615033
E-mail: tyulistyarini@yahoo.com

ABSTRACT

Janitri is one of the water springs located in Batu, East Java, which has been used by local people to irrigate their vegetable fields. In 2004, the restoration had done in the conservation area of Janitri by local governments, in collaboration with the Purwodadi Botanic Garden, as an effort to solve a landslide disaster. However, the restoration program not only saved people from the landslide but also increased the other ecosystem services in the Janitri water spring restoration area, in about six years after the restoration (2004-2010). Ecosystem services are the free services provided by ecosystems to the environment, in the form of regulating, supporting, provisioning and cultural services. The research was done to determined the difference of ecosystem services of the three different Land Use System (LUS), around the Janitri water spring, which are restored area, agricultural land, and mushroom farm area. The difference of these ecosystem services will illustrate the effectiveness of the restoration program. The parameter that been observed were soil biohydrophysics and carbon storage in each land use system. Data were analyzed statistically by MANOVA, PCA and cluster. The result showed that each land use system gives affect significantly on the ecosystem services. PCA analysis shows that forested area has the best ecosystem services. Based on cluster analysis, mushroom farm and agricultural land have more similar each other than to the forested area with distance no more than 30 by Euclidean similarity index. Restoration gives a positive impact to the environment indicates by better ecosystem services provided.

Keywords: ecosystem services, restoration, Sumber Brantas, Sumber Janitri, water springs.

INTRODUCTION

The dissimilarity of land use system becomes an important differentiator of the difference structure and function of each area. Land use change as a consequence, will change not only the structure of the landscape but also the function of it. The studies of land use change and land use coverage become one of a much-worked option to assess the ecosystem integrity. This work is done by valuing their ecosystem services (Hu *et al*. 2008; Burkhard *et al*. 2009; Martinez *et al*. 2009). Assessing the ecosystem services is become popular study research since it could give the complex information both ecologic and socio-economic information about the services provided by the area. This comprehensive research will also give more chance for trans-disciplinary study as environmental degraded problems not only give effect to the environment issue but also to the socio-economic value (Bukhard *et al*., 2009).

Ecosystem services based on Wunder and Thorsen (2014) is all of advantages that human can be obtained both on process and resources that available in the nature. The Millennium Ecosystem Assessment (MEA) (2005) categorize ecosystem services into four section, that is: Regulating services such as store carbon and control flooding; Provisioning services including supply clean water and wood; Supporting services such as preserve biodiversity and soil formation; Cultural services including their support on people recreation and education. Those all services that nature give for human will change in line with all of the activities that human done in the area, as well as the change in the land use systems.

Janitri water spring is located in the Sumber Brantas village, Batu city, surrounded by farm area. It is site in the altitude 1600 m asl, and categorized as a low mountain forest area (Whitten *et al*. 1999). Only about 16.60% from the total area is preserved as a remaining forest, while the other 83.4 % is utilized for farming, settlement and also mushroom industry (Yulistyarini and Fiqa, 2010). The forest remaining itself was a restoration product which is held in 2004. Restoration was done as a respond to the land slide and flood disaster in the Cangar forest area as a result of the logging activity in the forest

(Soejono, 2008). Some plants planted in the forested area as *Engelhardia spicata, Lithocarpus sundaicus, and Litsea diversifolia that are native species. Some other introduction plants such as Calliandra hautisoniana var. callothyrsus, Persea americana, Syzygium polyanthum, Acacia decurens, and Toona sureni also planted* in there. After six years of restoration program, monitoring was held to evaluate the restoration process that has been running. Restoration monitoring process generally done by valuing the ecosystem services produces in the area (Ruiz-Jaen and Aide, 2005; Benayas *et al*. 2009). Hence, it is important to evaluate the ecosystem services produce by such ecosystem around Janitri water spring to understand how the different of land use system give impacts on ecosystem services.

The research aimed is to study the difference value of ecosystem services such as infiltration rate (provisioning services) and above ground C-stock (regulating services), in three different Land Use System around the Janitri water spring. Those variables will give an overview how land use system might change the ecosystems. Furthermore, this research can also be used as an initial reference how to manage the forest area remaining surrounded by agricultural land and mushroom industry.

MATERIAL AND METHODS

Study Site

Study was done in Sumber Brantas village, Batu city, East Java province (between 07°74'96,0" S and 112°52'96,0" W). The topographical conditions of this area vary from flat, bumpy, hilly to mountainous, with 25 to 40% slope dominance. The dominant soil type in this area is Andisol, with medium to smooth soil texture and its soil solum dominated by dust. The average rainfall of Sumber Brantas village is 2500 mm year^{-1} and classified into wet climate type.

Janitri water spring was classified into Anjasmoro spring based on its location. Janitri water spring, like other springs in the upper mountains, is surrounded by an agricultural land, with vegetables as commodity, at a radius of 200 m from the water discharge (Yulistyarini *et al*., 2009). The water spring area large based on micro watershed delineation was about 20,82 ha, with three specific land use system (Fig. 1) which are agricultural land (13,47 ha) with vegetables as the commodity; mushroom farm 3,89 ha), with area covered by farming mushroom building and people settlements; and forested area (3,46 ha) with complete plant strata which is trees and sapling dominated by *Engelhardtia spicata*, and groundcover by *Penisetum purpureum* (Yulistyarini and Fiqa, 2010).

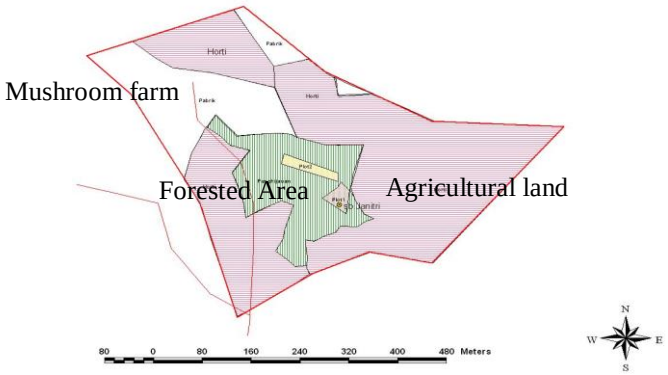


Figure 1. Three Land Use Systemsat around Janitri Water spring

Ecosystem services valuation

Ecosystem services measured in this research are rain infiltration rate which relating to the supply of clean water (provisioning services) and above ground C-stock which relating to climate regulation. Some soil biophysics characteristics which support the ecosystem services inside the area also determined such as soil saturated hydraulic conductivity (KSat) (cm.hour-1), soil aggregate stability (mm), soil carbon (%), soil density (g.cm^{-3}), and soil porosity (%). Biophysical variable was measured by taking soil samples in three areas at the three different depths that are 0-10 cm, 10-20 cm and 20-30

cm. Soil samplings were analyzed in Brawijaya University's Soil Laboratory. Infiltration rate was obtained by calculating the difference in rainfall with surface runoff and expressed in cm.hour^{-1}. The infiltration rate presented is the constant infiltration rate when the soil is in saturation condition (Widianto *et al.*, 2004).

Carbon storage on forested area was estimated from above ground pools; consist of trees (dbh > 30cm), saplings (dbh 5-30cm), ground covers (plant that dbh < 5cm i.e. shrubs, herbs, seedling, and woody climbers. There are six plots of 200 m^2 (40m x 5m) for measuring plants with dbh 5-30cm (saplings). While ground cover was measured in 12 sub plots of 1 m^2 (1m x 1m). The carbon stock calculating methods in above-ground was based on RaCSA (Rapid Carbon Stock Appraisal) methods (Hairiah *et al*., 2011).

Data collected than tabulated and analyzed statistically to find the difference among the three areas compared above. Data analyzed by MANOVA, Principle Component Analysis (PCA), and Cluster. Analysis was done by using PAST 3 statistic software.

RESULTS AND DISCUSSION

Statistically results showed that observation area significantly affects seven parameters that observed. It is proved that the three land use system showed different ecosystem services performances one to the others. However, not all the parameters showed the significantly difference among each other's.

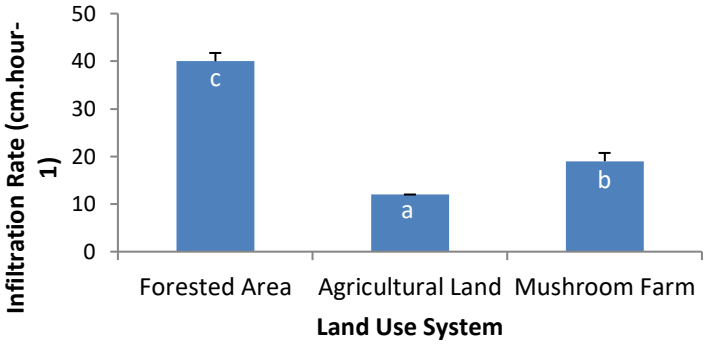


Figure 2. Infiltration rate in the three Land Use Systems (Note: Different notation shows different significantly with p<0,05)

Infiltration rate in each area is significantly different. Infiltration rate in forested area reached 40 ± 1,73 cm.hour-^{1}, which classified as very fast (>25 cm.hour^{-1}) (Lee, 1990). Infiltration rate in the mushroom farm and agricultural land show slightly different 19 ± 1,73 cm.hour^{-1} compared with 12 ± 0 cm.hour^{-1} (Figure 2). This infiltration rate will support the recharge of rain water into the soil.

Infiltration rate is affected by other soil biophysics characteristic. Soil saturated hydraulic conductance (KSat) in the forested area is the highest among the others. However, the statistical test show that KSat value of forested area 21,58 ± 11,21 cm.hour^{-1} and agricultural land 4,52 ± 2,74 cm.hour^{-1} is not significantly different, but significantly different from mushroom farm 0,4 ± 0,1 cm.hour^{-1} (Figure 3a). KSat soil is always proportional to soil porosity percentage. The higher soil porosity the higher KSat soil will be (Rieu & Sposito, 1991). KSat value of forested area is classified as fast since its value is more than 12,5 cm.hour^{-1} (Landon, 1984).

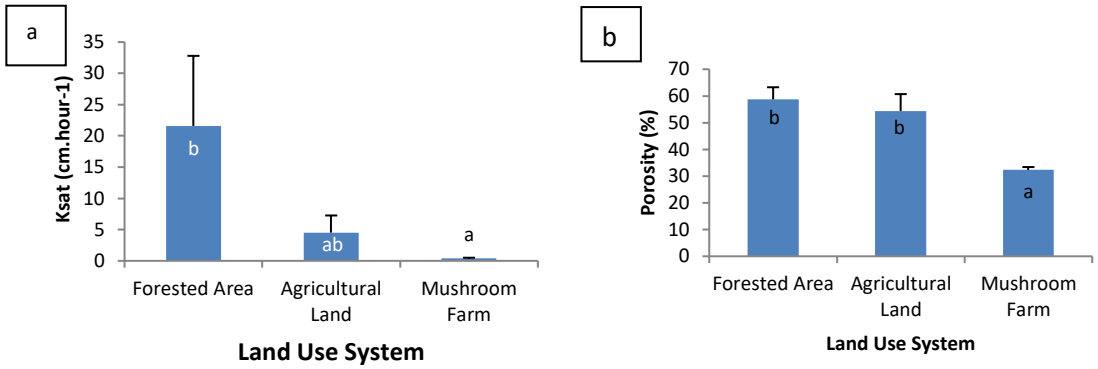


Figure 3. Soil saturated hydraulic conductivity (a) and Porosity (b) in three Land Use Systems around Janitri water spring (Note: Different notation shows different significantly with p<0,05)

Soil porosity in the forested area and in the agricultural land is higher than in the mushroom farm. Statistical result indicates that there is no difference porosity percentage in the agricultural land and forested area (Figure 3b). Forested area has a porosity percentage 58,71±4,55%, slightly different from agricultural land with value 54,38±6,32%, while porosity percentage in the mushroom farm only reached 32,3±1,1%.

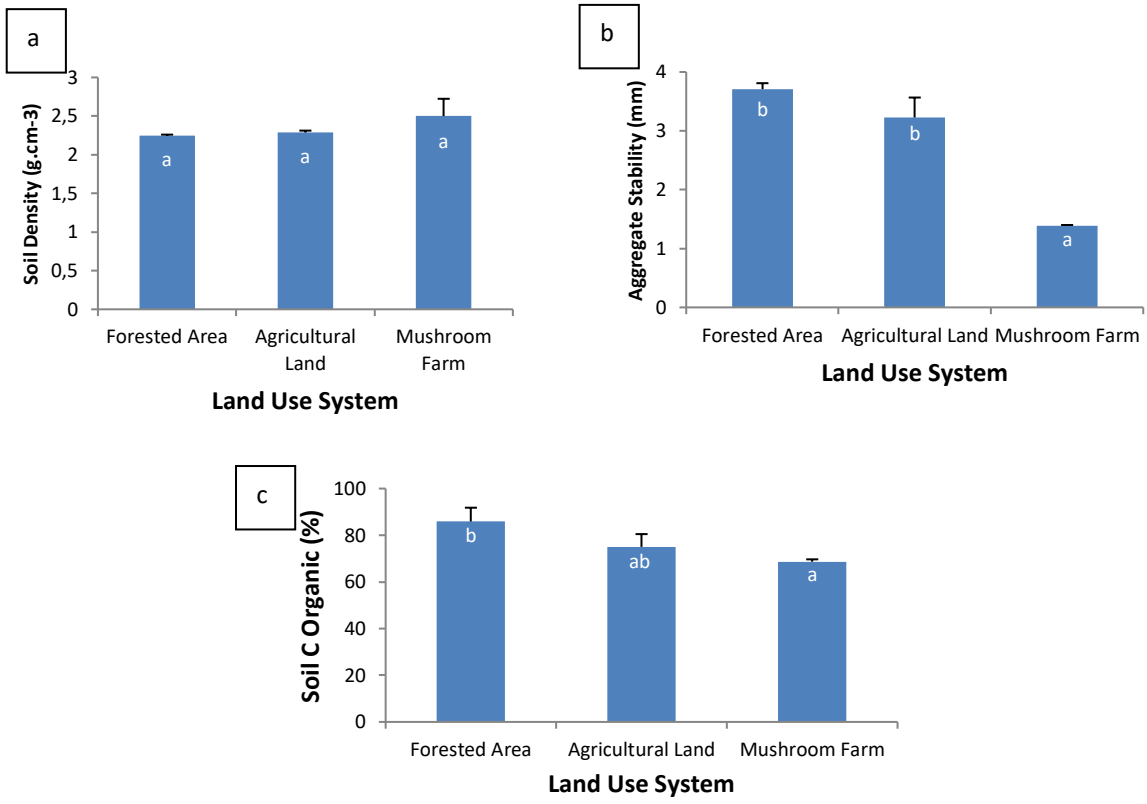


Figure 4. Soil density (a), Soil aggregate stability (b) and Soil C-organic (c) in three Land Use Systems around Janitri water spring (Note: Different notation shows different significantly with p<0,05)

Soil density will always inversely proportional with porosity, the lower soil density will cause the percentage of porosity increase (Suprayogo *et al*. 2004). A high value of soil density might be caused by soil compaction, as a result of human activity above it. Mushroom farm is the area with a lot of

activity on it; therefore the soil porosity in the area is low. Soil density, as one of the studied parameters showed that in the three areas observed no one of those areas showed different (Figure 4a). The highest value is belonging to mushroom farm (2,5 ± 0,22 $g.cm^{-3}$), followed by agricultural land (2,29 ± 0,025 $g.cm^{-3}$) and forested area (2,25 ± 0,015 $g.cm^{-3}$) respectively.

Soil aggregate stability in forested area and agricultural land is similar due to the statistical test 3,71 ± 0,1 mm and 3,23 ± 0,34 mm respectively, while in mushroom farm is only about 1,39 ± 0,01 mm (Figure 4b). Prasetya *et al*. 2012 also reported in the Pujon district, Batu City, the soil aggregate stability in the forest area is higher than in the agricultural area. This condition is affected by the composition of C-organic in the soil. The composition of C-organic in the soil can support the aggregate stabilization in the soil (Prasetya *et al*. 2012). This condition is appropriate with the research result in the study area, where soil C-organic compound in the forested area 85,9 ± 5,9 % is the highest percentage among the others though not significantly different to agricultural land if tested statistically (Figure 4c). In contrast, C-organic compound in the mushroom farm is the lowest (68,68 ± 0,99 %), insignificantly different with the soil C-organic compound in the agricultural land (75,01 ± 5,48 %).

The other ecosystem services variable to be compared is above ground C-stock in the three different area studies. Carbon stock is classified as regulating category in the ecosystem services by MEA. Study showed that above ground C-stock is strongly affected by the existence of trees as a land cover. Research result showed that above ground C-stock in the forested area is almost 6000 times as much C-stock as the agricultural land (135,94 ± 110,79 $ton.ha^{-1}$ as opposed to 0,024 ± 0,001 $ton.ha^{-1}$ for agricultural land). Mushroom farm above ground C-stock however only slightly different with the agricultural land, which is 0,012 ± 0,001 $ton.ha^{-1}$(Figure 5). However, the above ground C-stock in the forested area Janitri water spring is higher than in the coffee agroforestry around Krisik water spring (70 $Mg.ha^{-1}$) and in Ngantang (99-123 $Mg.ha^{-1}$) (Yulistyarini, 2013; Hairiyah and Rahayu, 2010).

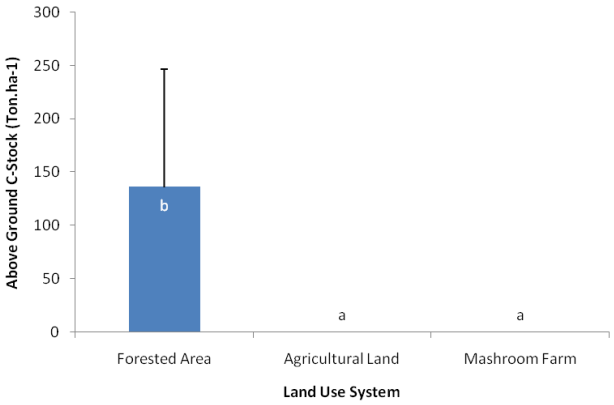


Figure 5. Above ground C-stock in three Land Use Systems around Janitri water spring

In general, Principle Component Analysis is shown that three group of study area are significantly different and located in the three different quadrant on the graphic (Fig. 6a). Forested area (FA) was considered as best ecosystem services due to the some parameters measured which is infiltration rate (IF), soil saturated hydraulic conductivity (KSat), above ground C-stock (AC), and soil C-organic compound (SC) has the highest value. Agricultural land has a good soil aggregate stability and porosity due to the human interventions to keep the stability of vegetables productivity in the farm, however its unnaturally process may limited. The opposite condition in the mushroom farm with the highest soil density value indicates that the area is disturbed a lot. Clustering analysis showed that agricultural land (AL) and mushroom farm (MF) is almost similar each other. It can be seen that the distance between them is not more than 30 by the Euclidean similarity index (Fig. 6b). Forested area categorized as a different group due to the ecosystem services performance that it shown. The condition may cause by the different vegetation covering the area. Land use system will influence what kind of vegetation covering the area, Wu and Hobbs (2002) said that both land use and land coverage will determine mot only the function but also the structure and the dynamic of the landscape. Vegetation is

an important part of the conservation. It provides litter to maintain the survival for other organism, and its canopy coverage will protect the soil from the rain-splattering blows that could damage the soil aggregates and may causes erosion (Puigdefabregas, 2005). Furthermore, the complex structure in the forested area will support not only the biodiversity, but also the other ecosystem services such as the soil formation and also to increase the C-stock in the area.

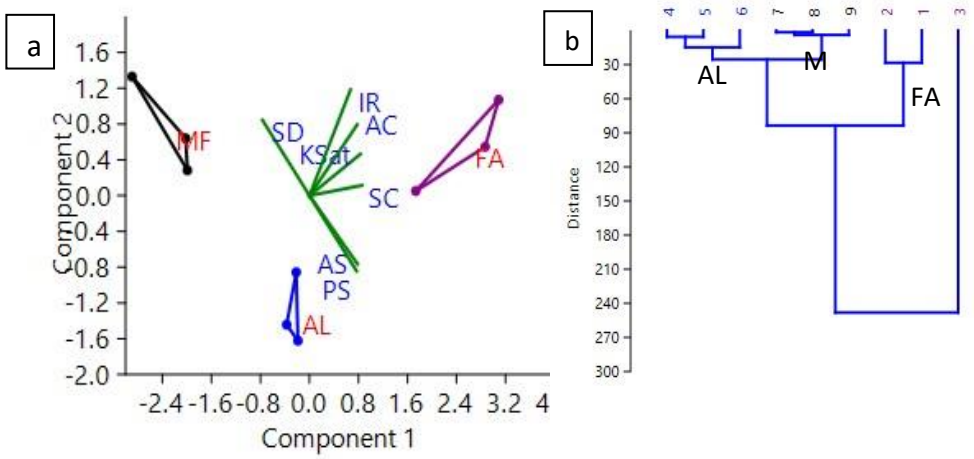


Figure 6. Group of three study area (Forested Area (FA), Agricultural Land (AL) and Mushroom Farm (MF)) based on Principle Component Analysis (PCA) (a) and Clustering (b)

Nevertheless, restoration is a long way process of land rehabilitation, six years process has already gives an improvement of the services provided by ecosystem. Forested area compared to two other land use systems, has a best ecosystem service based on variable measured. However, to maintain the stability of ecosystem services in the forested area, many efforts should be done not only by the local government but also by the people living around the Janitri water spring, especially to maintain the remaining forest and also to prevent the land use change around the Janitri water spring.

CONCLUSION

Forested area has already gives an improvement of the services provided by ecosystem, compared to two other land use systems (agricultural land and mushroom farm). Forested area has highest infiltration rate (40 ± 1,73 cm.hour-^{1}) and C- stock above ground (135,94 ± 110,79 ton.ha^{-1}).

REFERENCES

Benayas, JMR, AC Newton, A Diaz, JM Bullock. 1999. Enhancement of biodiversity and ecosystem services by ecological restoration: A meta-analysis. *Science*. 325: 1121-1124.

Burkhard, B, F Kroll, F Müller, W Windhorst. 2009. Landscapes 'Capacities to Provide Ecosystem Services – a Concept for Land-Cover Based Assessments. *Landscape Online* 15: 1-22.

Hairiah, K dan S Rahayu. 2010. Mitigasi Perubahan Iklim: Agroforestri kopi untuk mempertahankan cadangan karbon lanskap. Makalah dipresentasikan pada Seminar Kopi 2010. 4-5 Oktober 2010. Bali.

Hairiah, K, A Ekadinata, RR Sari, S Rahayu. 2011. Pengukuran Cadangan Karbon : dari Tingkat Lahan ke Bentang Lahan. Petunjuk Praktis. Edisi ke dua. Bogor World Agroforestry Center, ICRAF SEA Regional Office, Universitas Brawijaya. Malang. 66 p.

Hu, H, W Liu and M Cao. 2008. Impact of land use and land cover changes on ecosystem services in Menglun, Xishuangbanna, Southwest China. *Environment Monitoring Assessment* 146:147–156.

Landon, J.R. 1984. Booker Tropical Soil Manual. Antony Rowe Ltd. Chippenham Wiltshire.

Lee, R., 1990. Hidrologi Hutan. Gadjah Mada University Press. Yogyakarta.

Martinez, M L, OP Maqueo, G Vazquez, GC Campos, JG Franco, K Mehltreter, M Equihua, R Landgrave. 2009. Effects of land use change on biodiversity and ecosystem services in tropical montane cloud forests of Mexico. *Forest Ecology and Management* 258:1856–1863.

Millennium Ecosystem Assessment. 2005. Ecosystems and human well-being: synthesis report. Washington DC : Island Press.

Prasetya, B, S Prijono, Y Widjiawati. 2012. Vegetasi pohon hutan memperbaiki kualitas tanah Andisol-Ngabab. *Indonesian Green Technology Journal* Vol.1(1): 1-6.

Puigdefabregas, J. 2005. The role of vegetation patterns in structuring runoff and sediment fluxes in drylands. *Earth Surface Processes Landforms*. 30:133–147.

Ruiz-Jaen, MC and TM Aide. 2005. Vegetation structure, species diversity, and ecosystem processes as measures of restoration success. *Forest Ecology and Management*. 218:159–173.

Rieu, M and G Sposito. 1991. Fractal Fragmentation, Soil Porosity, and Soil Water Properties: I. Theory. *Soil Science Society* 55: 1231-1244.

Soejono. 2008. Panduan Pengenalan Vegetasi untuk Rehabilitasi Lahan di Sebagian Kawasan Tahura R. Soerjo Jawa Timur. UPT Balai Konservasi Tumbuhan Kebun Raya Purwodadi-LIPI. Pasuruan.

Suprayogo, D, Widianto, P Purnomosidi, RH Widodo, F Rusiana, ZZ Aini, N Khasanah, Z Kusuma. 2004. Degradasi sifat fisik tanah sebagai akibat alih guna lahan hutan menjadi sistem kopi monokultur: kajian perubahan makroporositas tanah. *Agrivita* 26 (1): 60-68.

Whitten, T., R.E. Soeriaatmadja, S.A. Afiff. 1997. Ekologi Jawa dan Bali. S.N. Kartikasari (Ed.). Terjemahan: S.N. Kartikasari, T.B. Utami, A. Widyantoro. 1999. Prenhallindo. Jakarta. p: 463-522.

Widianto, H. Noveras, D. Suprayogo, R.H. Widodo, P. Purnomosidhi dan M. van Noordwijk. 2004. Konversi Hutan Menjadi Lahan Pertanian : Apakah fungsi hidrologis hutan dapat digantikan sistem kopi monokultur? *Agrivita* 26 (1): 47-52.

Wu, J and R Hobbs. 2002. Key issues and research priorities in landscape ecology: An idiosyncratic synthesis. *Landscape Ecology* 17: 355-365.

Wunder S., Thorsen B.J. 2014. Ecosystem services and their quantification: What are ecosystem services? In: Thorsen B.J., Mavsar R., Tyrväinen L., Prokofieva I., Stenger A.(eds): The Provision of Forest Ecosystem Services: Quantifying and Valuing Non-marketed Ecosystem Services. Joensuu, European Forest Institute: 17–20.

Yulistyarini, T., Solikin, S. Sofiah, & R. A. Laksono. 2009. Karakterisasi beberapa mata air di Kota Batu dan vegetasi di sekitarnya. *Berkala Penelitian Hayati Edisi Khusus*. 3A :87–93.

Yulistyarini, T. & A. P. Fiqa. 2010. Struktur dan komposisi vegetasi pada lahan hutan tersisa di sekitar Mata Air Janitri, Sumber Brantas, Batu, Jawa Timur. Prosiding Seminar Nasional Basic Science VII. 20 Februari 2010. Universitas Brawijaya. I-429 sd 435.

Yulistyarini, T. 2013. Agroforestry kopi dan pengaruhnya terhadap layanan ekosistem di daerah resapan air Krisik (Ngantang, Kabupaten Malang). Prosiding Seminar Nasional Agroforestri. 21 Mei 2013. Balai Penelitian Teknologi Agroforestri. 242-245.

Secondary Metabolites of *Sarcotheca macrophylla* Blume. (Oxalidaceae): Potential Fruit as Natural Shampoo

Frisca Damayanti and Inggit Puji Astuti
Center for Plant Conservation - Botanical Gardens, Indonesian Institute of Sciences
Jl. Ir. H Juanda No. 13, Bogor 16003, West Java. Tel./fax: +62-251-8322187
Email: friscad@yahoo.com

ABSTRACT

Sarchoteca macrophylla Blume. is an endemic plant to Borneo, a member of Oxalidaceae family. This species is distributed throughout Borneo Island, exce pt for South Kalimantan. It is used by the people of Borneo as natural shampoo. This potential is made possible by the plant's secondary metabolite compounds, but there is no information about the compounds. Herein, the study aims to establish the secondary metabolite compounds of *S.macrophylla* as natural shampoo. Qualitative approach with color visualization method was employed. The results show that *S.macrophylla* contains secondary metabolites, such as flavonoid, tannin and saponin. The secondary metabolite compounds, especially saponins, are potential as natural shampoo because they have excellent foaming agents that can form very stable foams. In conclusion, *S.macrophylla* can be used as a natural shampoo ingredient.

Key words: color visualization, saponins, *Sarcotecha macrophylla*, secondary metabolites, shampoo

INTRODUCTION

Shampoo is one of the cosmetics needed by humans because it cleans the hair and scalp from all of dirt, such as in the form of oil, dust, and dead skin cells (Pertiwi *et al*., 2014; Kadhim and Saad, 2011). Most people feel that more foam will be more effective in cleaning up the dirt, although the foam in the shampoo is generally produced by a synthetic foam booster. Unfortunately, the use of synthetic agents in the body in large doses can cause irritation (Pertiwi *et al*., 2014). Therefore, finding the potential plant as a natural foam booster is very important. Herbal shampoo is believed to be safer to use and has lower side effects than synthetic agents.

Sarcotheca macrophylla is an endemic plant to Borneo. It is distributed in West Kalimantan, Central Kalimantan, and East Kalimantan. There is no record that this species is distributed in South Kalimantan (Astuti *et al*., 2013). Kenyah Dayak tribe people call this plant "Pengo". Other local names for this plant are *belimbing manik*, *kajukim*, *kerumbai merah*, *min*, and *ramajan*. Almost of all body parts of *S.macrophylla* can be used. One of the benefits of this plant is the fruits can be used as natural shampoo. Local people have used the fruit by grounding or squeezing and rubbing it on to their heads (Astuti *et al*., 2013). However, there has been no study of the secondary metabolites contained in these plants to prove its potential.

The study was conducted to determine the secondary metabolite compounds, especially saponins, of *S. macrophylla* to prove its potential as a natural shampoo. Detection of other metabolites such as alkaloids, flavonoids, and tannin is also required. The secondary metabolites have antioxidant, antibacterial, and anti-allergic functions (Aksara *et al*., 2013; Roy, 2017; Panche *et al*., 2016). The functions of the compounds can support the evidence that the fruit of *S. macrophylla* can be used as a material of natural or herbal shampoo.

MATERIAL AND METHODS

Sampling

The study used the fruits of *S.macrophylla*, taken from vak. XXIV.B. 151-151a, Center for Plant Conservation - Botanical Gardens, Indonesian Institute of Sciences. The sample taken consisted of ripe and red fruits. The collected fruits were cleaned from unwanted foreign materials. The fruits that had been picked from the tree were immediately analyzed for the content of secondary metabolites.

Color visualization methods

Qualitative method with color visualization was used. The secondary metabolites which were analyzed included alkaloid, flavonoid, tannin, and saponin.

Alkaloid

Sample was weighted by 1 gram, added with a few drops of NaOH. Then, it was ground by adding 5 ml $CHCl_3$ and filtered. The filtrate was added with H_2SO_4 2M. The acid layer was to be divided into three. Then, the sample was measured using three kinds of reagents, namely Dragendorf, Mayer, and Wagner.

Flavonoid

Sample was weighted by 1 gram, added with enough Aquadest. It was heated for 5 minutes and filtrated. The filtrate was added with Mg powder, HCl:EtOH (1:1), and Amyl Alcohol.

Tannin

Sample was weighted by 1 gram, added with enough Aquadest. It was then heated for 5 minutes and filtrated. The filtrate was added with 3 drops of $FeCl_3$ 10%.

Saponin

Sample was weighted by 1 gram, added with enough Aquadest. Then, it was heated for 5 minutes and filtrated. The filtrate was shaken strongly and let sit for a few seconds to detect the stability of the foam.

RESULTS AND DISCUSSION

To analyze the secondary metabolite compound in the fruit of *S.macrophylla*, we used a qualitative method with color visualization. The secondary metabolites analyzed were alkaloid, flavonoid, tannin, and saponin. The results of alkaloid analysis are shown in the Figure 1.

Figure1. Results of alkaloid analysis

In alkaloid analysis, three types of reagents were used, namely Wagner, Mayer, and Dragendorf. Each reagent has an indicator to determine a positive or negative result. With Wagner reagent, positive results are characterized by the presence of brown sediment. The result was that the tested sample had no sediment. It means the alkaloid was not detected in the Wagner method. With Mayer reagent, positive results are characterized by the appearance of white sediment. The result was there was no sediment in the measured sample. It means the alkaloids were not detected by the Mayer method. In the alkaloid analysis using the dragendorf reagent, positive results are characterized by the appearance of orange sediment. The result was there was no sediment in the tested sample. It means the alkaloids were not detected by the Dragendorf method. Overall, alkaloid was not detected in all alkaloid analyses (Figure 1). It indicates the sample of *S.macrophylla* fruit did not contain alkaloid compounds. Negative results can be caused by small amount of the alkaloid content in the fruits, so it was not detected by any type

of analysis. Therefore, an analysis with greater sensitivity is needed. Moreover, the negative results can be caused by the good environmental condition during the time of sampling. The amount of secondary metabolites will be affected by environmental condition. Greater amount of secondary metabolites will be produced when the plants are in a stress condition. Secondary metabolites are needed in plant's defense against biotic and abiotic stresses (Ramakrishna and Ravishankar, 2011). The results of flavonoids, saponin, and tannin analysis are shown in the Figure 2.

Figure 2. Results of flavonoid, tannin, and saponin analysis

In the flavonoid analysis, positive results are characterized with red-orange layers on the sample solution. The result showed that there was an orange layer in the sample solution (Figure 2). This indicates the sample of *S.macrophylla* contained flavonoid compound. In the tannin analysis, positive results are characterized by the changing color of the solution to a blackish green. The positive results were visible in the sample's color that changed to blackish green (Figure 2). This indicates the sample of *S.macrophylla* contained a tannin compound. In the saponin analysis, a positive result is indicated by stable foam in the solution formed for a few seconds. The result showed that there was stable foam in the sample solution for up to several seconds (Figure 2). This indicates that the sample of *S.macrophylla* contained a saponin compound. Summary of the results are presented in Table 1. As shown by Table 1, *S.macrophylla* contains secondary metabolite compounds such as flavonoids, tannins, and saponins. The compounds can prove the potential of the fruit as herbal shampoo.

Table 1. Result summary

Secondary metabolites		Indicator	Result	
Alkaloid	**Wagner**	Orange sediment	No sediment	**Negative**
	Mayer	White sediment	No sediment	**Negative**
	Dragendorf	Brown sediment	No sediment	**Negative**
Flavonoid		Red-Orange layer	Red-Orange layer	**Positive**
Saponin		Stable foam	Stable foam	**Positive**
Tannin		Blackish green	Blackish green	**Positive**

The requirements for herbal shampoo that can be used safely include having good foaming properties, not making allergies and irritation of the scalp, and able to prevent the growth of fungi that can cause the appearance of dandruff (Surani and Putriana, 2017). Flavonoids and tannins are chemical compounds that have antimicrobial, antioxidant, anti-allergic, and anti-irritant functions (Panche *et al*., 2016; Aksara *et al*., 2013; Yanti, 2014). Furthermore, tannin can damage the formation of fungal conidia that can inhibit reproduction of fungi (Adila *et al*., 2013), wheras saponins are foaming compounds and commonly used as ingredients for shampoo (Aghel *et al*., 2007). Saponins have detergent properties.

Saponins are excellent foaming agents, forming very stable foams. Because of their surfactant or detergent properties, they are commonly used in the industry for cosmetic products, such as shampoo. Moreover, the antifungal and antibacterial properties of saponins are important in cosmetic applications (Aghel *et al*., 2007). Recently, there has been increase in the use of natural products in cosmetics. The awareness for herbal cosmetics is increasing, because people believe that the products are safe and free from side effects (Arora *et al*., 2011). Besides *S.macrophylla*, many plants are reported to have beneficial use in shampoos, such as *Acanthophyllum squarrosum*, *Leucaena leucocephala*, *Berberis vulgaris*, *Lawsonia alba*, *Urtica dioica*, *Rosmarinus officinalis*, *Saponaria officinalis*, *Eucalyptus sp*., *Nigella sativa*, etc. (Aghel *et al*., 2007; Arora *et al*., 2011; Pertiwi *et al*., 2014).

In this study, we analyzed the content of secondary metabolites in *S.macrophylla* as shampoo material. Further research is required to strengthen scientific evidence of the potential of *S.macrophylla* as herbal shampoo, such as analyses of antimicrobial agent, anti-irritant function, viscosity level, and foam stability from *S.macrophylla* fruit extract.

CONCLUSION

S.macrophylla can be used as raw material of natural shampoo. It contains secondary metabolite compounds, especially saponin and other compounds such as flavonoid and tannin. It can also be concluded that the habit of the people of Borneo using *S.macrophylla* as a natural shampoo has been scientifically proven.

ACKNOWLEDGMENT

The authors would like to thank to the Center for Plant Conservation, Botanical Gardens - LIPI which has facilitated the authors in conducting and completing the research.

REFERENCES

Adila, R., Nurmiati, and Anthoni Agustien. 2013. Antimicrobial Test of *Curcuma* spp. On the Growth of *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. *J. Bio UA*. 2(1):1-7

Aghel, Nasrin., E.Moghimipour, and A.R.Dana. 2007. Formulation of a Herbal Shampoo using Total Saponin of *Acanthophyllum squarrosum*. *IJPR* 6(3) :167-172

Aksara, Riska., W.J.A. Musa., and L. Alio. 2013. Identifikasi Senyawa Alkaloid dari Ekstrak Metanol Kulit Batang Mangga (*Mangifera indica* L.). *Jurnal Entropi* 8(1): 514-519

Arora, Pooja., A. Nanda., M. Karan. 2011. Shampoo Based on Synthetic Ingredients Vis-A-Vis Shampoo Based on Herbal Ingredients: A Review. *International Journal of Pharmaceutical Sciences Review and Research* 7(1):41-46

Astuti, I.P., S. Rahayu, and W.U.Putri. 2013. *Sarcotheca macrophylla* Blume: an Endemic Plant Species of Borneo. *INAFOR* A-0135

Kadhim, r.b., and A.H. Saad. 2011. Formulation and Evaluation of Herbal Shampoo from Ziziphus spina Leaves Extract. *IJRAP* 2(6):1802-1806

Malangngi, Liberty P., M.S Sangi, and J.J.E. Paendong. 2012. Penentuan Kandungan Tanin dan Uji Aktifitas Antioksidan Ekstrak Biji Buah Alpukat (*Persea americana* Mill.). *Jurnal MIPA Unstrat Online* 1(1):5-10

Panche, A.N., A.D. Diwan, and S.R. Chandra. 2016. Flavonoids: an overview. J Nutr Sci. 5(47): 1-15

Pertiwi, Mega., H. Soetjipto, and S.Hartini. 2014. Optimalisasi Konsentrasi Ekstrak Saponin Daun Petai Cina (Leucaena leucocephala (Lam.) De Wit.) sebagai Agensia Pembusa Alami Sampo. *Proceeding Seminar Nasional Kimia dan Pendidikan Kimia* VI 253. ISBN:979363174-0: 253-261

Ramakrishna, A., and G.A. Ravishankar. 2011. Influence of Biotic Stress Signals on Secondary Metabolites in Plant. *Plant Signal Behav* 6(11): 1720-1731

Roy, Arpita. 2017. A Review on the Alkaloids an Important Therapeutic Compound from Plants. *IJPB* 3(2):1-9

Surani, F., and N.A. Putriana. 2017. Evaluasi Berbagai Sediaan Shampi Herbal Antiketombe dan Antikutu: Review Artikel. Farmaka 15(2):218-232

Yanti, Mella. 2014. Isolasi dan Identifikasi Senyawa Alkaloid dalam Ekstrak Daun Sirsak Hutan (*Annona glabra*). Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural Institute.

Utilization of Ornamental Plants of the Bogor Botanic Garden's Nursery

[1]Reni Lestari, Kartika Ning Tyas and Sopian
Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences.
Jl. Ir. H. Juanda No. 13, Bogor 16122, West Java, Indonesia. Tel./fax.: +62-251-8322187
*Email:reni_naa@yahoo.com; reni001@lipi.go.id

ABSTRACT

One function of a Botanic Garden other than conservation, research and environmental education is tourism. The garden collections could be displayed base on the taxonomical classification, bioregion, thematic or combination of those patterns. To attract visitors, the gardens used to exhibit many interesting areas or views. The areas could contain plant collections or many other ornamental plants. Some interisting areas of Bogor Botanic Gardens (BBG) are for example Teijsmann Garden, Bhineka/Soedjana Kasan Garden and Mexican Garden. To manage those interesting areas, the garden should provide many ornamental plants that prepared by the nursery of the garden or other sources. The nursery provided also ornamental plants requested by other botanic gardens that owned by local goverment in Indonesia. There were 27 new botanic gardens established in Indonesia or still in the process of establishing.The aims of this paper was to study the management and works of the nursery of BBG in term of the ornamental plants provision. This paper used data sources from the garden and nursery works, i.e. the species of ornamental plants provided by the BBG nursery during 2015-2016, and the data of requested ornamental plants from the nursery of BBG. Data from the observation on species of ornamental plants displayed at some areas of BBG was also collected. Ideally the nursery of BBG should manage the whole ornamental species planted at BBG as well as the ornamental plant species requested by other botanic gardens, other institution and for other purposes. There were some challanges faced by the nursery in managing the works for ornamental plants provision.

Key Words: Bogor Botanic Gardens, nursery, ornamental plants

INTRODUCTION

Botanic garden sare institutions holding documented collections of living plants for the purposes of scientific research, conservation, display and education (Jackson and Sutherland, 2000). According to the Presidential of the Republic of Indonesia Decree No 93 of 2011, five functions of a botanic gardens are conservation, research, environmental education, tourism and ecosystem services. The garden collections could be displayed base on the taxonomical classification, bioregion, thematic or combination of those patterns. The gardens used to exhibit many interesting areas or landscapes to attract the visitors. The areas could comprise of plant collections including many ornamental plants. The wealth of botanical knowledge can be seen in many of its historical and existing traditional gardens including in the US, UK and China (Cannon and Shian Kua, 2017) or even in many other countries of the worlds (Spencer and Cross, 2017; Krishnan and Novy, 2016).

Some examples of interesting and historical areas of Bogor Botanic Gardens (BBG) were Teijsmann Garden, Bhinneka/Soedjana Kasan Garden, Mexican Garden, Astrid Avenue, Aquatic Garden and Medicinal Plant Garden. To manage those interesting areas continuously, the garden should provide many ornamental plants from the nursery of the garden or other sources.

Management of the nursery of BBG was operated under Subdivision of Registration and Nursery of *Ex Situ* Plant Conservation Division. Beside supporting ornamental plants for BBG demand, the nursery provided also ornamental plants requested by other institusions including botanic gardens that owned by local government (Regional Gardens) or university in Indonesia.

There were more than 25 new botanic gardens established or still in the process of establishment in more than 17 provinces in Indonesia at present. The regional garden could be owned by a province or by a district or city government or university. BBG under Indonesian Institute of Sciencehas duty as advisor, mentor or supervisor of the process of establishing new botanic gardens in Indonesia (Presidential Decree No 93 of 2011). Before process of establishing new Botanic Garden, LIPI usually signed an MoU with the Local Government and BBG signed an Agreement with related Department

of the Local Government. LIPI did not work alone in doing that task, but worked together with Ministry of Public Works and Housing, Ministry of Environment and Forestry, and Ministry of Home Affairs of Indonesia.

At the Agreement between BBG and Local Government regarding establishment of new botanic gardens, BBG had important roles in assisting Regional Botanic Gardens in term of management of the gardens, management of plant collections, management of the garden nursery, registration/database of plant collection and training of human resources. Therefore, the nursery of BBG also provided ornamental plants that requested by Regional Botanic Gardens to begin or create new parks or gardens over there.The aims of this paper is to study the management and works of the nursery of BBG in term of the ornamental plants provision.

MATERIAL AND METHODS

This paper used data sources from the garden and nursery works from 2014-2016, i.e. the species of ornamental plants provided by the BBG nursery and its propagation methods, as well as the data of ornamental plants from the nursery that had been utilized by BBG or other Indonesian botanic gardens. Observation on the species of ornamental plants display at some BBG interesting areas was also conducted. Some references were used to accomplish the study, especially to identify the plant names (Gledhill, 2008; Min *et al.*, 2006; Sari *et al.*, 2010).

RESULTS AND DISCUSSION

Ornamental plant species provided by the nursery of the gardens and the propagation methods

Ornamental plants provided by the BBG nursery usually as a result of the coordination with the garden/park unit of the BBG plant collection management.The plant list could be seen at Table 1. The BBG nursery unit and the garden/park management unit initially bought the parent of the ornamental plants from other nurseries and then the BBG nursery propagated them. The plant species chosen are those that have interesting leaf or flower or plant habitus. Most of the BBG ornamental plants were propagated by cutting and splitting as could be seen at Table 1 (Harttman *et al.*, 2000; Tebbit, 2005). This propagation methods could easily be done for those ornamental plants and resulted many new plant numbers. On the other hand, seed was used as propagation material in palm and some woody ornamental plant (Table 1). Rootone F, that contain auxin, was used to speed up propagation process, especially for woody plants cutting. Auxinis a plant growth regulator that plays important roles in root induction and growth (Davies, 2004). The media for propagated plants comprised of mixing of husk, soil and manure (2:1:1).This media has high porosity and holding water capacity that supported plant growth.

Table 1. Stock of ornamental plant at Bogor Botanical Gardens nursery in early 2014, 2015 and 2016

No	Species	Family	Number of plant specimen			Propagation methods
			2014	2015	2016	
1.	*Aglaonema decorum*	Araceae	-	30	20	Cutting
2.	*Aglaonema commutatum*	Araceae	-	25	15	Cutting
3.	*Alternanthera tenella*(red)	Amaranthaceae	120	500	180	Cutting
4.	*Alternanthera tenella*(green)	Amaranthaceae	-	-	100	Cutting
5.	*Ardisia crispa*	Primulaceae	-	-	50	Cutting

No	Species	Family	Number of plant specimen			Propagation methods
			2014	2015	2016	
6.	*Aglaonema decorum*	Araceae	-	30	20	Cutting
7.	*Begonia sp.*	Begoniaceae	-	90	25	Cutting
8.	*Billbergia pyramidalis*	Bromeliaceae	80	50	45	Cutting
9.	*Bromelia* sp.	Bromeliaceae	70	50	105	Cutting
10.	*Calathea bella*	Marantaceae	100	60	105	Split, rhizome
11.	*Calathea loeseneri var metalica*	Marantaceae	-	50	135	Split, rhizome
12.	*Calathea luthea*	Marantaceae	16	18	18	Split, rhizome
13.	*Calathea majestica*	Marantaceae	10	-	-	Split, rhizome
14.	*Calathea micans*	Marantaceae	200	200	105	Split, rhizome
15.	*Calathea musaica*	Marantaceae	40	25	15	Split, rhizome
16.	*Calathea picturata*	Marantaceae	50	50	30	Split, rhizome
17.	*Calathea vaginata*	Marantaceae	50	-	-	Split, rhizome
18.	*Chrysalydocarpus lutescens*	Arecaceae	-	14	14	Seed
19.	*Carex morrowii*	Liliaceae	500	2000	1735	Split, rhizome
20.	*Chlorophytum bichetii*	Anthericaceae	300	250	280	Split, runner
21.	*Chlorophytum* sp. (green)	Anthericaceae	120	100	174	Split, runner
22.	*Chlorophytum* sp. (yellow)	Anthericaceae	-	50	35	Split, runner
23.	*Clerodendrum thomsoniae*	Verbenaceae	-		60	Cutting
24.	*Codiaeum variegatum*	Euphorbiaceae	700	650	270	Cutting
25.	*Cordyline fruticosa*	Asteliaceae	250	200	124	Cutting
26.	*Cordyline fruticosa* (dwarf)	Asteliaceae	50	30	15	Cutting
27.	*Costus curvibracteatus*	Costaceae	120	80	50	Cutting
28.	*Costus erythrophyllus*	Costaceae	190	50	60	Cutting
29.	*Costus woodsonii*	Costaceae	100	120	35	Cutting
30.	*Crinum asiaticum.*	Amaryllidaceae	50	100	35	Bulb
31.	*Ctenanthe oppenheimiana*	Marantaceae	60	5	50	Cutting
32.	*Cuphea hyssopifolia*	Lythraceae	200	150	15	Cutting
33.	*Cyclanthus bipartitus*	Cyclanthaceae	-	10	12	Cutting
34.	*Cynometra cauliflora*	Caesalpineaceae	-	-	5	Seed
35.	*Dianella ensifolia*	Phormiaceae	200	50	50	Split
36.	*Dieffenbachia amoena.*	Araceae	150	80	94	Cutting
37.	*Dracaena compacta*	Dracaenaceae	20	20	10	Cutting
38.	*Dracaena godseffiana*	Dracaenaceae	-	-	25	Cutting
39.	*Dracaena reflexa*(Srilangka)	Dracaenaceae	90	30	34	Cutting
40.	*Dracaena reflexa*(India)	Dracaenaceae	80	9	90	Cutting
41.	*Dracaena reflexa*(Jamaica)	Dracaenaceae	-	230	20	Cutting
42.	*Dracaena sanderiana* (White)	Dracaenaceae	100	250	135	Cutting
43.	*Dracaena sanderiana*(Green)	Dracaenaceae	100	30	50	Cutting
44.	*Dracaena sp. Kuning*	Dracaenaceae	-	20		Cutting
45.	*Echinodorus grandiflorus*	Alismataceae	10	10	10	Split
46.	*Evodia suaveolens*	Rutaceae	12	19	51	Seed, Cutting
47.	*Epipremnum giganteum*	Araceae	-	-	50	Cutting
48.	*Eucharis grandiflora*	Amaryllidaceae	70	30	5	Bulb
49.	*Eugenia macranta*	Myrtaceae	-	10		Cutting

No	Species	Family	Number of plant specimen			Propagation methods
			2014	2015	2016	
50.	*Aglaonema decorum*	Araceae	-	30	20	Cutting
51.	*Excoecaria cochinchinensis .*	Euphorbiaceae	50	-	99	Cutting
52.	*Excoecaria cochinchinensis variegata*	Euphorbiaceae	-	150		Cutting
53.	*Ficus deltoidea.*	Moraceae	-	-	6	Cutting
54.	*Heliconia caribaea*	Heliconiaceae	50	-	-	Rhizome
55.	*Heliconia chartaceae var. Sexy Pink*	Heliconiaceae	50	-	-	Rhizome
56.	*Heliconia latispatha*	Heliconiaceae	50	-	-	Rhizome
57.	*Heliconia indica*	Heliconiaceae	-	-	50	Rhizome
58.	*Hemigraphis alternata*	Acanthaceae	-	-	170	Cutting
59.	*Hibiscus rosasinensis*	Malvaceae	50	50	50	Cutting
60.	*Hippeastrum reticulatum*	Amaryllidaceae	150	100	105	Bulb
61.	*Hymenocallis littoralis*	Amaryllidaceae	50	50	15	Bulb
62.	*Hymenocallis speciosa*	Amaryllidaceae	70	100	20	Bulb
63.	*Ixora coccinea*	Rubiaceae	700	150	70	Cutting
64.	*Ixora coccinea var. coccinea*	Rubiaceae	45	30	27	Cutting
65.	*Ixora grandiflora*	Rubiaceae	-	-	5	Cutting
66.	*Kopsia fruticosa*	Apocynaceae	-	60	31	Cutting
67.	*Kopsia pauciflora*(green)	Apocynaceae	-	-	8	Cutting
68.	*Malphigia coccigera*	Euphorbiaceae	80	45	25	Cutting
69.	*Neomarica gracilis*	Iridaceae	100	100	65	Bulb, Split
70.	*Neomarica longifolia*	Iridaceae	100	50	75	Bulb, Split
71.	*Ophiopogon jaburan*	Asparagaceae	400	80	10	Split
72.	*Ophiopogon jaburan variegata*	Asparagaceae	-	70	45	Split
73.	*Ophiopogon japonicus*	Asparagaceae	300	300	150	Split
74.	*Orthoshipon aristatus.*	Lamiaceae	50	400	260	Cutting
75.	*Osmoxylon lineare*	Araliaceae	10	95	120	Cutting
76.	*Pachystachys lutea*	Acanthaceae	20	5	33	Cutting
77.	*Palisota bracteosa*	Commelinaceae	-	10	6	Cutting
78.	*Pandanus pygmaeus*	Pandanaceae	70	-	-	Cutting, Split
79.	*Pedilanthus tithymaloides*	Euphorbiaceae	200	294	179	Cutting
80.	*Philodendron selloum*	Araceae	7	6	156	Cutting
81.	*Phyllanthus myrtyfolius*	Phyllantaceae	-	-	50	Cutting
82.	*Phormium tenax var.Red*	Hemerocallidaceae	6	-	-	Cutting
83.	*Piper sarmentosum*	Piperaceae	-	-	30	Cutting
84.	*Polyscias balfouriana*	Araliaceae	30	20	10	Cutting
85.	*Ravenia spectabilis*	Rutaceae	15	10	3	Cutting
86.	*Rhapidopora aurea*	Araceae	400	200	195	Cutting
87.	*Rhapis exelsa*	Arecaceae	12	20	20	Seed
88.	*Rhoeo discolor*	Commelinaceae	300	300	175	Split, Rhizome
89.	*Rhopaloblaste ceramica*	Arecaceae	-	-	10	Seed
90.	*Sansevieria trifasciata*	Asparagaceae	-	15	20	Cutting
91.	*Schefflera arboricola*	Araliaceae	10	5	7	Cutting

No	Species	Family	Number of plant specimen			Propagation methods
			2014	2015	2016	
92.	*Aglaonema decorum*	Araceae	-	30	20	Cutting
93.	*Spathiphyllum cannifolium*	Araceae	350	85	300	Split, cutting
94.	*Spathiphyllum wallisii*	Araceae	150	150	180	Split, cutting
95.	*Spathiphyllum* sp. (dwarf)	Araceae	30	25	20	Split, cutting
96.	*Spathiphyllum* sp.variegata	Araceae	-	-	30	Split, cutting
97.	*Spathoglottis plicata*	Orchidaceae	20	568	267	Split, cutting
98.	*Stachyphrynium jagorianum*	Marantaceae	150	150	60	Split, Rhizome
99.	*Syzygium oleana*	Myrtaceae	-	-	10	Seed, Cutting
100.	*Tabernaemontana corymbosa* (variegata)	Apocynaceae	-	-	35	Seed, Cutting
101.	*Tabernaemontana* sp.	Apocynaceae	16	-	-	Seed, Cutting
102.	*Zamioculcas zamiifolia*	Araceae	-	-	20	Cutting
103.	*Zephyranthes candida*	Liliaceae	-	-	15	Tubers
104.	*Zephyranthes rosea*	Liliaceae	20	-	10	Tubers
Total specimen			**8319**	**9438**	**7788**	

The amount of numbers and species of ornamental plants provided by the nursery of Bogor Botanic Gardens increased from 69 species (29 family) in 2014 to 73 spesies (29 family) in 2015 and 96 species (36 family) in 2016 (Table 1). Those ornamental plants (101 species) were propagated by cutting (67 species), split (26 species), rhizome (15 species), bulb (7 species), runner (3 species), seed (8 species) and tubers (2 species) (Table 1). The most ornamental plant species propagated and provided by the nursery from 2014 until 2016 were *Carex morrowii* (4235 plants), *Codiaeum variegatum* (1620 plants), *Spathoglottis plicata* (920 plants), *Ixora coccinea* (855 plants), *Chlorophytum bichetii* (830 plants) and *Rapidopora* sp. (795 plants) (Table 1). Those plants were usually used as filler plants, border plants or ground cover. They are easy to be propagated and interesting ornamental plants due to its colour or shape of the vegetative plant parts.

The ornamental plants utilized during 2014-2016

Beside to fulfill requested of ornamental plants from BBG itself, the BBG nursery was also supplied demanded ornamental plants from other institutions, such as other botanic gardens in Indonesia or other government institutions in Bogor. The other important uses of ornamental plants propagated by the nursery were as the sources of material for environmental education as well as material for rooms decorating for some activities such as seminar or other important meetings.

According to the Presidential Decree number 93/2011, BBG become a mentor or supervisor of botanic gardens owned by local government (Regional Gardens) or by Universities in the establishment process. Therefore BBG used to develop a Collaboration Agreement with Local Government or Universities in Indonesia regarding the establishment of new botanic garden. The ornamental plants being sent to other Indonesian botanic gardens become the implementaton of the agreement. Those ornamental plants usually were utilized to add the ornamental plant collections in their nursery or to make or revitalize their parks.

Table 2. Number of ornamental plant specimen utilized during 2014-2016.

No.	Name of Botanic Gardens	Number of plant specimen			Total plant specimen
		2014	2015	2016	
1.	Bogor	3547	3956	9554	17057
2.	Baturraden	-	2622	2468	5090
3.	Batam	581	1130	884	2595
4.	Liwa	595	1245	65	1905
5.	Enrekang	931	190	699	1820
6.	Banua	-	376	1384	1760
7.	Pare-pare	-	1562	121	1683
8.	Katingan	-	950	400	1350
9.	Kuningan	641	392	-	1033
10.	Lombok	-	423	-	423
11.	Balikpapan	420	-	-	420
12.	Kendari	-	-	250	250
13.	Sambas	-	105	100	205
14.	Samosir	-	-	65	65
Total plant specimens utilized		**6715**	**12951**	**15990**	**18599**

The highest number of ornamental plants from the BBG nursery were those that requested by BBG (17.057 specimens), following by Baturraden Botanic Garden (5.090 specimens), Batam Botanic Garden (2.595 specimens), and Liwa Botanic Garden (1.905 specimens) (Table 2). On the other hand, the least number of requested ornamental plants from Bogor was those demanded by Samosir Botanic Garden, i.e. 65 specimens. The most number of requested plants from Baturraden Botanic Garden might due to the location that could be accessed by road trip from BBG. Those plants requested were usually because of their interesting appearence, adaptable and easy to grow at the gardens. The most requested plant species from Regional Botanic Gardens were *Carex morrowi*, *Codiaeum variegatum* and *Alternanthera tenella* (complete data not presented).

The ornamental plant species display at some areas of Bogor Botanic Gardens

Bogor Botanic Gardens have some interesting gardens, i.e Teijsmann Garden, Bhinneka/Soejana Kasan Garden, and Mexican Garden (Figure 1). BBG kept maintain the historical theme of these gardens until now. This commitment to maintain the gardens as heritage are also be done by other gardens for example in Australia (BG of Adelaide, 2003; Board of the BG and State Herbarium of South Australia, 2013) and New Zealand (Wellington Botanic Garden, 2014).

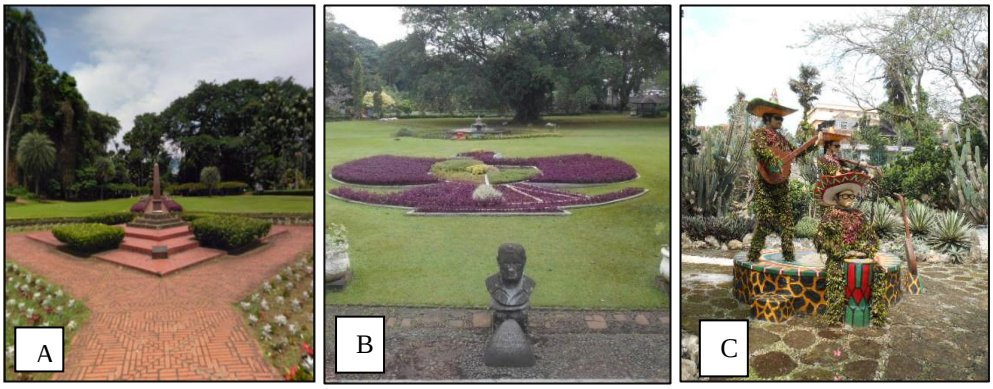


Figure 1. Some gardens in Bogor Botanic Gardens, (A) Teijsmann garden, (B) Bhinneka/Soejana Kasan Garden, and (C) Mexican Garden.

Teijsmann Garden of BBG was made to commemorate Johannes Elias Teijsmann, a garden expert of the Palace of General Governor Johannes van den Bosch. He arranged BBG plant collections in 1831 by grouping them according to the plant family and collected Indonesian plants and other countries of the world (Rijnberg, 1992).Teijsmann Garden is a formal garden that has a simetric landscape (Figure 1A). As many as 28 of ornamental plant species composed the garden (Table 3).

Bhinneka garden of BBG is also called as Sudjana Kasan Garden. There was a statue of Mr. Sudjana Kasan who became the director of BBG in 1959-1969. The concept of this garden is an English garden, because this garden has a pond as a focal point, a lawn as an open area and trees as a fence (Figure 1B).The ornamental plants in the lawn of this garden composed an Indonesian national emblem "Garuda Pancasila" that in real bring the Indonesian motto "Bhinneka Tunggal Ika" (Unity in Diversity).

Mexico Park is an area for succulent and Euphorbiaceae collections (Rijnberg, 1992). The concept of the park is adapted to the plant ecosystem of its collection, namely dry areas (Figure 1C). Most of the plant that composed this garden were BBG collection plants. Some plant families of this garden were Agavaceae, Asphodeloideae, Bromeliaceae, Cactaceae, Crassulaceae,and Euphorbiaceae (Table 5).

Table 3. The ornamental plant species cultivated at "Teijsmann" Garden of Bogor Botanic Gardens

No	Plant Species	Family
1.	*Acalypha macrophylla*	Euphorbiaceae
2.	*Adenium sp.*	Apocynaceae
3.	*Aerva sanguinolenta*	Acanthaceae
4.	*Aglaia* sp.	Meliaceae
5.	*Alternanthera tenella*	Amaranthaceae
6.	*Antigonon leptopus*	Polygonaceae
7.	*Begonia sp.*	Begoniaceae
8.	*Bromelia sp.*	Bromeliaceae
9.	*Calathea loeserneri var. metalica*	Marantaceae
10.	*Calathea sp.*	Marantaceae
11.	*Carex morrowii*	Liliaceae
12.	*Centaurea cineraria*	Asteraceae
13.	*Cordyline fruticosa*	Asteliaceae
14.	*Costus curvibracteatus*	Costaceae
15.	*Dieffenbachia amoena*	Araceae
16.	*Evodia suaveolens*	Rutaceae
17.	*Exoecaria cochinchinensis*	Euphorbiaceae
18.	*Gerbera jamesonii*	Asteraceae
19.	*Heliconia sp.*	Heliconiaceae
20.	*Ixora coccinea*	Rubiaceae
21.	*Mucuna novo-guineensis.*	Papilionaceae
22.	*Ophiopogon jaburan*	Asparagaceae
23.	*Pachystachys lutea*	Acanthaceae
24.	*Pseuderanthemum reticulatum*	Acanthaceae
25.	*Psychotria* sp.	Rubiaceae
26.	*Rhoeo discolor*	Commelinaceae
27.	*Spathiphyllum cannifolium*	Araceae
28.	*Spathiphyllum sp.*	Araceae

Table 4. The ornamental plant species cultivated at "Bhinneka" Garden of Bogor Botanic Gardens

No	Plant Species	Family
1	*Spathiphyllum cannifolium*	Araceae
2	*Acalypa macrophylla*	Acanthaceae
3	*Adenium* sp.	Apocynaceae
4	*Aerva sanguinolenta*	Acanthaceae
5	*Bauhinia* sp.	Caesalpineaceae
6	*Bougainvillea* cultivar	Nyctaginaceae
7	*Casuarina* sp.	Casuarinaceae
8	*Codiaeum variegatum*	Euphorbiaceae
9	*Cordyline fruticosa*	Asteliaceae
10	*Cordyline fruticosa*(dwarf)	Asteliaceae
11	*Costus curvibracteatus*	Costaceae
12	*Cuphea hyssopyfolia*	Lytheraceae
13	*Dianella ensifolia*	Phormiaceae
14	*Dieffenbachia amoena*	Araceae
15	*Dieffenbachia camilla*	Araceae
16	*Excoecaria cochinchinensis*	Euphorbiaceae
17	*Gardenia jasminoides*	Rubiaceae
18	*Hemigraphis alternata*	Acanthaceae
19	*Hibiscus rosa-sinensis*	Malvaceae
20	*Hymenocallis speciosa*	Amaryllidaceae
21	*Iresine herbstii*	Acanthaceae
22	*Ophiopogon jaburan*	Asparagaceae
23	*Ophiopogon japonicus*	Asparagaceae
24	*Osmoxylon lineare*	Araliaceae
25	*Philodendron selloum*	Araceae
26	*Psychotria* sp.	Rubiaceae
27	*Ravenia spectabilis*	Rutaceae
28	*Ruellia barbadine*	Acanthaceae
29	*Tabernaemontana corymbosa*	Apocynaceae

The prominent ornamental plants grew at some gardens of BBG were those of species *Aerva sanguinolenta, Ixora coccinea, Rhoeo discolor, Alternanthera tenella, Spathyphyllum cannaefolium* (Teijsmann garden (Figure 1.A. and Table 3)), species of *A. sanguinolenta, Opiopogon jaburan, O. japonicus, Cuphea hyssopifolia, R. discolor* (Bhinneka/Soejana Kasan Garden (Figure 1.B. and Table 4)) and species of *Plumeriarubra, P. sericifolia, Jatropha multifida, Bromelia and Opuntia* (Mexican garden (Figure 1.C. and Table 5)). Most of the plants were provided by the nursery of the garden. The other plants were prepared by the BBG parks unit that bought the plants from other nursery. Those plants were selected according to the theme of the parks.

If compared the list of plants at Table 3, 4 and 5 with the list of plants at Table 1, as many as 10 out of 28 plants at Teijsmann Garden, 15 out of 29 plants at Bhinneka Garden and 57 out of 68 plants at Mexican Garden were not available at the nursery. Most ornamental plants at Mexican Garden included as garden's collection, on the other hand all ornamental plants at Teijsmann Garden and Bhinneka Garden (Table 3 and 4) were not garden's collection and only for garden decoration. Ideally all ornamental plants at the garden should be managed and prepared by the garden's nursery, especially those that has function for garden decoration. Example of those ornamental plants that not yet provided

by the nursery of BBG were *A. leptopus*, *Centaurea cineraria*, *Gerbera jamesonii*, *Pseuderanthemum reticulatum*, *A. sanguinolenta*, *Codiaeum variegatum*, *Hemigraphis alternata* and *Iresine herbstii*.

Table 5. The ornamental plant species cultivated at "Mexican" Garden of Bogor Botanic Gardens

No	Plant Species	Family
1.	*Agave alibertii*	Agavaceae
2.	*Agave amaniensis*	Agavaceae
3.	*Agave americana*	Agavaceae
4.	*Agave atrovirens*	Agavaceae
5.	*Agave cernua*	Agavaceae
6.	*Agave giesbrechtii*	Agavaceae
7.	*Agave latifolia*	Agavaceae
8.	*Agave polyacantha*	Agavaceae
9.	*Agave potatorum*	Agavaceae
10.	*Agave rigida*	Agavaceae
11.	*Agave rubescens*	Agavaceae
12.	*Agave sisalana*	Agavaceae
13.	*Agave sp.*	Agavaceae
14.	*Agave vivipara*	Agavaceae
15.	*Aloe bainesii*	Asphodeloideae
16.	*Aloe excelsa*	Asphodeloideae
17.	*Bromelia sp.*	Bromeliaceae
18.	*Brunfelsia americana*	Solanaceae
19.	*Calathea picturata*	Marantaceae
20.	*Carex morrowii*	Liliaceae
21.	*Cereus hexagonus*	Cactaceae
22.	*Cereus jamacaru*	Cactaceae
23.	*Cereus marginatus*	Cactaceae
24.	*Cereus peruvianus*	Cactaceae
25.	*Cereus xanthocarpus*	Cactaceae
26.	*Codiaeum variegatum*	Euphorbiaceae
27.	*Cordyline sp.*	Asteliaceae
28.	*Euphorbia antiquorum*	Euphorbiaceae
29.	*Euphorbia barnhartii*	Euphorbiaceae
30.	*Euphorbia cattimandoo*	Euphorbiaceae
31.	*Euphorbia cooperi*	Euphorbiaceae
32.	*Euphorbia gardeniaefolia*	Euphorbiaceae
33.	*Euphorbia lactea*	Euphorbiaceae
34.	*Euphorbia neriifolia*	Euphorbiaceae
35.	*Euphorbia nivulia*	Euphorbiaceae
36.	*Furcraea foetida*	Agavaceae
37.	*Furcraea pubescens*	Agavaceae
38.	*Furcraea selloa*	Agavaceae
39.	*Furcraea sp.*	Agavaceae
40.	*Furcraea tuberosa*	Agavaceae
41.	*Heliconia sp.*	Heliconiaceae

No	Plant Species	Family
42.	*Hibiscus sp.*	Malvaceae
43.	*Jatropha multifida*	Euphorbiaceae
44.	*Jatropha podagrica*	Euphorbiaceae
45.	*Kalanchoe integra*	Crassulaceae
46.	*Kalanchoe mortagei*	Crassulaceae
47.	*Ophiopogon japonicus*	Asparagaceae
48.	*Opuntia brasiliensis*	Cactaceae
49.	*Opuntia candelabriformis*	Cactaceae
50.	*Opuntia robusta*	Cactaceae
51.	*Opuntia schumannii*	Cactaceae
52.	*Opuntia vulgaris*	Cactaceae
53.	*Opuntia zacuapanensis*	Cactaceae
54.	*Pereskia aculeata*	Cactaceae
55.	*Pereskia grandifolia*	Cactaceae
56.	*Pereskia sacharrosa*	Cactaceae
57.	*Plumeria rubra*	Agavaceae
58.	*Plumeria sericifolia*	Agavaceae
59.	*Plumeria sp.*	Agavaceae
60.	*Rhoeo discolor*	Commelinaceae
61.	*Sansevieria trifasciata*	Agavaceae
62.	*Yucca aloifolia*	Agavaceae
63.	*Yucca elephantipes*	Agavaceae
64.	*Yucca gloriosa*	Agavaceae
65.	*Yucca guatemalensis*	Agavaceae
66.	*Yucca sp.*	Agavaceae
67.	*Yucca treculeana*	Agavaceae
68.	*Yucca x gigantea*	Agavaceae

Challenges of the garden's nursery

In the future, as has been mentioned at previous part of the paper, ideally the nursery of BBG should manage the whole ornamental species planted at the garden as well as the ornamental plant species requested by other botanic gardens, other institution and for other purposes. There were some challenges in managing the nursery of BBG in the future, i.e. space forplant stock and work place, human resource numbers and capacity, coordination with other units of the gardens, get more parent plants and other specific plants media, nursery facilities and also database management. At this time, the nursery are in progressing of preparing and designing more space of workplace and plant stock placement as well as fulfill nursery facilities needed.

CONCLUSION

The nursery of Bogor Botanic Gardens played important roles to propagate and provide ornamental plants for the gardens, other institutions and also for environmental education material at the gardens. Ideally, the nursery of BBG should manage the whole ornamental species planted at BBG as well as the ornamental plant species requested by other botanic gardens, other institutions and for other purposes. There were some challenges faced by the nursery in term of managing the works for ornamental plants provision. Some other important ornamental plant species were also need to be

complemented to the list of the nursery, such as *Antigonon leptopus, Centaurea cineraria, Gerbera jamesonii, Pseuderanthemum reticulatum, Aerva sanguinolenta, Codiaeum variegatum, Hemigraphis alternata* and *Iresine herbstii*

ACKNOWLEDGMENT

The authors would like to thank to all of the Nursery staff of Center for Plant Conservation Botanic Gardens, Bogor for supporting the data for this paper, and Ms. Dina Safarinanugraha, SP., for the discussion regarding typical gardens at BBG.

REFERENCES

Board of the Botanic Gardens and State Herbarium. 2013. *Collection Policies*. Board of the BGA and SH, South Australia, Australia.

Botanic Gardens of Adelaide. 2003. *Wittunga Botanic Gardens Plant Collections Policy*. Department for Environment and Heritage. Government of South Australia.

Cannon, C.H. and C.S. Kua. 2017. Botanic gardens should lead the way to create a "Garden Earth" in the Anthropocene. *Plant Diversity* 39: 331-337.

Davies, P.J. 2004. The plant hormones: Their nature, occurrence and function. In Davies, P.J (Ed): Plant hormones: Biosynthesis, Signal Transduction, Action! 3rd edition. Kluwer Academic Publisher. London. P 1-15.

Gledhill, D. 2008. *The Name of the Plant*. Cambridge University Press. Cambridge.

Government of the Republic of Indonesia. 2011. *Presidential Decree concerning Botanic Gardens*. Presidential Decree No. 93 of 2011.

Harttman H.T., D.E.Kester and F.T. Davies. 2000. *Plant Propagation, Principles and Practice.* Prentice-Hall International Inc.New Jersey.

Krishnan, S. and A. Novy. 2016. The role of botanic gardens in the twenty-first century. Centre for Agriculture and Bioscience Reviews 11(23): 1-10.

Min, B.C, K. Omar-Hor and O.Y.C. Lin. 2006. 1001 *Garden Plants in Singapore*. National Park Board. Singapore.

Rijnberg, T.F. 1992. *Lands plantentuin, Buitenzorg* 1817-18 mei-1992 Kebun Raya Indonesia, Bogor. CV Wira Pratama Jakarta, Indonesia.

Sari, R., Ruspandi and S.R. Ariati (Eds). 2010. *An Alphabetical List of Plant Species Cultivated in Bogor Botanic Gardens*. Indonesian Institute of Sciences, Center for Plant Conservation Bogor Botanic Gardens. Indonesia.

Spencer, R. and R. Cross. 2017. The origins of botanic gardens and their relation to plant science, with special reference to horticultural botany and cultivated plant taxonomy. *Muelleria* 35: 43-93

Tebbit, M.C. 2005. *Begonias: cultivation, identification, and natural history*. Timber Press. Brooklyn.

Wellington Botanic Garden. 2014.*Botanic Gardens of Wellington Management Plan*. Wellington City Council. New Zealand

Jackson, R.S.W and L.A. Sutherland. 2000. *International Agenda for Botanic Gardens in Conservation*. Botanic Gardens Conservation International, UK.

The Assessment of Tree Health Status in Strategic Location of Bogor Palace

Rizmoon Nurul Zulkarnaen*, Hendra Helmanto, Fitri Fatma Wardani, Mujahidin, AnggaYudaputra
Center For Plant Conservation Bogor Botanic Garden, Indonesian Institute of Sciences
*email : rizmoon.zulkar@gmail.com

ABSTRACT

Nowadays, Bogor Palace was used as a residence of the President of Indonesian accordance of that concern, tree health assessment becomes a critical aspect in order to know the status of old trees in Bogor Palace. The information regarding of tree health status is quite necessary to find out the appropriate ways in monitoring purposes. The objective of this study is that to assess the tree health status in the strategic location of Bogor Palace. The purposive sampling method was applied to determine the trees health status on the strategic location where are often passed by the president and state guests. Visual and Sonic Tomograph methods are used to inspect the health status of selected trees. The total number of selected trees were 16 trees. Based on the observation method, the result showed that 62.5 % of trees were categorized as healthy trees, 18.75 % of trees were suffered from minor damages and 18.75 % of trees were major damages. Identification of decay percentage by PiCUS® 3.0 Sonic tomograph showed that decay on the tree *Albizia saman* 1.5%, *Canarium vulgare* 42.29% and 50.01%, *Agathis borneensis* 12.03% and 25.07%. The benefit of this study can provide recommendations for tree management to the managers of the Bogor Palace. In consequence, the result of this study can be implemented to reduce risk in the management of tree health status in Bogor Palace

Keywords: Bogor Palace, Sonic Tomograph, Tree Health, Visual Method.

INTRODUCTION

Trees are an important aspect of human life. The existence of the trees has an economic value, social, and aesthetic beauty. However, the older the tree which will be increasingly vulnerable to pests and diseases that can cause rotting and dead trees. Economically, the tree having moldiness also will reduce economic value (Liang *et al*., 2008).

Decay that occurs in the tree usually starts from the middle (Heart Decay), whereas decomposition starts from the outside/bark. If the decay occurs from the middle (Heart Decay) will reduce more than doubled the volume of timber (Tainter and Baker, 1996). According to Leininger *et al*. (2001), Logging timber in the United States each year are estimated every 236,000 m3 of harvested wood there are approximately 71,000 m3 of decay wood. Therefore, inspection and monitoring decay trees is useful for early detection of decay that occurs in the tree because it is not only economically beneficial but also safety aspect, Specifically for public places, streets, and tourist attractions.

Bogor Palace is one of the places with some of the old trees. many of those allegations have been experiencing rot and disease or pest infestations. Inspection and monitoring of tree health status in the Bogor palace need to be conducted as the basis of information in tree risk management. Inspection and monitoring trees health status in accordance with to silviculture understanding consist of several steps: controlling facilitating, protecting and saving (Nyland, 2002).

Health inspection and monitoring activities of trees done by two approaches: visually and tomography techniques. According to Wang (2009) in recent years, tomograph was successfully developed to detect damage to the tree (wood decay) showing success in detecting the decay of trees, especially for urban trees.

This study was aimed to identify trees health status in a strategic location of Bogor Palace by visual and sonic tomograph. The location was chosen because it was a location that became a historic place and became the residence of President. In addition, the location is also often a place for state meetings.

MATERIALS AND METHODS

a. Study site

This research was conducted in Bogor Palace on December 2015 (Figure 1).

Figure 1. Location of Bogor Palace

b. Tools and materials

PICUS Sonic tomograph was equipped with a sensor module, electronic hammer and a rubber mallet (Figure 2). The laptop was used to run Picus application, camera, binoculars, and a rolling meter. Trees in the backyard of Bogor were selected.

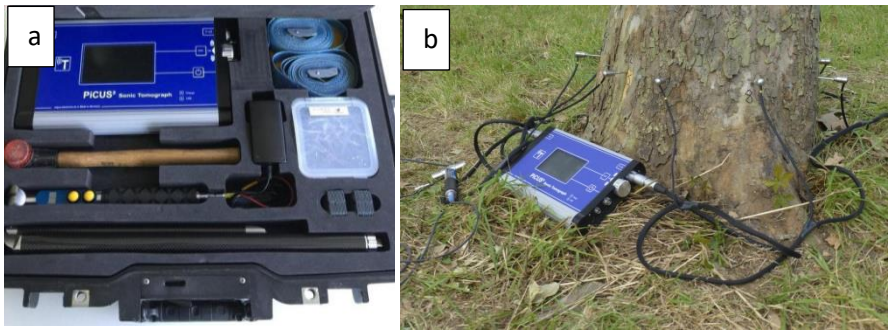


Figure 2. a. PICUS tools b. Use of PICUS on tree

c. Implementation

Trees selection Purposive sampling design was applied to select trees through visual observation that consists of tree damage appearance, the causes of damage, and the possible risk that would emerge if the trees collapse. To determine the health status of the main trunk, a rubber hammer was used with the simple way by hitting it on the trunk. A healthy and solid rod will reflect the sound like a wall when tapped. Whereas stem decay/loss/damage will reflect a loud sound like bamboo when tapped.

d. Classification tree health status

The classification of tree health status was figured out in Table 1 which described completely by Forest Health Monitory (FHM) (Mangold, 1997). Tree health value will be obtained by processing the data using formulas In the following:

$$\mathrm{NIK} = \sum_{i=1}^{16} (\mathrm{xi.\,yi.\,zi})$$

NIK : Value Index Tree Damage
Xi : Value weights on the type of damage
Yi : The value of the weight on the trees damaged
Zi : Value weights on the severity of the damage

Table 1 Classification of health of the tree by tree damage index

Classification tree health	index value of damage to trees
Healthy	0-5
Minor damage	6-10
Middle damage	11-15
Major damage	≥16

e. Detection of decay tree with PICUS® 3.0 Sonictomograph

Decay detection was performed by a PICUS® 3.0 Sonic tomograph. Installation of sensor devices was performed at different heights according to the height of the trunk that indicated damage. The number of sensors was used toadjust to the diameter of the tree (at least 6 sensors). Data were obtained from the sound wave velocity with a tap of an electronic hammer on the nail. The tapped nails will generate the sound wave which will propagate into the trunk of the tree and wave propagation time from point of origin to the point of receiver / other sensors (Argus, 2015). Through this software, the data speed of propagation of the sound wave is transformed into a tomogram image (2D image). It can calculate the density of trees and tree geometry by combining the sound wave velocity that has been recorded during the measurement. The percentage of tree decay analysis was performed by ImageJ software. This device can accurately describe the percentage based on the color degradation of results from PICUS® 3.0 Sonic tomograph. Procedures to collect data using PICUS® 3.0Sonictomograph can see in Figure 3.

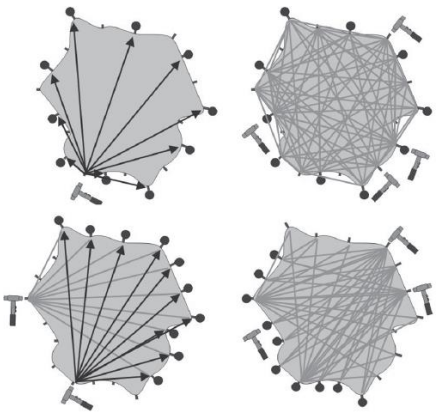


Figure 3. Data collection method using PICUS 3.0 Sonic tomograph

The results of measurement using a PICUS® 3.0Sonic tomograph will display a color that indicates the degradation level of trunk density. the color degradation was composed of several colors as below (Figure 4). Each of these colors depicts the density of the stem to stem rot (Table 2).

Figure 4. Degradation solid -decay color

Table 2.Color Description of measurement with PiCUS® 3.0Sonic tomograph

No	Colour	Description
1.	Black	Solid 100%
2.	Brown	Solid
3.	Green	there are indications of decay
4.	Purple	Starting decay
5.	Blue	Decay

RESULTS AND DISCUSSION

The visual assessment of sample trees

Based on visual inspection and monitoring the health status of the tree, were obtained which there are 16 selected trees (Table 1). In the case of visual observation showed that some of the selected trees may experience suffered from damage and surpassed the canopy burden. Insect outbreak, disease, and other abiotic factors can trigger tree damaging due to physiological process disorder (Mariyanti, 2011). Those factors may lead to the lowering of survival rate and health status of trees.

Trees which were located along the road in Bogor Palace consisted of 44% of the trees in the healthy category, 22% in the category of minor damage and 33% category of major damage.

Table 3. The result of visual assesment

No	Tree Spesies	Tree status	Description
1	*Albizia saman* (Jacq.) Merr.	Healthy	Good Vigor, sturdy stems
2	[1]*Canarium vulgare* Leenh.	Minor damage	Crown not balanced, 5% of dry branches, the main trunk allegedly decay, holes in the roots
3	[2]*Canarium vulgare* Leenh.	Healthy	Good vigor
4	[3]*Canarium vulgare* Leenh	Healthy	Good vigor
5	[3]*Canarium vulgare* Leenh.	Healthy	Good vigor
6	[4]*Canarium vulgare* Leenh.	Mayor damage	Low vigor, cancer stem and the base of the roots
7	*Albizia saman* (Jacq.) Merr	Healthy	Good vigor
8	*Ficus benjamina* L.	Healthy	Good vigor
9	*Pinus merkusii*Jungh. & de Vriese.	Minor damage	Sloping tree, on a dry branch
10	*Cocos nucivera*L.	Healthy	Good vigor
11	[5]*Canarium vulgare* Leenh.	Minor damage	Broken branches
12	[1]*Agathis borneensis* Warb.	Healthy	Good vigor
13	[2]*Agathis borneensis* Warb.	Healthy	Good vigor, discovered termites *Macrotermes* sp
14	*Pterocarpus indicus*	Mayor damage	Stem decay, there is Ganoderma, 15% of dry branches
15	*Swietenia macrophylla* King.	Mayor damage	The main stem is decay, 10%of dry branches
16	*Ficus elastica*	Healthy	Good vigor

Status Decay Trees

According to the measurement of sample trees by PICUS 3.0 Sonic Tomograph indicated there are five trees have had decayed (Figure 5).

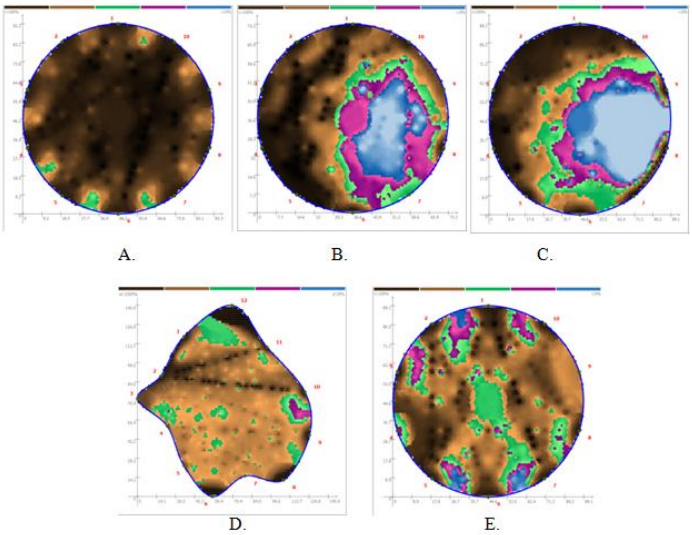


Figure 5. A. *Albizia saman* (Jacq.) Merr .; B. *Canarium vulgare* Leenh.; C. *Canarium vulgare* Leenh.; D. *Agathis borneensis* Warb.; E. *Agathis borneensis* Warb

In detecting the decay of trees using non-destructive methods can be done with the help of technology tomograph include electric, ultrasonic and georadar. Tomograph technology which includes electric, ultrasonic and georadar have been developing to detect the decay of trees as non-destructive methods. But according to Nicolotti *et al.* (2013), the most effective technology for detecting decay of the tree is that using ultrasonic tomography. This type of technology can determine the position of anomalies and estimate the size and shape of the tree trunk. The principle working of ultrasonic tomograph is the vibration velocity of the sound wave which are interpreted through the degradation of color.

Najafi *et al*. (2009) revealed that the research to detect decay on Fagusorientalis using the speed of sound wave to show results that correlate positively and a significant reduction to the raising dimensions of decay. the sound wave propagation speed of the measurement sample trees can be seen in Table 4.

Table 4. The value of the speed propagation of sound waveand sample trees condition

Code	Tree Species	Vaverage (m/s)	V minimal (m/s)	Decay wood (%)	Solid wood (%)
A	*Albizia saman* (Jacq.) Merr.	1874	942	1,5	98,5
B	*Canarium vulgare* Leenh.	1504	620	42,29	57,71
C	*Canarium vulgare* Leenh.	1540	271	50,01	49,89
D	*Agathis borneensis* Warb.	1545	440	12,03	88,97
E	*Agathis borneensis* Warb.	1501	856	25,07	74,93

*Description: V = sound wave velocity

Decay area on the wood is an area that delineated with another color excluded black and brown (Figure 3). The delineation of decay area on wood in accordance to shortly sound wave velocity. This case can be seen at the *Canarium vulgare* with an average velocity of 1540 m/s, the lowest 271 m/s

indicate that the decay area around 60%. Wang *et al*. (2004) stated that decay wood has sound wave velocity longer than healthy wood.

In the practice of tree, management is done differently based on the level of health of each. The healthy trees usually do not need much intensive but still see where the tree grows whether it is harmful or not (anthropogenic). Trees with minor damage are done by lightly pruning the canopy. Trees with middle damage are usually carried out with moderate to heavy pruning on the canopy to reduce canopy loads. Trees with mayor damage have to cut the stem.

Puspitasari (2014) stated that based on the proportion of solid wood, the percentage of solid wood> 75% included in the category of healthy trees, 50-75% in medium category and <50% is included in diseased trees. Therefore the sample trees in Bogor Palace were categorized in the healthy trees, except the tree *Canarium vulgare* the proportion of its solid wood approximately 40% and 60% in the medium category. Cutting the stem vertically in the same of original point is one of the ascertained methods that can be used to prove the accuracy of tree decay. This statement is literally supported with research that done by Gilbert and Smiley (2004) about the accuracy of tomogram in determining of the deteriorated area was obtained with comparing the actual location of the deteriorated area in vertically stem cutting and tomogram image.

Recommendations

The monitoring and management of risk trees are quite important to be able to prevent the devastating damage of trees. Pruning of branches is a precaution risk to reduce the canopy. Hariyadi *et al.* (2011) revealed that the main purposes of pruning are that to optimize light penetration and navigate the growth and development of branch and bud. It was implemented in pruning in Jatropha curcas L. Furthermore, eradication of damaged region of trees is another way in maintenance and management cases. Trees protection from insect outbreak is needed to avoid further damages. For instance, termites attack are found in *Agathis* sp that allegedly invaded the inner of the trees. In order to minimize the number of termite population by placing trap which is able to kill termite.

CONCLUSION

The assessment of tree health status in strategic location of Bogor Palace revealed that 62,5% in healthy trees status, 18.75% in suffered minor damage trees and 18.75%the trees were mayor damaged.Identification of decay using a PICUS® 3.0 Sonic tomograph showed decay on the tree *Albizia saman* 1.5%, *Canarium vulgare* 42.29%, and 50.01%, *Agathis borneensis* 12.03% and 25.07%. Trees that have been indicated decay need to get monitoring and proper treatment because it could collapse at any time. Therefore, we recommend mitigation measures in the form of load reduction of the tree canopy and routine inspection to determine the development of the percentage of decay, so that recommendation can be more accurate and precise in tree risk management.

ACKNOWLEDGMENTS

We thank Bogor Presidential Palace authority which has given permission to conduct research at the Bogor Presidential Palace. A lot of thanks was also delivered to the Tree risk management team of LIPI-Botanic Gardens.

REFERENCES

Duryat, Gitosaputro, S., dan Melya, R. 2014. Analisis status dan pemetaan kondisi kehealthyan pohon penghijauan di Kota Bandar Lampung. Laporan Penelitian. Universitas Lampung. Lampung.

Gilbert E, Smiley ET. 2004. Picus sonic tomography for the quantification of decay in white oak (Quercus alba) and hickory (Carya spp.). Journal of Arboriculture 30: 277-281.

Hariyadi, Purwoko BS, Raden I. 2011. Pengaruh pemangkasan batang dan cabang primer terhadap laju fotosintesis dan produksi jarak pagar (*Jathropa curcas* L.). Jurnal Agronomi Indonesia 39 (3): 205-209.

Liang, S., Wang, X., Wiedenbeck, J., Cai, Z. and Fu, F., 2008. Evaluation of acoustic tomography for tree decay detection. In Proceedings of the 15th International Symposium on Nondestructive Testing of Wood-Session 1: 49-54.

Mangold R. 1997. Overview of the Forest Health Monitoring Program.USDA Forest Service, USA.

Mariyanti. 2011. Evaluasi Kehealthyan Pohon Ornamental Di Kota Bogor Menggunakan Metode Visual Dan Gelombang Ultrasonik [*Skripsi*]. Institut Pertanian Bogor. Bogor.

Nicolotti G, Socco LV, Martinis R, Godio A, Sambuelli L (2003) Application and comparison of three tomographic techniques for detection of decay in trees. J Arboric 29:66 – 78.

Nyland, R. D. 2002. Silviculture : Concepts and Application second edition. McGraw-Hill. New York.

Puspitasari, D.A.I. 2014. Evaluasi Kesehatan Pohon Peneduh Jenis Angsana (*Pterocarpus indicus* Willd.) secara Sonic Tomography di Kota Surabaya. [*Skripsi*]. Institut Pertanian Bogor. Bogor.

Saeed Kazemi-Najafi, Ali Shalbafan and Ghanbar Ebrahimi. 2009. Internal decay assessment in standing beech trees using ultrasonic velocity measurement. European Journal of Forest Research 128: 345–350.

Tainter, EH. and EA. Baker. 1996. Principles of Forest Pathology. John Wiley and Sons. Inc. New York.

Wang X, Divos F, Pilon C, Brashaw KB, Ross JR, Pellerin FR. 2004. Assessmentof decay in standing timber using stress wave timing nondestructive evaluation tools. Forest Service. United States (US): United States Department of Agriculture 149–171.

Wang, X., Wiedenbeck, J. and Liang, S., 2009. Acoustic tomography for decay detection in black cherry trees. Wood and Fiber Science, 41(2): 127-137.

Characterization and potency of kimpul taro (*Xanthosoma sagittifolium* (L.) Schott) from Enggano island as a source of carbohydrate

[1)]Titi Juhaeti and Peni Lestari
Botany Division, Centre Research for Biology
Indonesian Institute of Sciences,
Jalan Raya Jakarta Bogor km 46 Cibinong. Phone: 021-8765056,
[*]email: tihaeti@yahoo.com

ABSTRACT

Kimpul taro (*Xanthosoma sagittifolium* (L.) Schott) with purple colour on almost all of its parts was collected during flora exploration to Enggano Island in April 2015. The rejuvenation of its collected cormels has been done since May 2015 in the Botany Division Garden Facility, Research Centre for Biology, Indonesian Institute of Sciences (LIPI) in order to characterize its morphology, growth and cormel production. This study was an important step to develop kimpul taro as a carbohydrate source for food diversification. Due to limited number of samples, the plant characterization was done to 12 samples of 4 month-old and 6 samples of 14-month-old taro plants. The plant characterization was done based on "Guidelines for Characterization and Evaluation of the Germplasms of Talas Kimpul" (Ministry of Agriculture, 2002). The analysis of nutrition potency was done at the Biochemistry Laboratory of Bogor Agricultural University (IPB). The taste/organoleptic characterization was done at the Physiology Laboratory, Research Centre for Biology, Indonesian Institute of Sciences. The results showed that taro kimpul was potential to be developed as a source of carbohydrate because its cormel and leaf stalks can be consumed with delicious taste, does not cause itching when consumed, and is nutritious. Good cultivation practices are needed to increase cormel production. This taro can be cultivated even on light shading area. The results of proximate analysis showed carbohydrate content of cormel 52.896%, fat 1.858%, protein 11.895 %, calcium 954.42 mg/100g, phosphor 656.682 mg/100g and oxalic acid 21.4 mg/kg. Meanwhile, carbohydrate content of leaf stalks was 55.482 %, fat 1.568 %, protein 3.894 %, calcium 1085.16 mg/100g, phosphor 695.728 mg/100g and oxalic acid 24.3mg/kg. As a conclusion, kimpul taro is potential to be developed as a carbohydrate source for nutritious food.

Key words: Characterization, carbohydrate source, Enggano Island, kimpul taro, proximate analysis.

INTRODUCTION

Food diversification is currently an important issue in Indonesia. The Indonesian government has been developing food diversification programme since 2002, as stipulated by Government's Regulation No. 68 of 2002 regarding Food Security (PERMENTAN, 2013). This programme is considered an alternative pathway to reduce the level of food insecurity and improve food security of Indonesia. The specific programme of "One Day No Rice" was then applied in some regions in Indonesia (Subagiyo, 2010). Some cereal and tuber commodities come to be alternative foods to reduce the rice and wheat consumption. Diversification is defined as an attempt to increase the diversity of agricultural products, both horizontally and vertically. According to Nurmiyati (2009), vertical diversification means the development of production after harvest, including the processing of agricultural products and waste. Meanwhile, diversifying horizontally means increasing the variety of agricultural commodities developed.

Developing the concept of diversification horizontally will reduce the risk of crop failure. This is because the magnitude of the risk of crop failure is spread across several commodities. The failure of the harvest on one commodity can be offset by the success of other commodity harvest. Exploration of potential food resource in Indonesian areas can be an alternative to support the government's food diversification programme.

LIPI Exploration Programme to Enggano Island, Bengkulu Province in 2015 was an effort to explore the local potentials, including food resources. From the exploration some local food commodities were collected, one of which was taro. The specific characteristic of this plant is its purple colour, light to dark, in almost all parts of the plant, including the cormel, leaf stalk and leaves. Local people had no local name for this plant, but the identification results in the Research Centre for Biology, LIPI, indicated it was *Xanthosoma sagittifolium* L. Schott. The Indonesian name for this species is *kimpul taro*. Based on field observations, this plant is limitedly consumed by the local people in Apoho

Village, Enggano Island. On the other hand, local people in other areas, such as East Java (Sulistyowati et al., 2014), West Java and Jayawijaya (Papua Province) consume kimpul taro raw.

Characterization study becomes important in order to follow up the collected samples as a result of exploration. This is the first step in the program of developing wild plants into cultivated plants before the study of its cultivation technique and its potential introduction to the wider community. The characterization study was carried out to examine the morphological characters of kimpul taro of Enggano, its nutritional potential and cultivation effort. It aimed to explore the potential of the kimpul taro as a carbohydrate source. The results of characterization can be used as a reference and breeding material in the next development of this commodity.

MATERIAL AND METHODS

Exploration was conducted in Enggano Island, Bengkulu Province, from April to May 2015. Sampling was done using line transect method combined with interviews to the local people. Transects were carried out on the Apoho village road. The target sample was a population of taro plants, because some local people stated that they rarely consumed this plant. The taro species was only found at two points in Apoho village. A transect was made by tracing the road as far as 100 meters and the width of the right and left areas of the road were 10 meters at that point, respectively. By walking along the transect line, the population of the taro plant, the size of the taro plant and the surrounding species were recorded (Enggano Exploration Report, 2015). After that, samples of planting materials were taken for further characterization at the Research Centre for Biology, Indonesian Institute of Sciences. Environmental conditions were observed in the sampling site, including sampling site's coordinate, altitude, humidity, air temperature, and soil pH. The data were measured using GPS, thermohygrometer, and pH meter, respectively.

The samples collected from the site consisted of 20 planting materials (cormel G1) taken from only two transect sample sites in the Apoho village because the target species was not found in other sites. The planting materials were planted in the field experiment of Research Centre for Biology, Indonesian Institute of Sciences. The study was conducted in the period of May 2015 - July 2016. Cormels were planted in Botany Division Garden Facility. The planting plots were previously fertilized by organic fertilizers (compost and manure) without any chemistry fertilizer.

The samples for characterization observation comprised of 12 four-month-old and 6 14-month-old taro plants. The plant characterization included: morphological characterization of whole plant parts; proximate analysis; oxalate content, mineral analysis (Fe, and Ca); amino acids analysis; and hedonic tests. Morphological characterization was done in accordance with the "Characterisation and Evaluation Manuals of Talas Germplasm" (Kusumo et al., 2002) on leaf, petiole, stem, and cormel. Proximate, oxalate, Fe, calcium, and amino acid analyses were done both on the petiole and cormels. Both cormel and petiole can serve as food. However, in this study we only did hedonic test on the cormel because of limited samples of petiole.

The phytochemical analyses, including proximate, amino acid, oxalate, Ca and Fe analyses, were performed at the Chemistry Department Laboratory, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University. The hedonic test was conducted to 20 respondents in the Botany Division, Research Centre for Biology.

RESULTS AND DISCUSSION

Conditions of the Exploration and Rejuvenation Sites

In Enggano Island, kimpul taro plants were found at two transect points. The plants lived on the edge of a swamp. They grew with other taro species (*Colocasia esculenta*). This location had a clay soil texture, soil pH of 6.8 and soil moisture of 60%. Rudyatmi and Rahayu (2014) describe that taro is adaptive to grow in marginal locations, one of which is flooded land. They also explain that the plants are able to grow at high saline and shaded locations, too. Taro plants can grow under low light intensity because they can maintain their chlorophyll content (Suketi et al., 2001) and their stomatal density (Djukri, 2003) remains high in low light-intensity environments.

The location where kimpul taro grew on Enggano Island was at 14 m above sea level (asl), on microclimate light intensity of 31900 lux, air temperature 34.6°C and air humidity 99%. In some literature, it is reported that taro crops can grow up until 1,300 masl (Hasan & Arif, 1999; Prihatman, 2000; Sulistyowati et al., 2014). At this location, rainfall in dry months is <200 mm, while in wet months 222.8 mm. The kimpul taro plants found in Apoho Village, Enggano Island, had a dark green leaf character. This kind taro is typically characterized by purple red lumps, starting from the base to the tip of petiole.

Morphological Characters

The samples collected were replanted in the field experiment of Research Center for Biology, LIPI, from the cormel propagation. The rejuvenation site was in Cibinong Science Centre, West Java Province. The site had an environmental condition almost similar to the original habitat of taro plants in Enggano Island. The research site at Cibinong is on 200 meters above sea level (Syafei, 2014). Rainfall in wet months is about 400-450 mm/month, while rainfall in dry months is 80-100 mm/month (Syafei & Hidayati, 2014). Daily temperature in the study area is around 30-33°C during the day and 24-25°C at night with 70-100% air humidity.

The taro plant characteristics after being replanted in the Research Centre for Biology, LIPI were almost same as those of the kimpul taro in its habitat (Table 1). The rejuvenation and characterization were done in accordance with the "Characterization and Evaluation Manual of Talas Germplasm" (Kusumo et al., 2002).

Table 1. Morphlogical characters of kimpul taro from Enggano Island

No	Morphological characters	4 month-old taro					14 month-old Taro
		1	2	3	4	5	
1.	Growing type						
	Ascalauscent	√	√	√	√	√	√
2.	Length/height of above-ground stem (cm)	0	0	0	0	0	10
3.	Diameter of above-ground stem (cm)	-	-	-	-	-	7,5
4.	Inner petiole colour						
	Light red	√	√	√	√	√	√
7.	Bulbilus on leaf axillary						
	0 = none	√	√	√	√	√	√
8.	Bulbilus along stem						
	0 = none	√	√	√	√	√	√
9.	Whole plant height (cm)	-	-	-	-	-	142,5
10.	Petiole						
	Sheathing below	√	√	√	√	√	√
11.	Leaf position						
	Cup form	√	√	√	√	√	√
12.	Leaf edge						
	Full, wavy	√	√	√	√	√	√
13.	Leaf shape						
	Sagittate-ovate (lobus> ¼ length of leaf)	√	√	√	√	√	√
15.	L/W leaf ratio						
	Length (cm)	79	81,4	72	72	75	68
	Width (cm)	51	49	46	49	49	43,4
16.	Leaf edge colour						
	Purple (outer), green (inner)	√	√	√	√	√	√
17.	Leaf sinus denuding						
	Blade (>5 mm)	√	√	√	√	√	√
18.	Shiny leaf surface						
	0 = none	√	√	√	√	√	√
19.	Glaucous colour of leaf surface						
	0 = none	√	√	√	√	√	√
20.	Colour of upper leaf surface						
	Reddish/green violet	√	√	√	√	√	√

No	Morphological characters	4 month-old taro					14 month-old Taro
		1	2	3	4	5	
21.	Colour of bottom leaf surface						
	Light green	√	√	√	√	√	√
22.	Leaf variety						
	0 = none	√	√	√	√	√	√
23.	Pubescence						
	0 = none	√	√	√	√	√	√
24.	Leaf vein						
	On leaf bottom	√	√	√	√	√	√
25.	Length of leaf petiole (cm)	145	108	102	105	105	86
26.	Colour of leaf petiole (2/3 upper part)						
	Reddish	√	√	√	√	√	√
27.	Colour of 1/3 bottom of leaf stalks						
	Red/violet	√	√	√	√	√	√
	Green with red line/violet						
28.	Glaucous on upper petiole						
	0 = none	√	√	√	√	√	√
29.	Length of sheathing petiole						
	1/3-2/3 of petiole	√	√	√	√	√	√
30.	Colour of edge of sheathing petiole						
	Same as petiole and sheath	√	√	√	√	√	√
31.	Harvest time of cormel						
	7-12 months.	√	√	√	√	√	√
32.	Cormel form						
	Ellipse	√	√	√	√	√	√
33.	Cormel size (at harvest time)						
	5 = middle	√	√	√	√	√	√
34.	Colour of outer cormel						
	Dark brown	√	√	√	√	√	√
35	Colour of inner cormel						
	Light red/ pale red	√	√	√	√	√	√
36.	Outside of cormel						
	Fibrous	√	√	√	√	√	√
37.	Colour of cormel apex						
	Light red/red	√	√	√	√	√	√
38.	Cormel apex position						
	Under ground	√	√	√	√	√	√
39.	Stolon						
	None	√	√	√	√	√	√
40.	Rhizome						
	0 = none	√	√	√	√	√	√
41.	Number of suckers	5	5	5	5	5	5
	Latex						
	Few	√	√	√	√	√	√
42.	Latex consistency						
	Sticky	√	√	√	√	√	√
43.	Latex colour						
	White/creamy	√	√	√	√	√	√
44.	Parts of plant producing latex						
	+ = present	√	√	√	√	√	√
	Stem	√	√	√	√	√	√
	Leaf	√	√	√	√	√	√

No	Morphological characters	4 month-old taro					14 month-old Taro
		1	2	3	4	5	
	Cormus or cormel	√	√	√	√	√	√
45.	Angle of primary lateral vein from midrib	60^0	60^0	60^0	60^0	60^0	60^0
46.	Number of coupled primary lateral veins	6-7	6-7	6-7	6-7	6-7	6-7
47.	Vein position						
	Almost same along leaf edge	√	√	√	√	√	√
48	Number of vein groups						
	>2	√	√	√	√	√	√
49.	Colour of vein on upper side of leaf						
	Light green, darker than leaf	√	√	√	√	√	√
50.	Colour of vein on underside of leaf						
	red/violet	√	√	√	√	√	√
52.	Transverse sliced form of sheathing petiole						
	Closed	√	√	√	√	√	√
53.	Cormel numbers/plant	12	12	12	12	12	12
	Cormel length	14,6	6,5	12	7,9	6	14,4
54.	Cormel weight (gr)	115	119,1	67,8	52	105,9	35,6
55.	Cormel diameter	3,94	3,87	3,81	3,55	3,91	3,13

The results of the rejuvenation (first generation G1) are showed on Table 1. The first generation (G1) plants have a growth type of ascalauscent, with a plant height of about 1.5 meters (Figure 1a). Petiole colour is purple, starting from the base to the tip. Leaf shape is sagittate with wavy edges (Figure 1b). The leaves are bowl-shaped (cupped up). The upper surface of the kimpul taro leaves of Enggano is purplish green, while the lower surface is bright green (figure 1c).

Stems in 4-month-old plants are generally still hidden in the midrib, so they were not identified yet. However, 14-month-old talas kimpul ungu has reached the size of 10 cm stem above the soil surface (Figure 1). The result of the transverse incision of the stem shows that the inside colour of the stem is reddish (Figure 1e). The skin of kimpul taro cormel is dark brown, with the tip of the cormel being red. The cormel cross section shows the pale red/pale flesh (Fig. 1f).

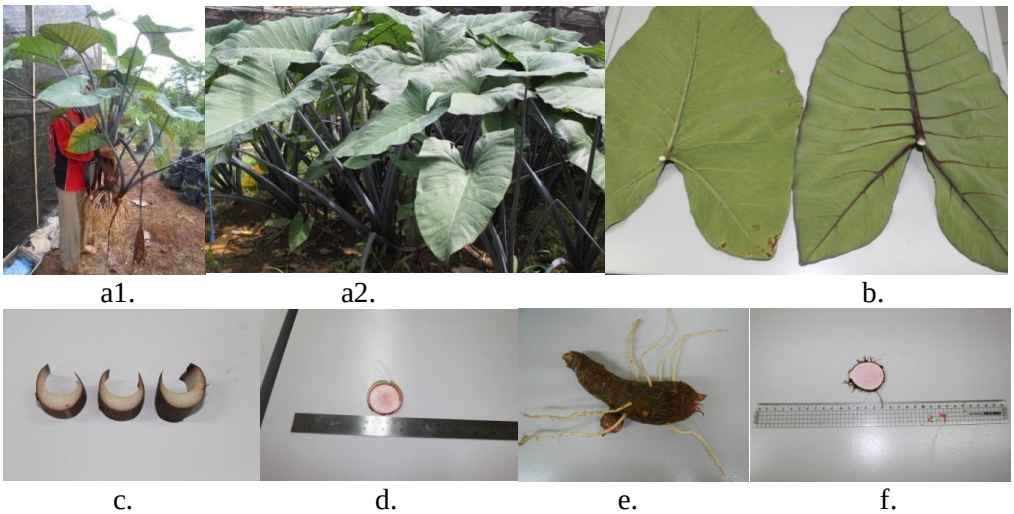


Figure 1. a: 1-2: plant habitus, b: old leaf (left), young leaf (right), c: inner colour of petiole, d: stem colour, e: cormel, f: inner colour of cormel

Phytochemical Characters of Kimpul Taro

All parts of kimpul taro plants: the leaf, stem, or cormel can be used as food (edible). Therefore, proximate analysis was performed on all three sections. In addition to the proximate test, hedonic test, or the level of consumer preference, was conducted only to the cormel. The result of proximate analysis showed that the carbohydrate portion in cormel of kimpul taro from Enggano Island was 52.89%. Carbohydrate content was also found in the stem and leaves (Table 2). Protein content in cormel was 11.8%, and its fat content was 1.85%. In addition to macro nutrient content, kimpul taro also contained amino acids with varied compositions (Table 3).

Carbohydrate content in kimpul taro flour was smaller than that of white rice (78.9%) and maize (72.4%). But the content of protein and fat in cormel of kimpul taro from Enggano was greater than that of white rice (6.8%, 0.7%) (Barus, 2005; anonymous, 2014). With such data, this commodity can be an appropriate food for children and emergency food.

Table 2. Proximate content of every part of kimpul taro from Enggano

Content	Unit	Leaf	Petiole	Cormel
Water	%	4.31	4.16	4.86
Ash	%	0.458	0.512	0.527
Protein	%	3.724	3.894	11.895
Crude fiber	%	0.547	0.842	0.932
Carbohydrate	%	52.642	55.482	52.896
Ca	mg/100g	989.62	1085.16	954.42
P	mg/100g	614.653	695.728	656.682
Oxalate acid	mg/kg	23.5	24.3	21.4

Note: The analysis was done at Biochemical laboratory, Department of Biochemical of IPB.

Table 3. Amino acid contents of kimpul taro from Enggano

No.	Amino acid	Concentration (%)
1.	Aspartic acid	0.695
2.	Glutamic acid	2.036
3.	Serine	0.304
4.	Glycine	0.198
5.	Histidine	0.242
6.	Arginine	0.264
7.	Threonine	0.305
8.	Alanine	0.366
9.	Proline	0.218
10.	Tyrosine	0.382
11.	Valine	0.554
12.	Methionine	0.290
13.	Cysteine	0.216
14.	Isoleucine	0.238
15.	Leucine	0.158
16.	Phenylalanine	0.384
17.	Lysine	0.276

Consumer Preference of Kimpul Taro

The analysis was not only done to the proximate properties, but also consumer preference of the cormel of kimpul taro. This was analysed through the hedonic test. The results showed that most respondents liked the cormel of kimpul taro from Enggano. As many as 63.1% of respondents said the cormel of talas kimpul was good and had a rather soft until soft textures. Some respondents stated that

the cormel of kimpul taro was itchy on the tongue (47.3%), while others stated it was not itchy (52.5%) (Table 4). In addition to the cormel, the petiole can also be consumed as vegetable because it tastes good and does not itch.

The itching when consuming taro is caused by oxalic acid. This compound is common in plant bulbs belonging to the Araceae family. This compound is soluble in water and alcohol. However, when binding to metals, such as calcium or magnesium, the compound will turn into an insoluble precipitate (Pancasasti, 2016). In the plant body, oxalic crystals serve as one of the plant defence mechanisms against herbivores (Lestari, 2010). When oxalate enters the human body, either as water-soluble or water-insoluble compounds, it can inhibit the digestibility of the body against calcium and can precipitate and form kidney stones (Pancasasti, 2016). Therefore, it is important to know the natural oxalic acid content of the commodities.

Test results on oxalic acid content showed that talas kimpul from Enggano had oxalic acid content of 21.4 - 24.3 mg/kg (Table 2). This value is below the permissible threshold of oxalic acid content in foodstuffs, i.e. 71 mg/kg (Sefa-Dedeh & Agyr-Sackey, 2004). Therefore, talas kimpul from Enggano is very safe to be consumed as food. In some other taro species, the oxalic acid content can reach 2891-6224 mg/kg in *X. undipes* (Pancasati, 2016), while other literature reported *X. undipes* to have an oxalate content between 3300-4400 mg/kg (Muttakin et al., 2015) and senthe's (*Alocasia macrorrhiza* L. Schott) oxalate content reached 648 mg/kg (Amalia et al., 2013).

Table 4. Hedonic test of taro cormel

Variable	Grade	Percentage (%)
Texture	Not delicate	5.2
	Rather delicate	26.3
	Normal	26.3
	Delicate	36.8
	Very delicate	5.2
Taste	Not delicious	5.2
	Rather delicious	10.5
	Normal	21.0
	Delicious	63.1
	Very delicious	0
Itch	Not itchy	31.5
	Rather itchy	36.8
	Normal	21.0
	Itchy	10.5
	Very itchy	0

CONCLUSION

Since it can be conveniently consumed by local people, kimpul taro from Enggano Island is potential to be developed as a source of carbohydrate for food diversification. The research has showed that kimpul taro oxalate content is low, does not cause itching when consumed, and has a good taste with rather delicate to delicate textures. For further utilization, more research is required on various aspects such as morpho-agronomic characterization, proximate analysis and analysis of other nutritional components, physiological research, and cultivation.

REFERENCES

Amalia R dan R Yuliana. 2013. Studipengaruh proses perendaman dan perebusan terhadap kandungan kalsium oksalat pada umbi senthe (*Alocasia macrorrhiza*(L.) Schott). *JurnalTeknologi Kimia danIndustri* 2(3):17-23.

Anonim. 2017. Pulau Enggano. http://www.ppk-kp3k.kkp.go.id/direktori-pulau/index.php/public_c/pulau_info/295. [Accessed 8 May 2017].

Anonim. 2014. Data kandungan gizi bahan pangan dan hasil olahannya. http://bkppp.bantulkab.go.id/filestorage/dokumen/2014/07/Data%20Kandungan%20Gizi%20Bahan%20Pangan%20dan%20Olahan.pdf [diunduh 8 Mei 2017]

Barus P. 2005.Studi penentuan kandungan karbohidrat, protein dan mineral dalam air rebusan beras sebagai minuman pengganti susu. *Jurnal Sains Kimia* 9(3):15-16.

Djukri. 2003. Seleksi tanaman talas (*Colocasia esculenta*) untuk adaptasi terhadap cekaman naungan. Disertasi. Program Pascasarjana. Institut Pertanian Bogor.162 hal.

Hasan dan Arif. 1999. *Talas* (*Colocasia esculentai* L. Schott). Institut Pertanian Bogor. Bogor

Kusumo S, M Hasanah, S Moeljopawiro, M Thobari, Subandriyo, AHardjamulia, A Nurhadi, H Kasim. 2002. *Buku Panduan Karakterisasi dan Evaluasi Plasma Nutfah Talas*. Komisi Nasional Plasma Nutfah. Departemen Pertanian. 94 p.

Lestari P. 2010. Preselection of caricature plant (*Graptophyllum pictum* (L) Griff) resistance trait to *Doleschalliabisaltide* Cramer (Lepidoptera: Nymphalidae). Thesis. Agricultural Faculty. Bogor Agriculture Institute.

Muttakin S, Muharfiza, S lestari. 2015. Reduction of oxalate levels in Bantenlocals taro through immersion salt water. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia* 7(1):1707-1710.

Nurmiyati. 2009. Karakterisasi kimpul (*Xanthosoma* spp) berdasarkan karakter morfologi dan analisis isozim. Tesis.Universitas Sebelas Maret. 74 hal.

Pancasasti R .2016. Pengaruh elevasi terhadap kadar asam oksalat talas beneng (*Xanthosoma undipes* K.Koch) di sekitar kawasan Gunung Karang Provinsi Banten. *Jurnal Ilmiah Setrum* 5(1):21-25.

Prihatman K.2000. Talas.http://www.ristek.go.id. [Accessed 1 May 2015]

Rudyatmi E dan ES Rahayu. 2014. Karakterisasi talas lokal Jawa Tengah (identifikasi sumber plasma nutfah sebagai upaya konservasi tanaman pangan altenatif). *Sainteknol* 12(1):1-8.

Sefa-Dedeh, S. And Agyir-Sackey, E.K. 2004. Chemical composition and the effect of processing o oxalate content of cocoyam Xanthosoma sagittifolium and Colocasia esculenta cormels. *Food Chemistry* 85: 479-487.

Suketi K, BS Purwoko, D Supandi, IH Somantri, IS Dewi, dan Minantyorini. 2001. Karakterisasi dan konservasi in vitro plasma nutfah serta seleksi adaptasi untuk mendukung tumpangsari. *Laporan Hasil Penelitian. Insitut Pertanian Bogor dan Badan Litbang Pertanian.35 hal.*

Sulistyowati PV, N Kendarini, danRespatijarti. Observation the existence of taro plant genus *Colocasia* and *Xanthosoma* in Kedung kandang subdistrict and Ampel gading subdistrict, Malang. *Jurnal Produksi Tanaman* 2 (2):86-93.

Syafei M, R Hidayati. 2014. The influence of altitude and monthly rainfall to diarrheal disease incidence rate (Case study: Bogor regency). *Journal Agromet Indonesia.* 28(1):33-39.

Syafei M. 2014. Penentuan tingkat kerentanan dan karakteristik curah hujan pada penyakit diare (studi kasus: Kabupaten Bogor). Skripsi.Fakultas Matematika dan Ilmu Pengetahuan Alam. Institut Pertanian Bogor. 38p.

Subagiyo, RZ. 2010. One day no rice. http://m.antaranews.com. [Accessed 18 October 2018].

Peraturan Menteri Pertanian [PERMENTAN]. 2013. Program Peningkatan Diversifikasi dan Ketahanan Pangan Masyarakat Bahan ketahanan Pangan Tahun Anggaran 2013. [Accessed 18 October 2018].

Sulistyowati PV, N Kendarini, Respatijarti. 2014. Observasi keberadaan tanaman talas-talasan genus *Colocasia* dan *Xanthosoma* di kecamatan kedung kandang kota malang dan kecamatan ampel gading kabupaten malang. *Jurnal produksi Pangan.* 2(2):86-93.

Morphology and Molecular Identification of Local Cultivars of Pisang Raja (*Musa* Spp.) from Yogyakarta, Central Java and East Java, Indonesia

Lia Hapsari[1], Fauziah[2], Trimanto[3]
[1,2,3]Purwodadi Botanic Garden – Indonesian Institute of Sciences
Jl. Surabaya – Malang Km 65, Pasuruan, 67163, East Java, Indonesia
Corresponding author: lia.hapsari@lipi.go.id; hapsari.lia@gmail.com

ABSTRACT

Pisang Raja is well-known banana cultivars in Indonesia. Particularly in Java, there are not less than 40 local cultivars of Pisang Raja were recorded which needs to be clearly classified and identified. Genomic group identifications were conducted both morphology and molecular to thirteen local cultivars of Pisang Raja originated from Yogyakarta, Central Java and East Java collection of Purwodadi Botanic Garden – Indonesian Institute of Sciences. Morphological identification was using taxonomic scoring of fifteen dianogstic characters between *M. acuminata* and *M. balbisiana* wild species as their ancestral parents, whereas molecular identification was using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism/PCR-RFLP of Internal Transcribed Spacer/ITS method. Result showed that genomic group of 13 Pisang Raja local cultivars based on morphology consists of 8 AAB, 3 ABB, and 2 AAA; whereas molecular result consists of 4 AAB, 3 ABB, and 6 AAA. About 4 out of 8 banana cultivars which identified as AAB morphologically were revealed as AAA molecularly. Molecular approach using PCR-RFLP of ITS to identify genomic group of Pisang Raja local cultivars provide more objective, consistent and reliable result, also less time-consuming than morphology. Nonetheles morphological description of bananas is still necessary to conduct as part of a longer-term systematic evaluation and selection basis for further utilization and development. Genomic group result of Pisang Raja local cultivars examined are as follows: Raja Bandung (ABB), Raja Siem (ABB), Raja Prentel (ABB), Raja Gintung (AAB), Raja Temen (AAB), Raja Lingi (AAB), Raja Marto (AAB), Raja Kenanga (AAA), Raja Ketan (AAA), Raja Nangka (AAA), Raja Pendek (AAA), Raja Talun (AAA) and Raja Warangan (AAA).

Key words: genomic group, identification, ITS, morphology, molecular, PCR-RFLP, Pisang Raja

INTRODUCTION

Pisang Raja is indigenous and well-known banana cultivar in Indonesia and Malaysia. It is also present in the Philippines, Thailand and Papua New Guinea with their own local names. (Espino *et al.* 1992; Lim 2012). It is characterized by its large fruit, thick coarse skin, orange; flesh creamy-orange, coarse texture, sweet taste; bunches bear 6-8 hands (Espino *et al.* 1992). Particularly in Java; there are many local cultivars of Pisang Raja, not less than 40 local cultivars of Pisang Raja were recorded from some previous inventory and diversity studies of bananas in Java by Jumari & Pudjoarinto (2000); Ekasari *et al.*, 2012; Ayuningtyas (2013); Indraswari (2014); Firdausi *et al.* (2015); Hapsari *et al.* (2017).

Pisang Raja plays important roles in Javanese cultural life, therefore become highly prized in the market. It is one among the obligatory components of offerings and decorations in the ritual ceremonial activities. Raja means king, symbolising hopes for the happy, successful prosperity of kings and to become a wise and righteous person. Pisang Raja used for rituals are limited to certain local cultivars *i.e.* Pisang Raja Sajen, Raja Temen and Raja Pulut (Hapsari *et al.* 2017). Fruit of Pisang Raja cultivars can be consumed both as dessert and processed, or considered as dual purpose banana. Banana fruits are nutritious food recommended for people at all ages, especially for baby, also diet food for adults but consumption must be limited for diabetic and kidney problem patients (Hapsari & Lestari, 2016). The carbohydrate levels of some Pisang Raja cultivars are following; Pisang Raja 28.95 %, Raja Siem 23.66 %, Raja Sere 23.97 %, Raja Pakuwan 20.49 %, Raja Nangka 27.94 % (Heyne, 1987) and Raja Bandung 31.13 % (Hapsari & Lestari, 2016)

In taxonomic view, Pisang Raja is a hybrid species and should correctly be written as *Musa* x *paradisiaca* L. or as *Musa acuminata* x *Musa balbisiana* (AAB group) since it is a triploid hybrid with

two genomic sets contributed by *M. acuminata* Colla (A genome) and one by *M. balbisiana* Colla (B genome). According to morphological characteristic using taxonomic scoring, Pisang Raja supposed to have intermediate characters of both ancestral parents (using 1-5 degree diagnostic) with score 26-46 (Simmonds & Shepherd, 1955; Simmonds 1959; Espino *et al.* 1992). However, morphological characteristics of the high diversity of Pisang Raja local cultivars in Java doesn't always resemble *Musa* AAB group. Therefore, it needs to be clearly classified and identified.

Identification and characterization using morphological attributes (whole-plant including floral and fruit) are found difficult, subjective and time consuming; however, it still important to perform. As a more reliable and fast alternative, various molecular techniques also have been used to study the genetic diversity and taxonomy of cultivated bananas to confirm the previous morphological identification. Some molecular techniques have been extensively used in identification and banana genetic analysis such as Amplified Fragment Length Polymorphism/AFLP (Wong *et al.*, 2002), Polymerase Chain Reaction-Restriction Fragment Length Polymorphism/PCR-RFLP (Nwakanma *et al.*, 2003; Ekasari *et al.*, 2012; Hapsari *et al.*, 2015), Random Amplified Polymorphic DNA/RAPD (Pillay *et al.* 2000; Sukartini, 2008, Poerba & Ahmad, 2010), DNA barcoding using some markers (Liu *et al.*, 2010; Hrˇibova´ *et al.*, 2011; Li *et al.*, 2013; Hapsari, 2015), microsatellites (Retnoningsih 2009; Windarti *et al.*, 2009; Lu *et al.*, 2011; de Jesus *et al.*, 2013; Poerba & Ahmad, 2013), etc.

The objective of this study is to identify the genomic group of 13 different local cultivars of Pisang Raja originated from Yogyakarta, Central Java and East Java using morphology and molecular assessed by PCR-RFLP of the Internal Transcribed Spacer/ITS; also study its phenetic and genetic relationship. PCR-RFLP of ITS is effective and more economically molecular technique to identify the genomic group on bananas cultivars than other techniques (Nwakanma *et al.*, 2003; Ekasari *et al.*, 2012); although it was not able to distinguish individual's homozygous diploid and triploid (Hapsari *et al.*, 2015). A proper and correct classification and characterization of local banana cultivars is important as basis information for further utilization and development, evaluation and conservation management, also for further banana breeding programmes.

MATERIALS AND METHODS

Plant materials

Plant materials examined in this study were thirteen local cultivars of Pisang Raja originated from Yogyakarta, Central Java and East Java collection of Purwodadi Botanic Garden-Indonesian Institute of Sciences (Table 1). Genomic group identifications were conducted both morphology and molecular. Plant part used for molecular analysis was fresh young cigar leaf (furled leaf) tissues of each cultivar.

Table 1. Plant materials Pisang Raja local cultivars examined in this study

No	Cultivar name	Vak location	Registration number	Locality
1	Raja Lingi	XXIV.E. 14-abc	P19760188	Yogyakarta
2	Raja Warangan	XXIV.D.48	P1994058	Yogyakarta
3	Raja Bandung	XXIV.D. 69-ab	P19940525	Yogyakarta
4	Raja Gintung	XXIV.D.88a	P19940533	Yogyakarta
5	Raja Siem	XXIV.D. 23-ab	P1975062	Prembun, Kebumen, Central Java
6	Raja Marto	XXIV.D. 57-a	Not available	Temanggung, Central Java
7	Raja Ketan	XXIV.D. 22-ab	P19760732	Siman, Ponorogo, East Java
8	Raja Nangka	XXIV.E. 7-bc	P19810567	Purwodadi, Pasuruan, East Java
9	Raja Kenanga	XXIV.E. 24-a	P19810568	Purwodadi, Pasuruan, East Java

No	Cultivar name	Vak location	Registration number	Locality
10	Raja Prentel	XXIV.E. 2-bc	P19940520	Nongkojajar, Pasuruan, East Java
11	Raja Pendek	XXIV.E.70	P2012030021	Pajarakan, Probolinggo, East Java
12	Raja Temen	At Nursery	P2012030017	Pasrepan, Pasuruan, East Java
13	Raja Talun	XXIV.D. 20b	P1972054	Purwodadi, Pasuruan, East Java

Morphological method

Taxonomic scoring for bananas method designed by Simmonds & Shepherd (1955) were conducted to identify the genomic group of Pisang Raja local cultivars. By using scoring method of fifteen characters, each of characters were scored depends on the diagnostic differences between M. acuminata and M. balbisiana wild species as their ancestral parents (Table 2). For each character in which the cultivar agreed with wild M. acuminata the score of 1 was given; for each character in which the cultivar agreed with M. balbisiana the score of 5 was given; and intermediate expressions of the character were assigned score of 2, 3 or 4 according to its intensity.

Identification of genomic group was then determined by its respective total score range. The scoring technique provides for a range of 15 (15 x 1) for wild M. acuminata and 75 (15 x 5) for wild M. balbisiana species. M. acuminata cultivars should have total scores of 15 to 25 while M. balbisiana cultivars ranged 70 to 75. The hybrid cultivars (M. acuminata x M. balbisiana) are expected to score between 26 to 69 points; it comprises of AAB (26-46), AB/AABB (47-49), ABB (59-63), and ABBB (67-69) (Simmonds & Shepherd, 1955; Silayoi & Chomchalow, 1987).

Molecular method

Molecular analysis was conducted at Laboratory of Plant Physiology, Tissue Culture & Microtechnique, Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia. Whole genome DNA from leaf tissues was isolated using Promega Wizard® Genomic DNA Purification Kit, Wisconsin, USA. Protocol of DNA isolation was conducted following its manufacturer's instructions for plants. Amplifications of ITS region were using primer pairs of ITSL (5'-TCG TAA CAA GGT AGG CGT TTC TG-3 ') as a forward primer and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') as a reverse primer (White et al., 1990; Hsiao et al., 1994; Nwakanma et al., 2003). The amplifications were performed at a volume of 15 µl consisting of 1,5 µl 25 ng DNA sample, 1,5 µl of 10 pmol forward and reverse primers, 1,5 µl of nuclease-free water and 7,5 µl DreamTaq Green PCR Master Mix (2x) from Thermo Scientific, California, USA. PCR thermal cycle protocol consisted of 1x initial denaturation (95 °C for 3 minutes); followed by 25x of denaturation (95 °C for 30 seconds), annealing (53 ºC for30 seconds), and extension (72 ºC for 30 seconds); and final extension (72 ºC for 7 minutes). Successful amplifications were confirmed by electrophoresis separation on 1,5% agarose gels, stained with 1 µg/ml of Ethidium bromide in TBE buffer, then visualised under UV light. DNA ladder Gene-ruler 100 bp (Thermo Scientific, California, USA) were used to estimate the sizes of amplified DNA fragments.

PCR products were then digested using RsaI restriction endonuclease enzyme (Thermo Scientific, California, USA). The digestion protocol consists of 10 µl PCR product, 18 µl nuclease-free water, 2 µl 10x Buffer Tango and 2 µl RsaI. Incubation was conducted at 37 °C water bath for overnight (± 12 to 16 hours). The digested DNA fragments were confirmed using electrophoresis separation on 2% agarose gels, stained with 1 µg/ml of Etbr in TBE buffer, then visualised under UV light. DNA ladder Gene-ruler 50 bp (Thermo Scientific, California, USA) were used to estimate the sizes of digested DNA fragments.

Table 2. Characters used in banana classification through taxonomic scorecard Simmonds & Shepherd (1955)

No.	Character	*Musa acuminata* (score 1)	*Musa balbisiana* (score 5)
1	Pseudostem color	More or less heavily marked with brown or black blotches	Blotches slight or absent
2	Petiolar canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin inclosed, not winged below, clasping pseudostem
3	Peduncle	Usually downy or hairy	Glabrous
4	Pedicels	Short	Long
5	Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
6	Bract shoulder	Usually high (ratio < 0.28)	Usually low (ratio > 0.30)
7	Bract curling	Bract reflex and roll back after opening	Bracts lift but do not roll
8	Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
9	Bract apex	Acute	Obtuse
10	Bract color	Red, dull purple or yellow outside; pink, dull purple or yellow inside	Distinctive brownish-purple outside; bright crimson inside
11	Color fading	Inside bract color fades to yellow towards the base	Inside bract color continuous to base
12	Bract scars	Prominent	Scarcely prominent
13	Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
14	Male flower color	Creamy white	Variably flushed with pink
15	Stigma color	Orange or rich yellow	Cream, pale yellow or pale pink

Phenetic and genetic relationship reconstruction

Clustering analyses were performed to reconstruct relationship pattern of Pisang Raja local cultivars both morphology and molecular. Prior to analysis, the scoring data of morphology characters (1-5) was transformed to 0-1 matrices. Whilst for molecular data, the band pattern of digested DNA fragments was quantified into numeric data score (absent=0; present=1). Clustering analyses both phenetic and genetic were conducted using Paleontological Statistics (PAST) software version 1.94b; with unweighted, paired group algorithm and Jaccard similarity measure (Real & Vargas, 1996).

RESULTS AND DISCUSSIONS

Morphological characteristics

Taxonomic scoring result showed that morphological characteristics of thirteen Pisang Raja local cultivars examined were varying. It comprises of three genomic groups, comprises of 8 AAB, 3 ABB, and 2 AAA (Table 3).

Pisang Raja local cultivars which identified as Musa AAB group including Raja Gintung, Raja Ketan, Raja Lingi, Raja Marto, Raja Nangka, Raja Talun, Raja Temen and Raja Warangan (Table 3). General characteristic of Musa AAB group is having intermediate characters, mostly scored 2-4 of their both ancestral parents, with two rows in each loculus of ovules. Key character of each cultivar are following: Pisang Raja Gintung has 1-5 seeds per fruit (Fig. 1-A) while Pisang Raja Ketan has persistent flower relicts on the fruit (Fig. 1-B). Pisang Raja Lingi has persistent neutral/male flowers and presence of withered bracts on the rachis (Fig. 1-C). Pisang Raja Marto has degenerating early or absent male axis (Fig. 1-D), Pisang Raja Nangka is included as plantain type (Valmayor et al., 2000); the fruit is starchy so that requires some cooking to become palatable (Fig. 1-E). Pisang Raja Talun has spiral bunch shape; all fruit is attached to a unique crown coiled around the stalk (Fig. 1-F). Pisang Raja Temen (temen=true) means the true Pisang Raja; it bears bunches with 6-10 hands with flesh creamy-orange and sweet taste (Fig. 1-G). It is popular cultivar mostly used for obligatory requirement in traditional rituals of Javanese culture (Hapsari et al. 2017). Pisang Raja Warangan has sharp curved and slightly ridged fruit shape (Fig. 1-H).

Pisang Raja Bandung, Raja Prentel and Raja Siem were identified as Musa ABB group (Table 3). General characteristic of Musa ABB group is close related to M. balbisiana, mostly scored 3-5, with four rows in each loculus of ovules and the fruits are mostly seeded 3-10 seeds per fruit. Key character of each cultivar are following: Pisang Raja Bandung has compact bunch and very waxy fruits (Fig. 2-A), Pisang Raja Sri has pseudostem height less than 2 m, small and slightly ridged fingers (Fig. 2-B), and Pisang Raja Siem has fruit with pointed shape and persistent style (Fig. 2-C).

Raja Kenanga and Raja Pendek were identified as Musa AAA group (Table 3). General characteristic of Musa AAA group is having characters close related to M. acuminata, mostly scored 1-2, with two rows in each loculus of ovules, curved fruit shape, sweet taste and aromatic fruits. Key character of each cultivar are following: Pisang Raja Kenanga has pseudostem height up to 3 m with green-yellow fruit at ripening (Fig. 2-D), whilst Pisang Raja Pendek has dwarf type plant appearance with yellow-green fruit at ripening (Fig. 2-E).

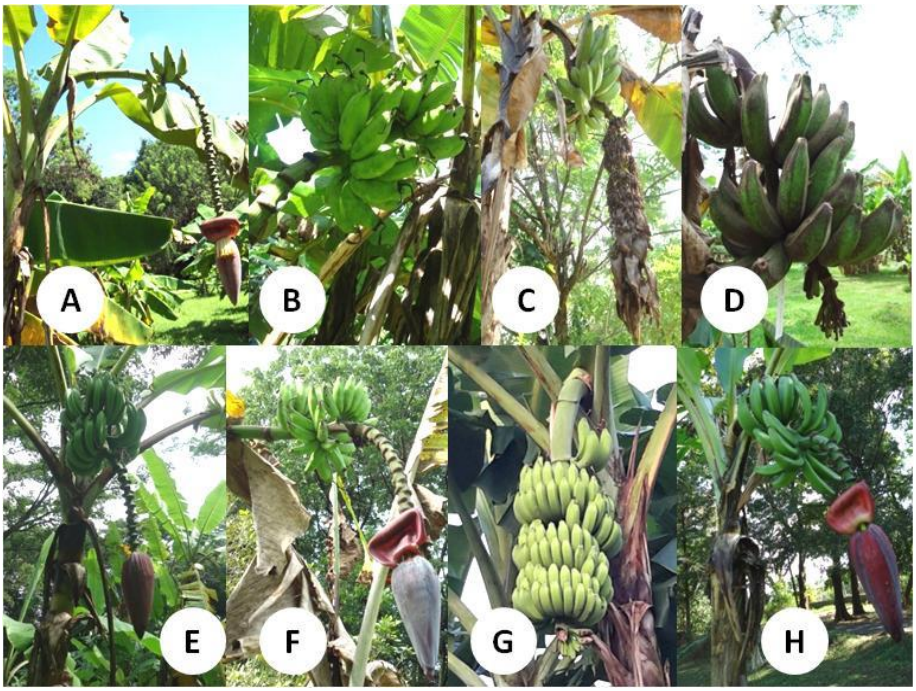


Figure 1. Plants and bunches appearance: A. Raja Gintung, B. Raja Ketan, C. Raja Lingi, D. Raja Marto, E. Raja Nangka, F. Raja Talun, G. Raja Temen and H. Raja Warangan

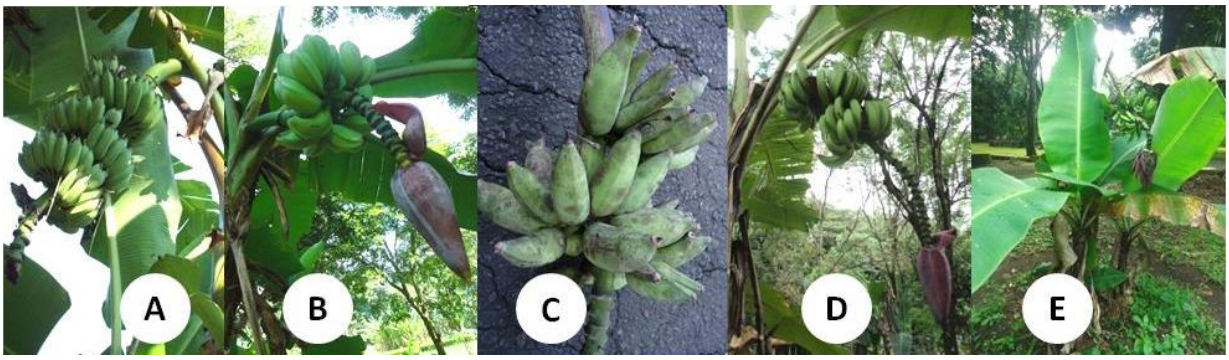


Figure 2. Plants and bunches appearance: A. Raja Bandung, B. Raja Prentel, C. Raja Siem, D. Raja Kenanga and E. Raja Pendek.

Table 3. Morphological characteristic scoring result of 13 Pisang Raja local cultivars

No	Character	Raja Bandung	Raja Prentel	Raja Siem	Raja Gintung	Raja Ketan	Raja Lingi	Raja Marto	Raja Nangka	Raja Talun	Raja Temen	Raja Waranga	Raja Kenanga	Raja Pendek
1	Pseudostem colour	4	4	4	3	1	2	2	1	2	2	2	1	1
2	Petiolar canal	5	4	5	2	4	4	3	2	3	3	2	2	2
3	Peduncle	4	4	2	3	3	2	3	2	3	3	2	1	1
4	Pedicel	5	4	5	4	2	3	3	2	3	4	3	1	1
5	Ovules	5	5	5	1	1	1	1	1	1	1	1	1	1
6	Bract shoulder	4	4	3	2	2	3	1	3	2	3	1	1	2
7	Bract curling	3	4	3	2	2	4	2	1	2	3	1	1	1
8	Bract shape	3	3	3	2	2	3	1	3	2	3	3	2	1
9	Bract apex	3	3	3	2	2	3	1	3	2	3	3	2	1
10	Bract colour	5	5	4	3	3	4	4	2	3	3	3	1	2
11	Bract colour fading	5	5	5	3	4	3	5	2	4	3	2	1	1
12	Bract scars	4	4	3	3	3	3	5	3	2	2	2	1	1
13	Free tepal of male flower	4	4	5	2	4	3	3	3	3	3	3	2	2
14	Male flower colour	5	3	5	3	2	2	3	3	3	3	3	2	1
15	Stigma colour	5	3	5	2	3	4	5	3	3	2	3	1	1
Total score		**64**	**59**	**60**	**37**	**38**	**44**	**42**	**34**	**38**	**41**	**34**	**20**	**19**
Genomic group		**ABB**	**ABB**	**ABB**	**AAB**	**AAB**	**AAB**	**AAB**	**AAB**	**AAB**	**AAB**	**AAB**	**AAA**	**AAA**

Molecular characteristic

Amplifications of ITS region to thirteen Pisang Raja local cultivars produced ±700 bp (Fig. 3-A) fragment that is equivalent in size to the ITS of most Angiosperms (Baldwin et al., 1995). The digestion of PCR products using RsaI showed 530 bp and 120 bp fragment unique to the A genome and two fragments of 350 bp and 180 bp that were specific to the B genome. The band expression is quantitative based on its genomes contribution, banana cultivars which possessed two sets of 'B' genomes showed stronger band intensity at 350 bp and 180 bp than those with single 'B' genome (Fig. 3-B). This study results are in accordance to some previous studies by (Nwakanma et al., 2003; Ekasari et al., 2012; Hapsari et al., 2015).

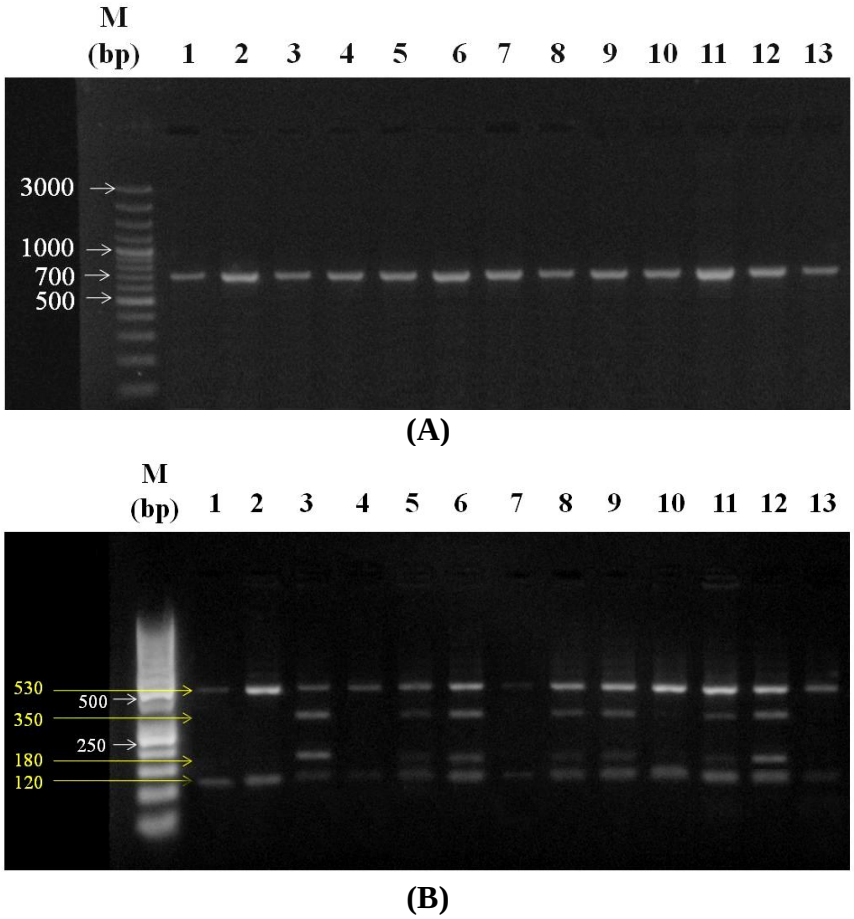


Figure 3. Visualization result on agarose gel: A. Amplification of ITS and B. Digestion of ITS PCR products using *Rsa*I. Remarks: 1. Raja Warangan, 2. Raja Kenanga, 3. Raja Prentel, 4. Raja Ketan, 5. Raja Marto, 6. Raja Siem, 7. Raja Pendek, 8. Raja Lingi, 9. Raja Temen, 10. Raja Nangka, 11. Raja Gintung, 12. Raja Bandung, and 13. Raja Talun.

Genomic group identification using PCR-RFLP of ITS to thirteen Pisang Raja local cultivars showed that it comprises of 3 ABB, 4 AAB, and 6 AAA. The finding of this study is about 4 out of 8 banana cultivars which identified as AAB morphologically were revealed as AAA molecularly i.e. Raja Ketan, Raja Nangka, Raja Talun, and Raja Warangan (Fig. 3-B). The final genomic group identification result were shown in Table 4.

Molecular approach using PCR-RFLP of ITS to identify genomic group of Pisang Raja local cultivars provide more objective result, consistent and reliable, also less time-consuming than morphology. PCR-RFLP method is simple and considerable less expensive with consistent compared to other molecular method. Nonetheles morphological description of bananas is still needed due to it is related with customer preference attributes of banana cultivars. Molecular method is needed to confirm the genomic constitution of banana cultivars for correct identification and classification

Table 4. Differences of genomic group identification result using morphplogy and molecular

No.	Cultivar name	Genomic group		Species name (final result)
		Morphology	PCR-RFLP ITS	
1	Raja Bandung	ABB	ABB	*M. acuminata* x *M. balbisiana* (ABB)
2	Raja Prentel	ABB	ABB	*M. acuminata* x *M. balbisiana* (ABB)
3	Raja Siem	ABB	ABB	*M. acuminata* x *M. balbisiana* (ABB)
4	Raja Gintung	AAB	AAB	*M. acuminata* x *M. balbisiana* (AAB)

No.	Cultivar name	Genomic group		Species name (final result)
		Morphology	PCR-RFLP ITS	
5	Raja Ketan	AAB	AAA	*M. acuminata* (AAA)
6	Raja Lingi	AAB	AAB	*M. acuminata* x *M. balbisiana* (AAB)
7	Raja Marto	AAB	AAB	*M. acuminata* x *M. balbisiana* (AAB)
8	Raja Nangka	AAB	AAA	*M. acuminata* (AAA)
9	Raja Talun	AAB	AAA	*M. acuminata* (AAA)
10	Raja Temen	AAB	AAB	*M. acuminata* x *M. balbisiana* (AAB)
11	Raja Warangan	AAB	AAA	*M. acuminata* (AAA)
12	Raja Kenanga	AAA	AAA	*M. acuminata* (AAA)
13	Raja Pendek	AAA	AAA	*M. acuminata* (AAA)

Phenetic and genetic relationship of 13 Pisang Raja local cultivars

Dendogram relationships based on morphology (phenetic) and molecular (genetic) of thirteen Pisang Raja local cultivars were clustered in three groups following its genomic group but in different pattern (Fig. 4). Phenetic relationship showed that AAA group were closer related to AAB group (similarity 12%) than to ABB group (similarity 8%). The morphological characteristics of AAB and AAA groups are quite similar so that become problems in taxonomic scoring, therefore their phenetic relationship were become sister group. Among AAB group, it was separated into two sub-groups i.e. sub-group I (Raja Marto, Raja Gintung, Raja Talun and Raja Ketan), and sub-group II (Raja Lingi, Raja Temen, Raja Nangka and Raja Warangan). Whilst, among ABB group, Raja Bandung were closer related to Raja Siem with similarity of 42% (Fig. 4-A). Meanwhile, genetic relationship showed that ABB and AAB groups were closer related (similarity 50%) than AAA group (similarity 34%). In addition, genetic relationship showed 100% similarity within group, due to the band patterns which are very specific to each genomic group (Fig. 4-B).

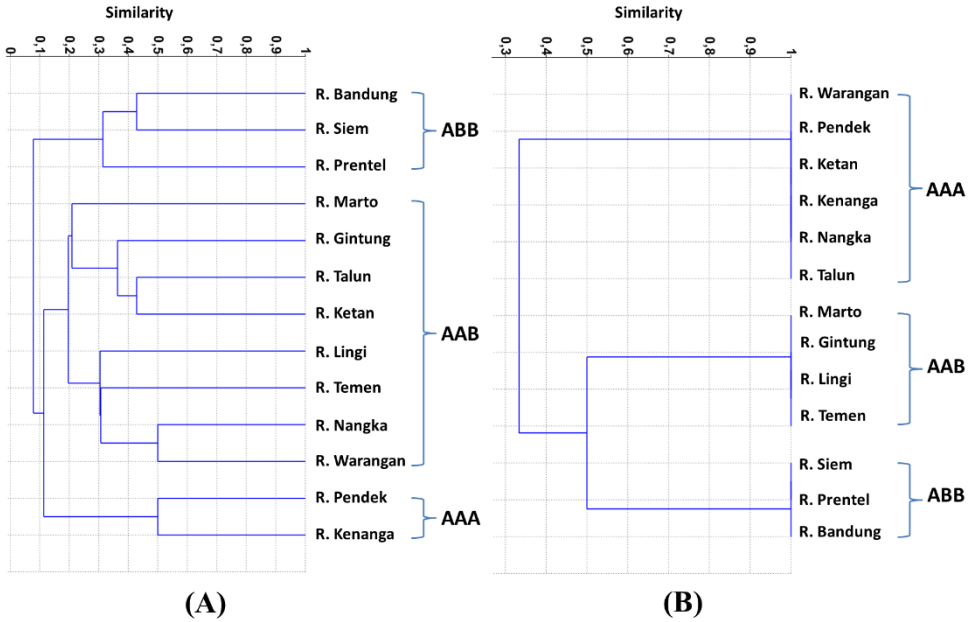


Figure 4. Dendogram phenetic (A) and genetic (B) relationship of thirteen Pisang Raja local cultivars

CONCLUSION

This study was clearly identified and classified the genomic group of thirteen Pisang Raja local cultivars. Genomic group result based on morphology was slightly different to molecular method, in

which about 4 out of 8 banana cultivars which identified as AAB morphologically were revealed as AAA molecularly i.e. Raja Ketan, Raja Nangka, Raja Talun, and Raja Warangan. Phenetic and genetic relationships were clustered in three groups following its genomic group. Dendogram of phenetic relationship showed that Musa AAA group was closer related to AAB group than to ABB group. Whilst, genetic relationship showed that Musa ABB were closer relatd to AAB group than AAA group.

Molecular approach using PCR-RFLP of ITS to identify genomic group of Pisang Raja local cultivars provide more objective, consistent and reliable result, also less time-consuming than morphology. However, both morphological description and molecular confirmation of genomic group of the rich local genetic variety of Indonesian banana cultivars is necessary to conduct as part of a longer-term systematic evaluation and selection basis for further utilization and development.

ACKNOWLEDGEMENTS

This study was part of DIPA thematic research project 2012-2015 of Purwodadi Botanic Garden – Indonesian Institute of Sciences entitled "Revitalization of the collection and diversity study of Musaceae in Purwodadi Botanic Garden". In addition, the molecular analysis was funded by Indonesia Endowment Fund for Education, Ministry of Finance, Republic Indonesia (LPDP RI) with Graduate Fellowship Grant number PRJ-541/LPDP/2013. Sincere thanks addressed to Ahmad Masrum (Field assistant) who assist authors during the morphological study at Musa field collections of Purwodadi Botanic Garden. And we thank to Didik Wahyudi for all the helps and valuable discussions during the molecular analysis at laboratory.

REFERENCES

Ayuningtyas PD. 2013. Ethnobotany study of bananas (*Musa* spp.) in Karanganyar, Purbalingga District. Thesis. Jenderal Soedirman University, Purwokerto. [Indonesian]

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on Angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

De Jesus ON, de Oliveira e Silva S, Amorim EP, Ferreira CF, de Campos JMS, de Gaspari Silva G, Figueira A. 2013. Genetic diversity and population structure of *Musa* accessions in ex-situ conservation. BMC Plant Biology 13:41. http://www.biomedcentral.com/1471-2229/13/41.

Ekasari TWD, Retnoningsih A, Widianti T. 2012. Analysis diversity of banana cultivars using marker PCR-RFLP of Internal Transcribed Spacer (ITS) DNA ribosom. *Jurnal MIPA* 35(1): 21–30. [Indonesian]

Espino RRC, Jamaludin SH, Silayoi B & Nasution RE. 1992. *Musa* L. (edible cultivars). *In*: Verheij EWM & Coronel RE. (eds.). *Plant Resources of South-East Asia No.2. Edible fruits and nuts*. Bogor. Indonesia: Prosea Foundation. Pp. 225–233.

Firdausi N, Hayati A, Rahayu T. 2015. Ethnobotany study and diversity of banana fruit (*Musa*ceae) in traditional society of Pandalungan, Krai Village, Yosowilangun, Lumajang District. *Biosaintropis* 1 (1): 26–34. [Indonesian]

Hapsari L, Wahyudi D, Azrianingsih R, Arumingtyas EL. 2015. Genome identification of bananas (*Musa* L.) from East Java Indonesia assessed with PCR-RFLP of the Internal Transcribed Spacers Nuclear Ribosomal DNA. *International Journal of Biosciences* 7 (3): 42–52.

Hapsari L. 2015. Genetic diversity and relationship of bananas (*Musa* L.) in East Java based on Internal Transcribed Spacer region DNA sequences. Thesis. Magister Program in Biology, Department of Biology, Faculty of Mathematic and Natural Sciences. Brawijaya University. [Indonesian]

Hapsari L & Lestari DA. 2016. Fruit characteristic and nutrient values of four Indonesian banana cultivars (*Musa* spp.) at different genomic groups. *Agrivita* 38(3):303–311.

Hapsari L, Kennedy J, Lestari DA, Masrum A, Lestarini W. 2017. Ethnobotanical survey of bananas (*Musa*ceae) in six districts of East Java, Indonesia. *Biodiversitas* 18: 160–174.

Heyne K. 1987. *Indonesia Useful Plants Volume I.* Jakarta. Indonesia: Yayasan Sarana Wana Jaya: 552–566. [Indonesian]

Hsiao C, Chatterton NJ, Asay KH, Jensen KB. 1994. Phylogenetic relationships of ten grass species: An assessment of phylogenetic utility of the Internal Transcribed Spacer region in the nuclear ribosomal DNA in monocots. *Genome* 37: 112–120.

Hřibová E, Ćížková J, Christelová P, Taudin S, de Langhe E, Doleźel J. 2011. The ITS1-5.8S-ITS2 sequence region in the *Musa*ceae: structure, diversity and use in molecular phylogeny. *PLoS ONE* 6(3): e17863. doi:10.1371/journal.pone.0017863.

Jumari, Pudjoarinto A. 2000. Phenetic relationship of banana cultivars in Java. *Biologi* 2(9): 531–542. [Indonesian]

LiL-F, Wang HY, Zhang C,.Wang X-F,.Shi F-X,.Chen W-N & Ge X-J. 2013. Origins and domestication of cultivated banana inferred from chloroplast and nuclear genes. *PLoS ONE 8*(11): e80502. doi:10.1371/journal.pone.0080502.

Lim TK. 2012. *Edible Medicinal and Non Medicinal Plants Volume 3, Fruits*. Pp 551-553. Springer Netherlands. DOI 10.1007/978-94-007-2534-8_72

Liu AZ, Kress JW & Li DZ. 2010. Phylogenetic analyses of the banana family (*Musa*ceae) based on nuclear ribosomal (ITS) and chloroplast (*trn*L-F) evidence. *Taxon* 59 (1): 20–28.

Lu Y, Zhang X, Pu J, Qi Y, Xie Y. 2011. Molecular assessment of genetic identity and genetic stability in banana cultivars (*Musa* spp.) from China using ISSR markers. *Australian Journal of Crop Sciences* 5(1): 25–31.

Nwakanma DC, Pillay M, Okoli BE, Tenkuano A. 2003. PCR-RFLP of the ribosom DNA Internal Transcribed Spacer (ITS) provide markers for the A and B genomes in *Musa* L. *Theor. Appl. Genet.* 108: 154–159.

Pillay M, Nwakanma DC, Tenkouano A. 2000. Identification of RAPD markers linked to A and B genome sequences in *Musa* L. *Genome* 43(5): 763–767.

Poerba YS, Ahmad F. 2010. Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers. *Biodiversitas* 11(3): 118-123. DOI: 10.13057/biodiv/d110303

Poerba YS, Ahmad F. 2013. Genetic variation analyses of *Musa* balbisiana Colla based on RAPD and ISSR markers. *Berita Biologi* 12(2): 259–267

Real R, Vargas JM. 1996. The probabilistic basis of Jaccard's index of similarity. *Systematic Biology* 45 (3): 380–385.

Retnoningsih A, Megia R, Hartana A. 2009. Microsatellite markers for classifying and analysing genetic relationship between banana cultivars in Indonesia. International Symposium on Banana: ISHS-ProMusa Symposium on Global Perspectives on Asian Challenges. Pp. 153-160.

Simmonds NW, Shepherd K. 1955. The taxonomy and origins of the cultivated banana. *Journal of Linnaeus Society (Botany)* 55: 302–312.

Simmonds NW. 1959. Bananas. New York, U.S.: Longman Inc Sukartini. 2008. Analisis jarak genetik dan kekerabatan aksesi-aksesi pisang berdasarkan primer Random Amplified Polymorphic DNA. *J. Hort.* 18(3): 261–266.

Valmayor RV, Jamaluddin SH, Silayoi B, Kusumo S, Danh LD, Pascua OC & Espino RRC. 2000. *Banana cultivar names and synonims in Southeast Asia*. Los Banos. Laguna. The Philippines: International Network for the Improvemnet of Banana and Plantain, Asia and the Pasific Office.

Wahyuningtyas W, Retnoningsih A, Rahayu ES. 2009. Genetic diversity of B genome bananas based on microsatellite markers. *Biosaintifika* 1 (1): 1–10. [Indonesian]

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* Innis MA, Gelfrand DH, Snisky JI, White TJ (Eds.). PCR protocols: A guide to methods and applications. Academic Press. New York, USA. Pp. 315–322.

Seed storage behavior of selected species from Gede Pangrango Mountain

[1]Irvan F. Wanda, [1]Dian Latifah, [2]Anggun R. Gumilang, [1]Mimin, [1]Harto, and [2]Cahyadi
[1]Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences (LIPI), Jl. Ir. H. Juanda No. 13 Bogor 16122, West Java, Indonesia. Tel./Fax. +62-251-8322187,
[2]Cibodas Botanic Gardens, Indonesian Institute of Sciences (LIPI), Sindanglaya Cipanas Cianjur 43253.
*email: ivan.wandassi@gmail.com.

ABSTRACT

It is known based on many previous researches that mountainous plants generally have recalcitrant seeds. This study was aimed to identify seed storage behaviour of several Gede Pangrango Mountain plants. The seed collection was conducted in Mount Gede Pangrango in July 2016 located in three sites which were Cibodas, Selabintana and Cimande. From exploration, there are 23 seed species and out of 23 seed species, only 14 species can be tested because of the limited number of seeds. The seed storage behaviour was assessed using a 100-seed test to investigate the seed viability after dessiccation. There are recalcitrant seeds on the mountain and some are orthodox seeds. The results show that from selected species, 50 % species has orthodox seeds and 50% species were recalcitrant. This finding is supported by a hypothesis that mosst of high-altitude seed species are recalcitrant. Hence, there is a suggestion that the seed conservation activities applying seed banking technology was recommended in mountainous regions of Indonesia such as Mount Gede Pangrango where excessive exploitation, climate change, volcano eruption and forest fires that often occur on several mountains in Java could be a threat towards plant extinction.

Keywords: Gede Pangrango Mountain, orthodox, recalcitrant, seed banking, seed storage behavior

INTRODUCTION

The diversity of plants in Indonesia, especially in Java, has decreased significantly at this time. Some of the contributing factors are various changes in pressure on plant populations in the forests to meet human needs and the increasing human population. In addition, other causes are plant breeding that can lead to genetic uniformity, land conversion causing habitat damage, climate change, invasion of exotic plants and unsustainable use of plant diversity. Facing these severe challenges, in situ conservation efforts of plants need to be integrated with ex situ conservation in order to race against time to prevent the extinction of plant species. Seed storage at seed bank facilities is one of the ex-situ conservation strategies for bioresources in the world's Botanical Gardens (Linington, 2003; Linington, 1997).

Gede Pangrango National Park (GPNP) in West Java, Indonesia has an area 15.000 hectares. GPNP covers three districts, namely Cianjur, Bogor and Sukabumi regencies, and is divided into three regional national park management Areas. To facilitate collection management, GPNP is divided into 22 smaller area conservation units called Resort (TNGP, 2015). The GPNP area is almost entirely a mountainous rainforest vegetation. GPNP topography varies from gently sloping to mountainous, with an altitude range between 1000-3000 m above sea level (asl). Van Steenis (1972) categorized the zones based on elevation and stated that mountainous zone is at the altitude of 1000-2000 m whereas subalpine is above 2400 m asl. The type of soil that dominates GPNP area is brown latosols, brown andosol and brown regosol associations, as well as gray and litosol regosol complex. The climate in the region GPNP is classified as Type A climate ranged between 11.30-33.30%, based on the classification of Schmidt and Fergusson with the air temperature ranged between 10°-18°C, relative humidity 80-90 % and the average annual rainfall of 3000-4200 mm (Arbiastutie *et al.*, 2017). Forest dominated by member of Fagaceae and Theaceae tree families. Mt. Gede Pangrango National Park is a core zone of Cibodas Biosphere Reserve with high valued conservation. There is lack of a comprehensive study on seed conservation in this area.

Based on a floristic and species composition research in Gede Pangrango National Park, there were three layers of floristics, whereas *Schima wallichii* and *Castanopsis javanica* were found on the

first layers (canopy). *Persea rimosa, Decaspermum fruticosum, C. javanica and Polyosma ilicifolia* were found in on the second layer (under canopy). *Saurauia pendula, Turpinia sphaerocarpa, Symplocos fasciculata*, and *Ficus ribes* were found on the third layer (Yamada, 1997). Some of those species in this area are known as endangered species (*C.argentea*), vulnerable (*Lithocarpus indutus)* and least concern (*C. Javanica* and *S. wallichii*) (WCMC, 1998; Barstow, 2018; Barstow *et al.*, 2018). These tree populations of Mt Gede Pangrango is threatened by invasive alien species, land alteration (houses and vegetable plantation), visitors (climber, hikers and other tourism activities). In addition, GPNP also a home for several endangered fauna such as Javan Gibbon (*Hylobates moloch*) and Javan Leaf Monkey (*Presbytis comata*), threated Leopard (*Panthera* pardus) and protected under Indonesian Law Pangolin (*Manis javanica*). This area also a home for endangered Javan Eagle (TNGP, 2015). Every destruction of tree population and environment will had negative impact for those animals. As an effort to conserve tree species of Mt Gede Pangrango National Park, seed conservation is one of the option. However, based on the Kew Seed Information Database, some species that found in GPNP are recalcitrant (*S. wallichii*) and some others are still in doubt (RBGK, 2017). In addition, there is not much information about the species of seeds from GPNP and their seed storage behaviour. The recalcitrance or seed storage behavior is important to be understood in an accordance with the seed conservation. Seed storage behavior is determined by the ability of seed to survive on minimum desiccation tolerance (Hong & Ellis, 1996). The seed storage behavior classifies the seeds into two categories: recalcitrant and orthodox. There is another category, intermediate, where rthe seeds that fall into this category are more tolerant to the desiccation than the recalcitrant seeds; but much less tolerant to the desiccation compared to the orthodox seeds and generally the intermediate seeds lose viability rapidly at low temperature (RBGK, 2017).

In order to identify the seed storage behavior of collected seeds of Mt. Gede Pangrango National Park, we have conducted a 100-seed test according to the Millennium Seed Bank Royal Botanic Gardens Kew procedures. The results of this test were important to understand the storage method that will be used for seed conservation in the Seed Bank of The Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences.

MATERIAL AND METHODS

Study site

The research on seed storage behavior of selected species from Gede Pangrango Mountain was carried out at the seed laboratory and the nursery greenhouse of The Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences in Bogor, Indonesia from July to Agust 2016.

Plant samples and sampling method

The fruit and seed collecting was carried out in Gede Pangrango National Park at three resorts, namely Cimande Resort, Selabintana Resort, and Cibodas Resort (Figure 1). These resorts were chosen as representative of two district (kabupaten) where GPNP located (Kabupaten Bogor and Cianjur) in the field. The seed collecting and exploration activity was based on plant collection by purposive random sampling. The sampling was started at the beginning of forest edge where species bearing mature fruit were found. The forest track was followed upward toward the altitude. The field activities consisted of fruit and seed collecting, identification, and making vouchers for herbariums.

Fruit and seed collection activity in the field was following MSB guidelines (RBGK, 2014). Seed collection methods were carried out by harvesting directly from fruit trees. The seeds selected with a total of more than 100 seeds were tested using 100-seed test followed the Millennium Seed Bank Royal Botanic Gardens Kew standard to investigate the seed viability after desiccation. Documentation was also carried out during exploration activities.

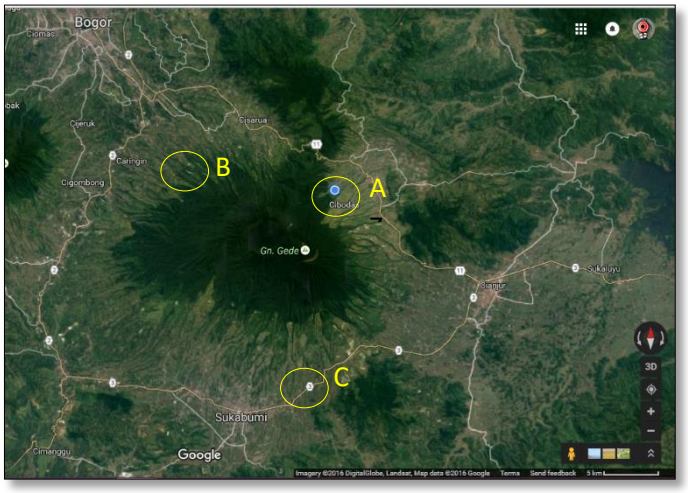


Figure 1. Location map of the seed collecting and exploration activity in Gede Pangrango National Park. A. Cibodas Resort, B. Cimande Resort and, C. Selabintana Resort.

Procedures

In order to identify the seed storage behavior of collected seed of Mt. Gede Pangrango National park, we had conducted a 100-seed test followed Millennium Seed Bank Royal Botanic Gardens Kew standard (Pritchard *et al.*, 2004; Gold and Hay, 2014). Firstly, the moisture content of 10 individual seeds was determined (ISTA, 2015). Then the initial germination tests were carried out at an optimum temperature for the study species using two seed lots/samples containing 13 seeds each. After that, the 32 seeds were dried by mixing them with an equal weight of silica gel in a suitable sealed container. At the same time, place 32 control seeds at high humidity in a sealed container, using moistened vermiculite to maintain humid conditions. Finally, both samples were held at 25^0C (Figure 2).

For the desiccated seed sample, the silica gel was changed and the seeds were weighed every 1-3 days, depending on the seed size. As the seeds dry and their weight decreases, the frequency of weighing can be reduced. When the seeds reach a constant weight at equilibrium, six seeds were taken out to determine the moisture content after desiccation. The germination tests were undertaken on two samples of 13 seeds from both the desiccated sample and the moist-stored control sample.

Figure 2. 100-seed test sample of Gede Pangrango National Park seed collection

Data analysis

The germination rates before and after desiccation in the 100-seed test supported by the initial seed moisture contents were used to determine the seed storage behaviour supported by the available information on the same taxa referred to Seed Compendium (Hong *et al.*, 1998) and Millenium Seed

Bank Kew's Seed Information Database (Liu *et al.*, 2008). The probability of the occurrence of species with orthodox and recalcitrant seeds was estimated by comparing descriptively the proportion of the recalcitrant and orthodox seeds using diagrams.

RESULTS AND DISCUSSION

There were twenty three (23) species seed collection from three locations within the Mt. Gede Pangrango National Park (GPNP) (Figure 3). In general, the species collected were species that bloom and have fruit throughout the year. Most types of targets were not successfully collected because this type of plant was not in the fruiting period when exploration was carried out. The Rubiaceae was the most collected family (5 species and 3 genus) followed by Arecaceae (4 species and 3 genus) (Figure 4). Of the 23 species of seeds, only 14 species can be tested for using 100-seed test because of the limited number of seeds. The number of seeds that do not reach 100 grains cannot be tested.

Figure 3. Some seeds collection from Gede Pangrango National Park: (1) *Calamus reindwardtii* Mart.; (2) *Pinanga javana* Blume; (3) *Cinchona succirubra* Pav. ex Klotzsch; (4) *Acronychia pedunculata* (L.) Miq.; (5) *Daemonorops* sp.; (6) *Lasianthus furcatus* (Miq.) Bremek.; (7) *Schima walichii* Choisy

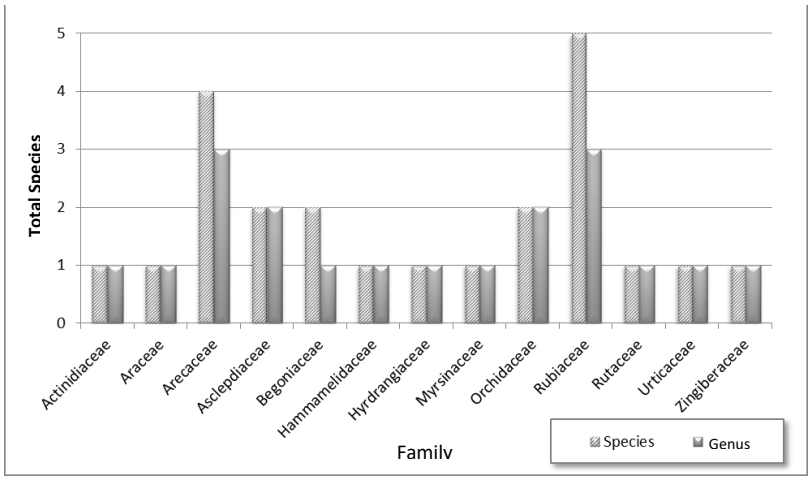


Figure 4. The number of species and genus of Gede Pangrango National Park seed collection in July 2016

The result showed that there were seven species of three surveyed locations at Gede Pangrango National Park (GPNP) classified as orthodox and other have recalcitrant character (Table 1). Plants that have an orthodox character ie *Pinanga javana* Blume, *P. coronata* (Blume ex Mart.) Blume,

Calamus reindwardtii Mart, *Vilebrunea integrifolia*, *Cinchona succirubra* Pav. ex Klotzsch, *Hedychium roxburghii* Blume dan *Strobilanthes cernua* Blume. While plants that have the character of recalcitrant is *Lasianthus rigidus* Miq., *L. furcatus* (Miq.) Bremek, *L. purpureus* Blume, *Daemonorops sp, Acronychia pedunculata* (L.) Miq dan *S. walichii* Choisy. Most orthodox seeds come from the family of Arecaceae and some of the Urticaceae and Zingiberaceae. Many tropical and subtropical tree species are known to produce recalcitrant seeds (Berjak and Pammenter, 2004), but there is still limited knowledge on the germination and storage behavior of such tree seeds, especially wild tree species. Recalcitrant seeds have high moisture content, estimated to be in the range of 30-70% at maturity and such seeds include rubber, cocoa, coconut, mango, and jack fruit (Chin, 1995). The study species with recalcitrant seeds (50% studied species: *L. rigidus* Miq., *L. furcatus* (Miq.) Bremek, *L. purpureus* Blume, *Daemonorops sp, A. pedunculata* (L.) Miq dan *S. walichii* Choisy) in Mt Gede Pangrango may also have varied seed moisture content ranging from 11,29-49,8% (Table 1). Many Rubiaceae and Rutaceae seeds are orthodox; but few can be recalcitrant or intermediate (Hong *et al.*, 1998). *L. rigidus* and *L. furcatus* (Rubiaceae) and *A. pedunculata* (Rutaceae) may exhibit intermediate character as the seed moisture content was low i.e. 11.97%, although it appears to be recalcitrant based on this research; however, it will require further investigation. *S. wallichii* shows recalcitrant character which may support Hong *et al.* (1998) who were doubt that this species is 'recalcitrant?'. Recalcitrant seeds germinate rapidly when sown fresh, but are sensitive to desiccation and freezing (Berjak and Pammenter, 2004; McDonald, 2004). This makes them difficult to store and some recalcitrant seeds perish at 26% seed moisture content when stored at 15°C temperature or below. This seed moisture content is promising for tropical rainforest species.

Table 1. Germination rate and seed storage behavior of collected species of Mt. Gede Pangrango National Park

No	Species	Family	Location	Initial Moisture Content (%)	Initial Germination Rate (%)	Germination Rate (after desiccation) (%)	Seed Storage Behaviour
1	*Pinanga javana*	Arecaceae	Selabintana	29.38	80.77	75.00	Orthodox
2	*Calamus reindwartii*	Arecaceae	Cimande	27.08	69.23	87.50	Orthodox
3	*Lasianthus rigidus*	Rubiaceae	Selabintana	12.05	15.38	6.25	Recalcitrant
4	*Daemonorops sp*	Arecaceae	Selabintana	49.8	0.00	3.13	Recalcitrant
5	*Villebrunea integrifolia*	Urticaceae	Selabintana	9.37	3.85	0.00	Orthodox*
6	*Acronychia pedunculata*	Rutaceae	Selabintana	11.97	0.00	0.00	Recalcitrant
7	*Pinanga coronata*	Arecaceae	Selabintana	29.17	92.31	78.13	Orthodox
8	*Schima wallichii*	Theaceae	Cimande	11.29	0.00	0.00	Recalcitrant*
9	*Lasianthus furcatus*	Rubiaceae	Selabintana	16.11	23.08	15.63	Recalcitrant
10	*Lasianthus furcatus*	Rubiaceae	Cibodas	48.41	3.85	18.75	Recalcitrant
11	*Cinchona sucirubra*	Rubiaceae	Cimande	47.37	0.00	0.00	Orthodox*
12	*Hedychium roxburghii*	Zingiberaceae	Cibodas	29.14	73.08	0.00	Orthodox*
13	*Strobilanthes cernua*	Acanthaceae	Cibodas	34.38	30.00	0.00	Orthodox*
14	*Lasianthus purpureus*	Rubiaceae	Cibodas	44.14	16.67	15.63	Recalcitrant

Note: *= seed behavior based on Hong *et al.* (1998)

Among all selected species studied, 50% were classified as orthodox seed, i.e *P. javana* Blume, *P. coronata* (Blume ex Mart.) Blume, *C. reindwardtii* Mart, *V. integrifolia*, *C. succirubra* Pav. ex Klotzsch, *H. roxburghii* Blume dan *S. cernua* Blume (Table 1). Orthodox seeds can be dehydrated to low moisture content without drastic effect on germination unlike recalcitrant seeds (McDonald, 2004). They are deliberately dried to very low moisture content to enhance storability and longevity. The true

orthodox seeds are known to withstand sub-freezing temperatures for long periods when dried to 10% moisture content (Bonner, 1990). Although classification this might be based on observations (without any seed drying tests), such seeds seem to clearly belong to the orthodox.

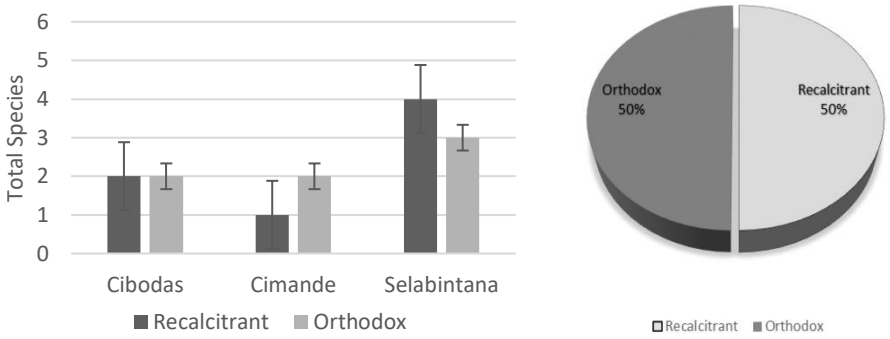


Figure 5. The proportion of recalcitrant and orthodox seeds of collected species at three sites of Mt. Gede Pangrango National Park (Cibodas, Cimande and Selabintana resort).

There are three study areas that is Selabintana, Cimande and forest of Cibodas.. Three orthodox seeds (3 species) were found in Selabintana, two species were from Cibodas and Cimande each (Table 1; Figure 5). In addition, the most recalcitrant seeds (4 species) were found in Selabintana, two species were found in Cibodas and one species was found in Cimande (Figure 5). In general, we found both seed storage behavior (recalcitrant and orthordox) but no intermediate behavior on these three sites.

Hong and Ellis (1996) stated that environment contribute to seed storage behaviour. Orthodox species found in the enviroment subjected to occasional or seasonal drought while recalcitran species usually found at moist ecosystem or high humidity. This may suggest that the probability of orthodox seeds collected from mountainous areas is quite large (approximately 50%) so that the seed conservation activities applying seed banking technology was recommended in mountainous regions of Indonesia such as Mt. Gede Pangrango where excessive exploitation, climate change, volcano eruption and forest fires that often occur on several mountains in Java could be a threat towards plant extinction. This National Park may cooperate with near-by Seed Bank which belongs to Cibodas Botanic Gardens ndonesian Institute of Sciences (LIPI).

CONCLUSION

Pinanga javana Blume, *P. coronata* (Blume ex Mart.) Blume, *Calamus reindwardtii* Mart, *Vilebrunea integrifolia*, *Cinchona succirubra* Pav. ex Klotzsch, *Hedychium roxburghii* Blume and *Strobilanthes cernua* Blume that have an orthodox character were found in Gede Pangrango National Park (GPNP). In addition, *Lasianthus rigidus* Miq., *L. furcatus* (Miq.) Bremek, *L. purpureus* Blume, *Daemonorops sp*, *Acronychia pedunculata* (L.) Miq dan *Schima walichii* Choisy which are recalcitrant seeds occurred here. The results show that 50% seeds tested were orthodox and 50% seeds were recalcitrant that come from GPNP. This may suggest that the probability of orthodox seeds collected from mountainous areas is quite large (approximately 50%) so that the seed conservation activities applying seed banking technology was recommended in mountainous regions of Indonesia such as Mount Gede Pangrango where excessive exploitation, climate change, volcano eruption and forest fires that often occur on several mountains in Java could be a threat towards plant extinction. This National Park may cooperate with Cibodas Botanic Gardens-Indonesian Institute of Sciences (LIPI) Seed Bank.

ACKNOWLEDGMENTS

This research was funded by DIPA/Indonesian Goverment Funding Scheme Year 2016-2017: (Thematic/Core Competence Scheme: Seed Conservation of Rare, Orchid and Potential Plants in Indonesian Seed Banks). Many thanks to Dr. Siti Roosita Ariati for giving permission to use the Nursery Unit of Registration and Nursery Department, Center for Plant Conservation, Botanic Gardens - LIPI. We also grateful for generous assistance from all team members of LIPI Botanic Gardens Seed Bank: Aulia Hasan Widjaya, S.Si, Muhammad Asep Lukmanul Hakim, Ahati, Sukron Ma'mun and Usman.

REFERENCES

Arbiastutie, A., D. Marsono, M.S. Hartati dan R. Purwanto. 2017. The potential of understorey plants from Gunung Gede Pangrango National Park (West Java, Indonesia) as servixs anticancer agents. *Biodiversitas* 18 (1): 109-115

Barstow, M. 2018. *Castanopsis javanica.The IUCN Red List of Threatened Species* 2018: e.T62004572A62004577. http://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T62004572A62004577.en. (accesed 26 October 2018).

Barstow, M and Kartawinata, K. 2018. *Castanopsis argentea. The IUCN Red List of threathened Species* 2018: e.T62004506A62004510. http://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T62004506A62004510.en. (accesed 26 October 2018).

Berjak, P., And N.W Pammenter. 2004. Biotechnological aspects of non-orthodox seeds: An African perspective. *South African Journal of Botany* 70(1): 102-108

Bonner, F.T. 1990. Storage of seeds: Potential and limitations for germplasm conservation. *Forest Ecology and Management* 35, 35-43

Chin, H.F. 1995. Storage of recalcitrant seeds. In: Basra, A.S. (Ed). *Seed Quality Basic Mechanisms and Agriculture Implications*. The Haworth Press, Inc. NY. USA. pp 209-222

Gold, K. and F. Hay. 2014. Identifying desiccation-sensitive seeds. *Technical Information Sheet of Millenium Seed bank Partnership of Royal Botanic Gardens Kew* 10: 1-4.

Hong, T. & R. Ellis. 1996. *A Protocol to Determine Seed Storage Behaviour*. Rome: IPGRI.

Hong, T.D., S. Linington and R.H. Ellis. 1998. *Compendium of Information on Seed Storage Behaviour*. The Trustees, Royal Botanic Gardens Kew, UK.

International Seed Testing Association (ISTA). 2015. *International Rules for Seed Testing*. International Seed Testing Association. Bassersdorf, Switzerland.

Linington, S. 1997. The Millennium Seed Bank Project. *Botanic Gardens Conservation News* 2(9): 34-35.

Linington, S. 2003. The Design of Seed Banks. In: R. Smith, J. Dickie, S. Linington, H. Pritchard, & R. Probert (Eds). *Seed Conservation-Turning Science into Practice*. Surrey: Royal Botanic Gardens, Kew. pp 165-177

Liu, K., R.J. Eastwood, S. Flynn, R.M. Turner, and W.H. Stuppy. 2008. *Seed Information Database* (release 7.1, May 2008) https://www.kew.org/data/sid. (accessed 1 May 2017).

McDonald, M.B. 2004. Orthodox seed deterioration and its repair. In: Benech- Arnold RL, Sánchez RA (Eds). *Handbook of Seed Physiology, Application to Agriculture*. Haworth Press, Inc. NY. USA. pp 273-298.

Royal Botanic Gardens Kew (RBGK). 2014. *Millennium Seed Bank Reources*. https://www.kew.org/science/collections/seed-collection/millennium-seed-bank-resources. Retrieved on 26 October 2018.

Royal Botanic Gardens Kew (RBGK). 2017. Seed Information Database: http://data.kew.org/sid/sidsearch.html (accesed 30 April 2017)

Taman Nasional Gunung Gede Pangrango (TNGP). 2015. *Tentang Taman Nasional Gunung Gede Pangrango (TNGP)*. https://www.gedepangrango.org/tentang-tnggp/ (accesed 11 October 2016).

Taman Nasional Gunung Gede Pangrango (TNGP). 2015. *Hewan*. https://www.gedepangrango.org/tentang-tnggp/hewan/ (accesed 26 October 2018)

Van Steenis, CGGJ. 1972. Flora Pegunungan Jawa. Pusat Penelitian Biologi LIPI. Bogor.

World Conservation Monitoring Center (WCMC). 1998. *Lithocarpus indutus*. The IUCN Red List of Threatened Species: e T31990A9668174. https://dx.doi.org/10.2305?IUCN.UK.1998. RLTS.T31990A9668174.en. (accesed 26 October 2018)

Yamada, I. 1997. *Monographs of The Center for South East Asian Studies. Tropical Rain Forest of Southeast Asian: A Forest Ecologist's View*. (P. Hawkes, Trans.) Honolulu: University of Hawa'i Press.

Effect of Seed Soaking in Water on the Seedling Viability and Vigour of Indonesian Local Yam Beans/Bengkuang (*Pachyrhizus erosus* L. Urb.)

[1]M. Muchlish Adie and [1]Ayda Krisnawati
[1]Indonesian Legumes and Tuber Crop Research Institute (ILETRI)
Jl. Raya Kendalpayak Km 8, PO Box 66 Malang 65101, East Java, Indonesia
Tel.: +62-341-801468, 801075, Fax.: +62-341-801496
*Email: mm_adie@yahoo.com

ABSTRACT

Root tuber of bengkuang or yam bean (*Pachirhizus erosus* L. Urb.) has high economic value in Indonesia. The seed supply is become the problem within yam bean development and related to plant population in the field. A total of five local yam bean originated from Bogor, Malang, Gorontalo, Kediri and Kepahiang (Bengkulu) was evaluated for their viability and vigour through seed soaking treatment. The field experiment was conducted in Malang from May – October 2016. The experiment was arranged in completely randomized factorial design, consists of soaking treatment as first factor and five local varieties as second factor. A total of 20 seeds was germinated with three replicates. The soaking treatment using distilled water for 0, 2, 4, 6, 8, and 10 hours, respectively. The average maximum growth potential of five local yam beans ranged from 87.33 – 94.33%, revealed a higher of the seed viability. A significant interaction of soaking duration and local variety was showed on characters of dry seedling weight. The seed soaking for 4 hours gave the highest speed of germination (4%/day) compared to those of without soaking as well as another soaking treatment. The without soaking treatment gave the highest maximum growth potential (94.33%) at 10 days after sowing. This fact shows that the seed soaking only accelerate the initial germination, especially on the seedling growth until 4 days. If the environmental factor, especially soil moisture is not a constraint, hence, the seed soaking is not necessary to obtain optimal plant population in the field.

Keywords: seed soaking, viability, vigour, yam bean

INTRODUCTION

In Indonesia, yam bean (bengkuang) has a high economic value and was generally planted once a year at the beginning of rainy season. Until now, there has not been certified seed growers that produces yam bean seeds, so the source of seed used by farmers generally comes from self-cultivation or uncertified seed traders.

The crop population becomes the determinant of crop productivity per unit area. Optimum plant population can be achieved if the seeds used have high vigor levels. Seed germination starts from the process of water absorption from the environment around the seed. Water is one of important factors of seed germination. The rate of water absorption by seed is determined by the level of seed permeability, especially the seed coat and the amount of water available in the seed environment. Seed imbibition is the first step in the process of biochemistry required for the process of germination (Bewly & Black, 1994; Asiedu *et al*., 2000). Efforts to accelerate seed germination can be performed in various ways such as hydropriming, halopriming, thermopriming, solid matrix priming, and biopriming (Ashraf & Foolad, 2005). Hydropriming is a technique of accelerating germination by soaking the seeds in aqueous solution. Various research results have revealed that the hydropriming treatment was considered quite practical and simple to increase the percentage and speed of germination, and also assessed to improve uniformity of sprout growth in stress conditions (Clark *et al*., 2001; Mavi *et al*., 2006; Berchie *et al*., 2010). Other research results (Dursun & Ekinci, 2010; Nawaz *et al*., 2013; Girolamo & Barbanti, 2012; Manonmani *et al*., 2014) also reported that priming in pre-sowing has an influence on the simultaneity of sprouts in a stress environment such as low and high temperature, salinity and matric stress.

Characteristics of yam bean seeds is coated by a layer of quite thick seed coat (testa). Seed coat has various functions i.e. as protection against the environment, especially temperature and humidity

during storage, and also acts as a protectant against microorganism (Duke & Kakefuda, 1981). The role of seed skin during germination is to protect against hydration stress and electrolyte leakage. In rice seeds, it was reported that soaking seed treatment in ascorbic or salicylic acid improved seed germination, seed and seedlings vigor under salinity levels comparing with the other antioxidants and distilled water (Polthanee & Tre-loges, 2002). Song (2011) found that hydropriming on *pansy* seeds (*Viola wittrockiana*) negatively affected the speed of germination, the percentage of germinating seeds and germination capacity. In corn and chickpeas, it was reported that the optimum duration of soaking seeds at 31, 25 and 15°C of soaking temperatures could be 6, 9 and 18 h respectively (Rahman *et al*., 2011). Differences in the duration of seed-soaking treatments to stimulate seed viability and vigor from the various studies above shows that each commodity and genotype has different response to the seed soaking treatment. Research on soaking seeds in the seeds of yam bean in Indonesia has not been reported. Farmers use seeds originating from crops of a year earlier and stored in the form of pods. Planting was perfomed by *tugal* planting system without seed treatment. The objective of this research was to determine the duration of soaking seeds in water from various local varieties of yam bean in Indonesia.

MATERIALS AND METHODS

Field research

The preparation of research material was done in Kendalpayak Research Station (Malang, East Java, Indonesia) from May to October 2016. Local varieties of yam bean used were from Bogor (West Java), Malang (East Java), Gorontalo (Sulawesi), Kediri (East Java), and Kepahiang (Bengkulu). Land used was lowland. Soil processing was done optimally. The planting media was 6 m long of ridge and the distance between the ridge was 0.5 m. The planting was using *tugal* and planted 1 seed/hole and covered with manure. Fertilization with a dose of 250 kg Phonska/ha and 100 kg SP 36 was given entirely at the time of planting. Plant maintenance in the form of weeding, irrigation, and control of pests and diseases were conducted optimally. Pods harvesting were done after the pods turned to brown/black and then dried by using sunlight. The seed processing was done after the pod has dried. Seeding was done manually by hand.

Seed vigor and viability testing

The seed viability and vigor test were carried out using sand medium, conducted in Indonesian Legume and Tuber Crops Research Institutes' greenhouse in November 2016. The sand used was sterilized using heat. The study was arranged in a completely randomized factorial design with three replications. The first factor was the duration of soaking in water consisting of 0, 2, 4, 6, 8, and 10 hours. The second factor was five local varieties of yam beans. The soaking of yam bean seeds was done by soaking the seeds using destilled water. Each treatment consisted of 20 yam bean seeds, and planted on sand medium with 5 cm distance between rows, 2 cm in a row, and 2 cm seed depth. Sand media was kept moist by spraying water as needed. Observations include normal and abnormal seed germination from the fifth day to the tenth day. On the tenth day observations were performed for all normal sprout germination including stem length, root length, stem dry weight, and root dry weight.

RESULT AND DISCUSSION

Yam bean was one of the legume root crops, and has profitable economic value. The yam bean was exclusively used for its tuberous roots (Sorensen, 1996), and nowadays the root and tubers of yam bean were second in importance for human nutrition after cereals. In Indonesia, yam bean is mostly cultivated in early of the rainy season, which was the same is done in yam bean production centers in Africa namely Ghana and Nigeria (Kiu *et al*., 2001).

Important issues in the cultivation of yam bean is the population of plants that are less than optimal as a result of the use of low seed quality because it was obtained from the harvest a year earlier, environmental stress, and the character of yam bean seeds which is coated by a relatively thick and hard seed coat. The same problem was also found in some countries of yam bean production

centers, i.e. the problems of simultaneity and uniformity of seed growth in the field (Qiu *et al*., 1995). Olisa *et al*., (2010) obtained that the average of germination percentage of yam bean seed in the field was only 79.56%.

Seed viability is critical phases in the life of a plant when they are the most vulnerable to injury, disease, and water stress (Raven *et al*., 2005). The vigour of seeds can be improved by techniques generally known as seed priming, which enhances the speed and uniformity of germination (Demir & Van de Venter, 1999). Seed priming is a controlled hydration process followed by re-drying that allows seeds to imbibe water and begin internal biological processes necessary for germination, but which does not allow the seed to actually germinate.

Maximum growth potential

The maximum growth potential was not affected by the interaction between duration of soaking with local varieties or single effects of local varieties and soaking duration, but the maximum growth potential was significantly influenced by the length of the soaking seed treatment (Table 1).

The highest maximum growth potential (94.33%) was obtained in the treatment without seed soaking. The soaking treatment for 2 to 6 hours gives a comparable maximum growth potential that was between 89.33 - 89.67%. Treatment of seed soaking between 8 - 10 hours showed a lower maximum growth potential. Based on this fact, in order to obtain maximum growth potential from the yam bean seeds, the treatment of seed soaking was not required.

Among the five local varieties tested, the without soaking treatment of varieties originated from Kepahiang had the highest maximum growth potential (98%) and the lowest was local variety from Bogor (85%).

Table 1. Maximal growth potential (%) of five local variety of yam beans on various seed soaking treatment

Local variety	soaking duration (hours)						
	0	**2**	**4**	**6**	**8**	**10**	**Average**
Bogor	85	85	75	75	72	83	80.00
Malang	97	93	95	88	92	88	93.00
Gorontalo	97	92	87	95	92	90	91.33
Kediri	95	90	93	90	87	85	90.00
Kepahiang	98	88	97	100	95	97	95.00
Average	94.33	89.67	89.33	89.67	87.33	88.67	89.83
Mean square :							
Seed soaking (S)				**			
Local variety (V)				ns			
S × V				ns			

** = significant at p=0.01, ns = not significant

Research on five local varieties originated from some regions in Indonesia showed that the seed soaking did not increase the maximum growth potential of the yam bean seeds. The highest maximum growth potential of 94.33% was obtained in the treatment without soaking (Table 1). In this study, the longer of seed soaking will decrease the maximum growth potential of yam bean seeds. Based on variable of the maximum growth potential, seed soaking in the water was not necessary for the yam bean seeds originated from local varieties of Indonesia.

The characteristic of yam bean seed is coated by a relatively thick and hard seed coat (testa). The seed coat plays an important role in the plant life cycle by controlling the development of the embryo and determining seed dormancy and germination (Moise *et al*., 2005), hence seed with thin seed coat will easily absorb water. Olisa *et al*., (2010) showed that scarification is not a practice to aid imbibition before germination in African Yam Bean (AYB) despite the hardness of the seed. Yam bean seed have hypogeal germination which occurs between the fourth and the seventh day after planting (Adewale, 2011).

Speed of germination

The speed germination was one of the benchmarks of the seed vigor growth. Seed soaking treatment significantly affected the speed germination of five local varieties of yam bean, but no significant effect was obtained between local varieties and the interaction between soaking treatment with local varieties (Table 2).

The highest speed germination was obtained in 4 hours of soaking treatment, i.e. 4.0%/day, which was not significantly different from the soaking for 6 hours. The lowest speed germination was obtained from the soaking treatment for 8 hours. The seeds from the local varieties of Malang, Kediri and Kepahiang have speed of germination above 4%/day on soaking treatment for 4 hours, while the speed germination of local varieties of Bogor and Gorontalo was only about 3.33%/day.

In the treatment without seed soaking, the seeds from the local varieties of Gorontalo provide the highest speed germination of 4.85%/day, even this was the highest in all soaking treatments. It appears that the speed germination parameters of seed in addition to being determined by environmental factors were also affected by seed genetic factors.

Table 2. Speed germination (%/day) of five local variety of yam beans on various seed soaking treatment

Local variety	soaking duration (hours)						
	0	**2**	**4**	**6**	**8**	**10**	**Average**
Bogor	3.33	2.12	3.33	3.03	1.82	2.73	2.73
Malang	3.64	3.33	4.24	3.33	4.24	3.94	3.79
Gorontalo	4.85	3.94	3.33	3.64	3.03	3.33	3.69
Kediri	3.64	3.94	4.55	3.03	3.33	3.64	3.69
Kepahiang	3.33	3.33	4.55	3.94	3.94	3.94	3.84
Average	3.76	3.33	4.00	3.39	3.27	3.52	3.55
Mean square :							
Seed soaking (S)				*			
Local variety (V)				ns			
S × V				ns			

** = significant at p=0.01, ns = not significant

Seed germination depends on both internal and external conditions. Seed priming treatments such as osmo-priming, hydro-priming, matrico-priming, hormonal-priming have been employed to accelerate germination, seedling growth and yield in most of the crops under normal and stress conditions (Basra *et al*., 2006). The pre-sowing treatments cause initiation of the early metabolic processes and the re-drying of seeds arrest, but do not reverse, the initial stages of germination so that on the availability of suitable conditions, the time taken to germinate is reduced (Hosseein *et al*., 2011). Seed priming is a controlled hydration process followed by re-drying that allows seeds to imbibe water and begin internal biological processes necessary for germination, but which does not allow the seed to actually germinate. Accelerating and homogenizing the germination process is a prerequisite for a good crop establishment, the efficient use of resources, and eventually to increase yields (Harris, 1996). The principle of priming is based on the fact that it is possible to hydrate seed in some ways at a moisture level sufficient to initiate the early events of germination but not sufficient to permit radical protrusion (Moradi & Younesi, 2009).

Stem and root length of yam bean sprouts

Root length was not affected by the interaction between soaking duration and local varieties of yam bean, as well as the single treatment effect of soaking duration and local varieties also had no significant effect on root length (Table 3). There was an indication that the longer the soaking treatment resulted in longer roots. Local varieties of Malang and Kepahiang have longer roots than the other three varieties of yam bean. The root of Bogor local varieties was the shortest (11.97 cm).

Table 3. Root length (cm) of five local variety of yam beans on various seed soaking treatment

Local variety	soaking duration (hours)						
	0	**2**	**4**	**6**	**8**	**10**	**Average**
Bogor	10.89	12.00	10.64	13.29	12.31	12.69	11.97
Malang	10.68	11.93	12.05	11.87	12.33	21.78	13.44
Gorontalo	10.70	11.79	12.85	12.80	13.08	12.70	12.32
Kediri	11.91	11.63	12.63	11.45	12.37	12.21	12.03
Kepahiang	10.79	13.57	14.03	15.73	12.73	13.80	13.44
Average	10.99	12.18	12.44	13.03	12.56	14.64	12.64
Mean square :							
Seed soaking (S)				ns			
Local variety (V)				ns			
S × V				ns			

ns = not significant

The character of the stem length was influenced by the difference in seed soaking treatment, but the effect of interaction between soaking duration and local varieties, as well as the effect of local varieties, was not significant to the stem length (Table 4). If we observed the phenomenon of the character of the root length and the stem length, it appears that both traits were opposite each other. The seeds soaking by using destilled water can increase the length of the roots with the longer the soaking time, on the contrary for the character of stem length was likely to be shorter.

The highest stem length was achieved in the treatment without soaking (7.0 cm) and the shortest was in the soaking treatment for 4 hours (6.42 cm). Based on the average stem length of five local varieties of yam bean, the local variety from Gorontalo has the longest stem (6.85 cm) and the shortest stem was the local variety from Bogor (6.47 cm).

Table 4. Stem length (cm) of five local variety of yam beans on various seed soaking treatment

Local variety	soaking duration (hours)						
	0	**2**	**4**	**6**	**8**	**10**	**Average**
Bogor	6.99	6.89	6.31	6.19	6.01	6.41	6.47
Malang	7.22	6.46	6.26	6.79	6.40	6.21	6.56
Gorontalo	6.89	7.27	6.83	6.86	6.40	6.85	6.85
Kediri	6.79	6.67	6.34	6.89	6.71	6.35	6.63
Kepahiang	7.12	6.68	6.37	6.73	6.71	6.64	6.71
Average	7.00	6.79	6.42	6.69	6.45	6.49	6.64
Mean square :							
Seed soaking (S)				*			
Local variety (V)				Ns			
S × V				Ns			

* = significant at p=0.05, ns = not significant

Stem and root dry weight of yam bean sprouts

The seed soaking of yam bean has no significant effect on the root dry weight character, and the character was significantly more affected by the different local varieties. There was also no significant interaction between soaking seeds duration with local varieties on root dry weight character (Table 5).

Local varieties from Bogor have the highest dry root weight (0.43 g) compared to the other four local varieties. Comparing with root length characters, it turns out that the Bogor local varieties have the shortest root (Table 3).

Table 5. Root dry weight (g) of five local variety of yam beans on various seed soaking treatment

Local variety	soaking duration (hours)						
	0	**2**	**4**	**6**	**8**	**10**	**Average**
Bogor	0.46	0.53	0.37	0.40	0.40	0.43	0.43
Malang	0.34	0.36	0.34	0.38	0.32	0.35	0.35
Gorontalo	0.39	0.40	0.36	0.41	0.33	0.39	0.38
Kediri	0.38	0.40	0.35	0.39	0.36	0.32	0.37
Kepahiang	0.41	0.36	0.38	0.39	0.38	0.38	0.38
Average	0.40	0.41	0.36	0.39	0.36	0.37	0.38
Mean square :							
Seed soaking (S)				ns			
Local variety (V)				*			
S × V				ns			

* = significant at p=0.05, ns = not significant

In this study, interaction between seed soaking with local varieties was only significant for the dry weight of the stem. Besides, there was also a significant effect of local varieties on the dry weight of the stem but the soaking treatment had no significant effect on the dry weight of the stem (Table 6).

The highest dry weight of stems varies between soaking treatments and varies between local varieties. The highest stem dry weight of Bogor local varieties was obtained at treatment without soaking (0.40 g), on local varieties of Malang obtained at the treatment for 6 hours (0.35 g), on local varieties of Gorontalo on soaking for 4 hours (0.37 g), on local varieties of Kediri on soaking for 2 hours (0.33 g) and the for local variety of Kepahiang was achieved on a soaking treatment for 10 hours (0.37 g). This shows that between local varieties have different genetic differences especially in the process of stem growth.

Table 6. Stem dry weight (g) of five local variety of yam beans on various seed soaking treatment

Local variety	soaking duration (hours)						
	0	**2**	**4**	**6**	**8**	**10**	**Average**
Bogor	0.40	0.39	0.33	0.32	0.32	0.39	0.36
Malang	0.33	0.31	0.30	0.35	0.34	0.33	0.33
Gorontalo	0.32	0.37	0.37	0.33	0.32	0.34	0.34
Kediri	0.32	0.33	0.29	0.33	0.33	0.33	0.32
Kepahiang	0.33	0.33	0.34	0.35	0.34	0.37	0.34
Average	0.34	0.35	0.33	0.34	0.33	0.35	0.34
Mean square :							
Seed soaking (S)				ns			
Local variety (V)				*			
S × V				*			

* = significant at p=0.05, ns = not significant

The effect of seed soaking treatment in water on seed germination and vigor was determined by the type of commodity as well as varied in accession in the commodity. The speed of germination was one of the benchmarks of seed vigor. The seed soaking for 4 hours gives the highest germination rate (4.0%/day), comparable with the germination rate (3.39%/day) which obtained by seed soaking for 6 hours. Seeds that have high vigor levels will provide uniformity growth of sprouts in the field. In this study, it was revealed that the longer seed soaking duration will result in longer root growth of sprouts but the development of stems will be shorter. The seed soaking has no significant effect on the dry weight of roots and stems. Interestingly, the significant interaction between soaking duration with local varieties was obtained only on the dry weight of the stem. Polthanee & Treloges (2002) reported the seed soaking on soybean seed before seeding had no significant effect on leaf area per plant, total top dry weight, root length density, pod number per plant, seed number per pod, 100-seed weight and seed yield of soybean. Soaking seed in ascorbic or alicylic acid improved seed germination, seed and seedlings vigor under salinity levels comparing with the other antioxidants and distilled water (Yousuf *et al*., 2010).

Accelerating and homogenizing the germination process is a prerequisite for a good crop establishment, the efficient use of resources, and eventually to increase yields (Harris, 1996). Seed priming is reported as an efficient method for increasing of seed vigour and improvement of germination and seedling growth (Ascherman-Koch *et al*., 1992; Jumsoon *et al*.. 1996). In cowpea, the seeds of cowpea could be primed (both hydro and osmopriming) for increased performance (Singh *et al.*, 2014).

Research conducted by Polhaupessy (2014) found that soaking by using GA on soursop seeds had a significant effect on the percentage of germination, length of germination and root length. Based on this research of soaking on yam bean seeds, if the environment for growing seeds were in optimal condition then seed soaking was not necessary.

CONCLUSION

The seed soaking treatment on local variety of yam bean originated from Indonesia did not increase the maximal growth potential. The seed soaking for 4 hours was able to increase the speed germination of local varieties of yam bean. In the optimal growth environment, the yam bean seed can be directly planting without seed treatment process prior sowing.

REFERENCES

Adewale, B. D. 2011. Genetic Diversity, Stability and Reproductive Biology of African yam bean, *Sphenostyli stenocarpa* (Hochst. ex A. Rich.) Harms. PhD Dissertation. Departement of Agriculture, University of Abeokuta, Nigeria.

Ascherman-Koch, C., P. Hofmann, A.M. Steiner. 1992. Presowing treatment for improving quality in cereals. I. Germination and vigour. *Seed Science and Technology* 20: 435-440.

Ashraf M. and M.R. Foolad. 2005. Pre-sowing seed treatment – A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Advances in Agronomy* 88: 223–271.

Asiedu, E.A., A.A. Powell and T. Stuchbury. 2000. Cowpea seed coat chemical analysis in relation to storage seed quality. *African Crop Science Journal* 8:283-294.

Basra, S. M. A., M. Farooq, R. Tabassum and N. Ahmed. 2006. Evaluation of seed vigour enhancement techniques on physiological and biochemical basis in coarse rice (*Oryza sativa* L.). *Seed Science and Technology* 34: 719-728.

Berchie, J.N., H. Adu-Dappah, J. Sarkodie-Addo, E. Asare, A. Agyemang, S. Addy and J. Donkoh. 2010. Effect of seed priming on seedling emergence and establishment of four bambara groundnut landraces. *Journal of Agronomy* 9:180-183.

Bewley, J.D. and M. Black. 1994. *Seed: Physiology of development and germination*. 2nd Ed. Plenum Press. London.

Clark, L.J., W.E. Whalley, J. Ellis-Jones, K. Dent and H.R. Rowse. 2001. On farm seed priming in maize: A physiological evaluation. Proceedings of the 7th Eastern and South Africa Regional Maize Conference. Feb 11-15. Kenya. pp.268-273.

Demir I. and H.A. Van de Venter. 1999. The effect of priming treatments on the performance of watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) seeds under temperature and osmotic stress. *Seed Science and Technology* 27: 871–875.

Duke, S.H. and G. Kakefuda. 1981. Role of the testa in preventing cellular rupture during imbibition of legume seeds. *Plant Physiology* 67: 449-456.

Dursun A. and M. Ekinci. 2010. Effects of different priming treatments and priming durations on germination percentage of parsley (*Petroselinum crispum* L.) seeds. *Agricultural Sciences* 1(1): 17–23.

Girolamo G. and L. Barbanti. 2012. Treatment conditions and biochemical processes influencing seed priming effectiveness. *Italian Journal of Agronomy* 7(2): 178–188.

Harris, D. 1996. The effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi arid Botswana. *Soil and Tillage Research* 40: 73-88.

Hosseein, A., Farahani and M. Karsa. 2011. Effect of hydro-priming on seedling vigour in Basil (*Ocimum basilicum* L.) under salinity conditions. *Advances in Environmental Biology* 5(5): 828-833.

Jumsoon, K., C. Jeuonlai, and J. Ywonok. 1996. Effect of seed priming on the germinability of tomato *(Lycopersicum esculentum* Mill.) seeds under water and saline stress. *Journal of the Korean Society for Horticultural Science* 37: 516-521

Kiu G.Y.P., H.M. Amoatey, D. Bansar and F.K. Kumaga. 2001. Cultivation and use of African Yam Bean in the Volta of Ghana. *Journal of Food Technology in Africa* 6:74-77.

Manonmani V., M. A. J. Begum, and M. Jayanthi. 2014. Halo priming of seeds. *Research Journal of Seed Science* 7: 1–13.

Mavi, K., S. Ermis and I. Demir. 2006. The effect of priming on tomato rootstock seeds in relation to seedling growth. *Asian Journal of Plant Sciences* 5:940-947.

Moise, J.A., S. Han, L. Gudynaite-Savitch, D.A. Johnson, and B.L.A. Miki. 2005. Seed coats: structure, development, composition, and biotechnology. *In Vitro Cellular and Developmental Biology - Plant* 41: 620–644.

Moradi, A. and O. Younesi. 2009. Effects of osmo- and hydro-priming on seed parameters of grain sorghum (*Sorghum bicolor* L.). *Australian Journal of Basic and Applied Sciences* 3(3): 1696-1700.

Nawaz J., M. Hussain, A. Jabbar, G.A. Nadeem, M. Sajid, M. Subtain, and I. Shabbir. 2013. Seed priming a technique. *International Journal of Agricultural and Crop Sciences* 6(20): 1373–1381.

Olisa, B.S., S.A. Ajayi and S.R. Akande. 2010. Imbibition and response of pigeon pea and African yam bean seeds to scarification. *Research Journal of Seed Science* 3: 150-159.

Polhaupessy, S. 2014. The effect of GA concentration with soaking time towards seedling germination of soursop. *Biopendix* 1(1): 71-76.

Polthanee, A. and V. Tre-loges. 2002. Effects of soaking seed and fertilizer placement on growth and yield of soybean grown after rice in the post-monsoon season in Khon Kaen Province. *Songklanakarin Journal of Science and Technology* 24:197-207.

Qiu, J., J.A. Mosjidis and J.C. Williams. 1995. Variability for temperature of germination in *Sericea lespedeza* germplasm. *Crop Science* 35: 237-241.

Rahman, M.M., K.U. Ahammad and M.M. Alam. 2011. Effect of soaking condition and temperature on imbibition rate of maize and chickpea seeds. *Research Journal of Seed Science* DOI:10.3928/rjss.2011.

Raven, P. H., F. E. Ray, and E.E. Susan. 2005. *Biology of Plants*, 7th Edition. New York: W.H. Freeman and Company Publishers. pp. 504–508.

Singh, A., R. Dahiru, M. Musa, and B.S. Haliru. 2014. Effect of Osmopriming Duration on Germination, Emergence, and Early Growth of Cowpea (*Vigna unguiculata* (L.) Walp.) in the Sudan Savanna of Nigeria. *International Journal of Agronomy.* https://doi.org/10.1155/2014/841238

Song, Z.Y. 2011. Effects of pre-sowing treatments on germination, vigour and health of pansy (*Viola* × *wittrockiana* Gams.) seeds. Master Thesis. Department of Seed Science and Technology, Poznań University of Life Sciences.

Sorensen, M. 1996. *Yam Bean Pachyrhizus DC*. International Plant Genetic Resources Institute. Rome, Italy.

Yousof, F. I. ; I. F. Mersal and A. A. M. EL-Emam. 2010. Effect of soaking rice (*Oryza sativa*, L.) seed in some antioxidants solutions on germination and seedling vigor under different salinity levels. *Journal of Plant Production* 1: 279 – 290.

Inflorescence Pruning of African Arrowroot (*Tacca Leontopetaloides*): Efforts to Improve the Quality of Tuber

Peni Lestari, Albert Husein Wawo[*)] and Ninik Setyowati
Botani Building, Research Center for Biology, Indonesian Institute of Science.
Jakarta-Bogor Street KM 46. Cibinong. Zip code 16911. Telp. 0251 87907572
Mailing Address: wawoal@yahoo.com [*)]

ABSTRACT

Since 2011, the Research Center for Biology has made attempts to raise awareness in the community of the potential of african arrowroot (*Tacca leontopetaloides*), especially for those living in coastal areas and small islands. African arrowroot tubers contain about 89.4% carbohydrates. It also contains chemical compounds that are efficacious for treatment of some diseases. In addition to the nutritional potential, african arrowroot plants are tolerant to dry conditions. Therefore, the plants can be made emergency food for people in small islands and coastal areas. One of the challenges in this crop cultivation is increasing the tuber crops. Herein, research to improve productivity of african arrowroot was done through cultivation. The experimental design was constructed using RCBD non-factorial design with 3 levels of treatment: flower pruning (topping), i.e. without topping; topping; and combining topping with organic fertilization. The organic fertilizer dose given was 1 kg/hole. Each treatment was replicated 3 times. The results showed that topping treatment could increase the production of african arrowroot tubers. The treatment combined with fertilization resulted in better growth and production. African arrowroot plant can be a superior commodity in coastal areas and small islands.

Keywords: Fertilization, Productivity, Pruning**,** *Tacca leontopetaloides*, Tuber.

INTRODUCTION

Carbohydrates are a source of nutrients that the body needs in large quantities, in addition to protein and fat (Perdana & Hardinsyah, 2013). Carbohydrates serve as a source of energy to perform daily activities. We can get carbohydrates not only from rice, but also from cereals, tubers, sugar, honey, and dried fruit (Hardinsyah *et al*, n.d.). The more varied sources of carbohydrates are consumed, the more beneficial for the body. Each food contains different nutrients at different portions. Thus, eating a variety of foods will provide more complete nutrition to the body.

Tacca leontopetaloides is one of the plants that can be used as a carbohydrate source. This species has some general names, including african arrowroot (Fitter & Hay, 1981), arrowroot, east indian arrowroot, and indian arrowroot (Burkill, 1985; Contu, 2013). Every 100 grams of african arrowroot tuber flour contains 89.4% carbohydrates. This value is greater than the carbohydrate content of brown rice (Mulyani & Sukesi, 2011). In addition, african arrowroot tuber contains 5.1% proteins, 0.2% fats, 2.1% celluloses, 3.2% ashes, 0.27% calcium, and 0.2% phosphorus (Wawo *et al*., 2014). Its tuber also contains α-sitosterol group compounds, alcohol, taccalin, alkaloids, and steroidal sapogenin (Habila *et al*, 2011). Not only carbohydrates, african arrowroot tubers also contain medicinal compounds. Borokini and Ayodele (2012) reported that the Hawaiian traditional people used african arrowroot tubers for the treatment of stomach bleeding and diarrhea. The raw african arrowroot tubers are mixed with water and red clay, and then consumed. They also reported that in the country of Ivory Coast boiled african arrowroot leaf is used to treat elephantiasis and edema.

This plant is also tolerant to dry condition. Therefore, it can be used as emergency food especially for people who inhabit small islands and arid regions. Actually, african arrowroot tuber is consumed as a food source in Indonesia, as it is processed (Ubwa *et al*, 2011; Sitohang, 2013) by some people in several regions in Indonesia, i.e. Garut, Bangka Belitung, Sumenep and the archipelago of Karimunjawa, especially during dry season and high tidal waves season, as well as the people in Mozambique (Bruschi *et al*, 2014). In India, african arrowroot is consumed as a vegetable after its tubers

are boiled with guava or tamarind leaves (Misra & Misra, 2014). African arrowroot tubers have a bitter taste. Borokini and Ayodele (2012) and Ndouyang *et al*. (2014) explain that the bitter taste is caused by the alkaloid content in the tuber. Ukpabi *et al*. (2009) specifically explained that the bitter flavor compounds are derived from phenols, namely taccalin (3,5,7,4-tetrahydroxy-flavilum-3xyloside).

The biggest challenge in the development of an underutilized commodity is to boost crop productivity. However, it is possible to produce the plant optimally when the land use, the quality of seeds, and cultivation techniques are proper. Various ways can be done to increase crop productivity, either through the approach of cultivation or superior varieties. Nature has created the perfect balance (Tuasikal 2009; holy quran al-Mulk: 3-5). Cultivation approach can also be done through the concept of the balance of source and sink. Source is the organs that produce or export photosynthesis products, while sink is the recipient of photosynthesis products. In many species, the leaves are the main source (Mastur, 2015), while the sinks are primarily storage organs such as seeds, stems, and tubers. Tubers and seeds are the main sinks of the african arrowroot plant. The absence of inflorescence stalk, the seed-forming organ, is expected to increase the productivity of african arrowroot tubers.

Leaf pruning has been done to some commodities, particularly horticultural commodities and several tuberous species (Susila, 2006; Panggabean *et al*., 2014; Wijaya *et al*., 2015; Atrisiandy 2015), with the aim to increase production. However, not much has been reported on tuberous plants. On a different note, Nusifera (2009) reported that trimming of the flower buds could also be performed on yam tubers to increase production.

MATERIALS AND METHODS

Time and place

The study was conducted from May 2013 to April 2014 in the District of Simpenan, Sukabumi. The location of the study has sandy soil texture, with daily temperatures of 30-40^{o}C and humidity of 45-50%.

Research Material preparation

Seed tubers that were used as research materials were peripheral tubers of african arrowroot plant resulting from an exploration in Sukabumi. The tubers that were used were 70-200 grams. They were sowed in a tub filled with sand. Sprouting tubers were moved into the field for treatment with inflorescence pruning. Planting in the field was done by immersing the tuber in a planting hole that had been filled with 1 kg of manure/hole. The plants were planted in mounds as high as 30 cm. Spacing between plants was 100 cm x 100 cm. The inflorescence pruning treatment was done before the first flower bloomed. Additional fertilization was carried out after treatment, with a dose of 1 kg of manure/hole. Watering was done once a day or as needed.

Harvest time was when the African arrowroot plant leaves began to turn yellow. It was carried out by digging up tubers. Canopy and tubers were cleaned from the rest of the soil and measured.

Experimental design

The experiment was constructed in the non-factorial Randomized Completely Block Design (RCBD) with 3 levels of inflorescence pruning treatment, namely: P1: without inflorescence pruning and without additional fertilization; P2: inflorescence pruning without additional fertilization; and P3: pruning inflorescence with extra fertilization.

Each treatment was replicated 3 times, with 5 individual plants/replications. The variables observed included plant growth (height of the plant canopy, crown's width, number of leaves, and stem diameter) and crop production (total tuber weight per plant, size of the largest and the smallest tuber, total number of tubers per plant, weight of stover, and harvest index). Plant canopy observations were made when the canopy reached its maximum size, while the crop production observations were made after the harvest. The harvest index used was that of Dianawati (2013), in which the result of the division of the tuber weights by the plant weight is multiplied by 100. The plant's weight is the total weight of the stover, roots, tubers, and stolon.

Data analysis

Data were analyzed using variance test. The data were then tested using Duncan's Multiple Range Test (DMRT). Both tests were performed at 5% alpha value. Testing was performed using SAS version 9.1 program.

RESULTS AND DISCUSSION

African arrowroot Plant characteristics

African arrowroot is a tuberous plant commonly grown in the rainy season and becomes dormant in the dry season (Fitter & Hay, 1981). The plant only produces few leaves. Petiole and inflorescence stalk grow from the stem in the soil (rosette) (Figure 1a). African arrowroot has single-type leaves. Sukabumi accession has green leaves in color, but some other accessions have dark-green leaves. The edge of the leaves is flat, while the tip is pointed. Leaf bones are submerged and yellowish in color. Petiole and inflorescence stems groove clearly without stripes. African arrowroot inflorescence is formed at the end of the inflorescence stalk in an umbellata type (Figure 1b), which consists of dozens of single flowers. African arrowroot flowers do not bloom simultaneously. Only a few fruits are formed from a single stalk of inflorescence (Figure 1c).

The Sukabumi accession generally produces only one tuber in each generation (Figure 1a). The tubers tend to be rounded shape. Stem-end portion is generally flat, while the blunt-end of the tuber is rounded. According to Syarif *et al*. (2014), tuber shape is also influenced by soil texture and species around where the plant grows. African arrowroot from Sukabumi has a smooth skin surface tuber, and bright white when harvested. This color is then changed to brown and to light brown after it is saved (Figure 1d).

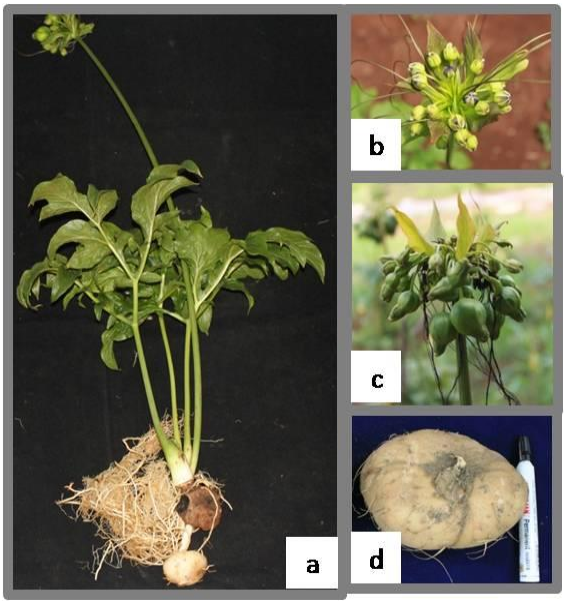


Figure 1. African arrowroot characteristics; a) whole part of plant; b) inflorescence; c) fruitset; d) tuber

African arrowroot is a tuberous plant that grows in coastal forest floor. Some studies indicate that the plant is distributed almost along the coasts of Indonesia (Setiawan, 2013; Syarif *et al*., 2014; Susiarti and Sulistiarini, 2015; Wawo *et al*., 2015), including Sukabumi Regency. In general, the african arrowroot plants cultivated in Sukabumi produce greater tuber size than if the same plant is cultivated outside their habitat. In the research of Wawo *et al*. (2015), the average production ofSukabumi accession cultivated in Cibinong was 182.90 grams. Meanwhile in this study, the average production of african arrowroot plant in Sukabumi was at least 670 grams.

The soil texture of the land under research in Sukabumi is sandy. Daily temperature in this area is around 30-40^{o}C and humidity of 45-50%, while the soil in Cibinong has a clay texture. This area had 32-33^{o}C daily temperature and humidity around 80-90% in 2013-2014. These differences in environmental conditions lead to differences in plant growth. Syarif *et al*. (2014) also confirm that different environmental conditions with different species compositions cause variations in the growth and production of african arrowroot in Java and the surrounding islands.

African arrowroot plant produces stolon, leaf shoot and inflorescence buds simultaneously at the beginning of its growth (Wawo *et al*., 2015), whereas flowers are one of the strong sinks for catching result of photosynthesis. Therefore, pruning inflorescence stalk is expected to augment the flow of photosynthesis result to tubers, thus increasing the production of tubers.

Plant growth

In general, both the control plants and the pruned ones had a similar canopy growth. The manure addition after treatment of inflorescence pruning did not increase plant growth significantly, but did so with the number of leaves. The number of leaves plays an important role in the growth and production of african arrowroot since the species only produces a few leaves per plant (Figure 2).

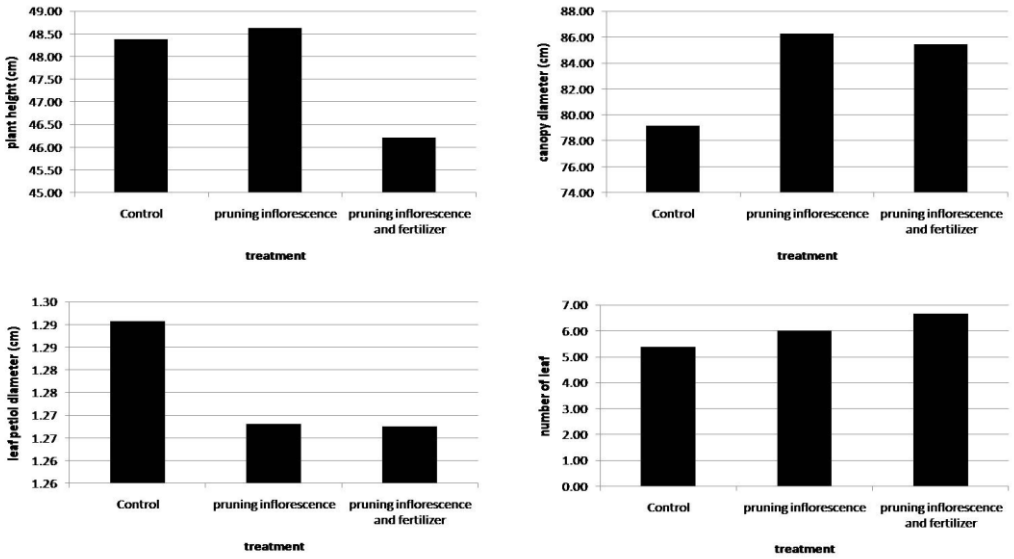


Figure 2. Plant growth for various pruning treatments

Nevertheless, the data on the growth of african arrowroot plant illustrate that the treatment did not increase plant height of the pruned plant or other growth variables, except the number of leaves. This can be explained as follows. African arrowroot produces leaves one by one. The second leaf will grow after the first leaf unfolds. The third leaf will grow after the second leaf, and so on. In this study, the pruning of inflorescence stalk was done before the first flower buds bloomed. At that time, the first leaf unfolded, but the second leaf had not grown yet. As a note, in general the first leaf is the highest and largest leaf on the african arrowroot plant. That is why pruning inflorescence stalk no longer affected the height of the plant and the diameter of the leaf petiole.

Furthermore, the african arrowroot leaves seemed not only to serve as a source, but also as a powerful sink, because after the inflorescence stalk was removed, the plant tended to form leaves rather than produce tuber. Leaves are important organs in photosynthesis. The greater the leaf number, the higher the photosynthesis activity, resulting in more assimilation (Pertamawati 2010; Suminarti 2010; Ariany *et al*., 2013; Purnamasari, 2016), and higher productivity.

Plant production

Treatment of pruning inflorescence could increase the production of african arrowroot tubers by weight (866 grams) more than the control (670 grams), although the number of tuber produced was lower (3.9 tubers) than controls (4.3 tubers). The addition of manure after pruning will no longer increase the production of african arrowroot tubers (815.2 grams); instead, it will expand the number of tubers produced (5.1 tubers) (Figure 3).

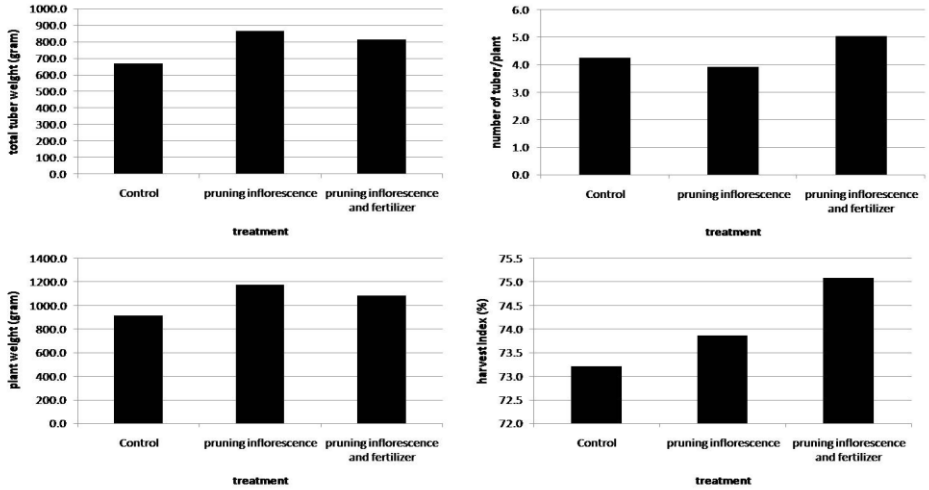


Figure 3. Total plant production in various treatments

In harvest time, african arrowroot treated with pruning produced a greater number of tubers than the control. The productivity index rose from 73.2 in control to 73.9 in pruning treatment. Interestingly, the weight gap between the largest tuber (802.8 gram) and smallest one (23.3 gram) was wide. The gap indicates that there were a few small tubers that could not be used both as propagation material and for consumption. Tuber size uniformity can be improved by the addition of organic fertilizers.

There were variations in the size of tubers in each treatment. The tuber sizes in the inflorescence pruning treatment ranged from 23.3 grams to 802.8 grams. This gap is less than the control (9 grams - 589.2 grams). However, the addition of organic matter after pruning tended to minimize the gap in size (33.6 grams - 620.6 grams) (Figure 4)

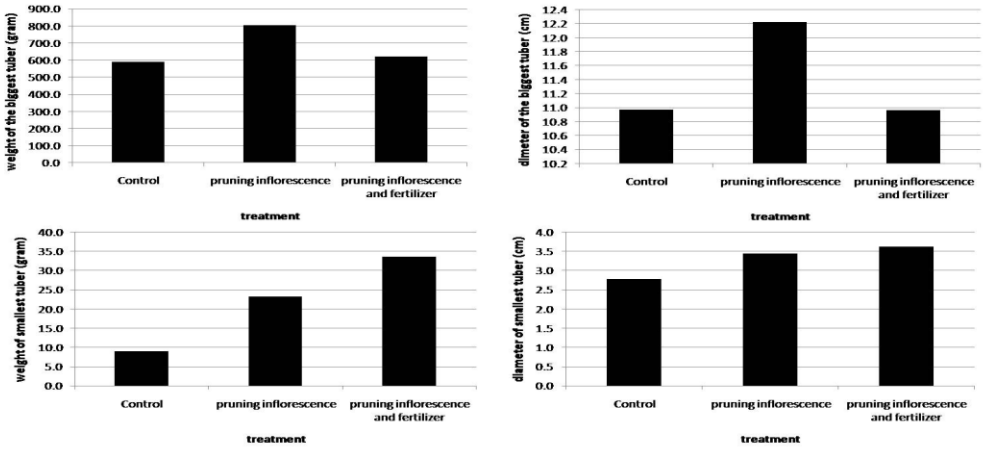


Figure 4. Tuber size (weight and diameter) in various treatments

The manure addition usually does not directly increase crop production. However, it can increase the weight of small tubers. So, there are more large-sized tubers for processing into starch. Small tuber, namely 33 grams to 100 grams in weight, can be used as seed in the next season. That is why the production index increased to 75.1 after the plants in the present study were fertilized, although the biggest tuber had a lower weight compared to the tuber treated with pruning without manure addition.

Several studies have shown the use of cow manure can increase the production of agricultural commodities (Evanita *et al*., 2014; Neltriana, 2015). Manure has a C/N ratio of 11.3 which indicates the level of decomposition of organic material into a form that is available to plants is very high. Therefore, nitrate will be immediately available to plants. Manure does not only play a role in providing nutrients, but also in improving soil physical properties, such as permeability, porosity of the soil, soil structure, water holding capacity, and soil cations (Gonggo, 2005). The data show that the fertilizer increased african arrowroot canopy growth and proved that the plant was actually responsive to fertilization. Pate *et al*. (2014) and Aziz and Susanto (2015) evinced that the application of manure combined with chemical fertilizers can spur the growth and production of african arrowroot. In line with these studies, Syarif (2015) also reported that adding potassium fertilizer by 20 g/pot increased the growth of african arrowroot.

CONCLUSION

This study concludes that inflorescence pruning treatment of african arrowroot can increase tuber production, whereas a combination of inflorescence pruning treatment and organic fertilization can improve the tuber size uniformity of african arrowroot.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the head of Research Center for Biology for the permit and research fund on african arrowroot. We also would like to thank Mr. Apip Tusnadi, who helped us during the research in Sukabumi.

REFERENCES

Anonim. 1997. *Alquran.* CV. Pusafrican arrowroot Mantiq. Solo. 604 hal.

Ariany, S.P, N Sahiri, A Syukur. 2013. Pengaruh kuantitas cahaya terhadap pertumbuhan dan kadar antosianin daun dewa (*Gynura pseudochina* (L.) DC) secara in vitro. *eJurnal Agrotekbis* 1(5):413-420.

Aziz SA dan R Susanto. 2015. Are *Tacca leontopetaloides* (Linn.) O. Kuntze mini-tuber growth affected by media composition and tuber size. *Journal Tropical Crops* 2(1):1-9.

Borokini TI dan AE Ayodele. 2012. Phytochemical screening of Tacca leontopetaloides (L.) Kuntze collected from four geographical locations in nigeria. *International Journal of Modern Botany* 2(4):97-102.

Bruschi P, M Mancini, E Mattioli, M Morganti, and MA Signorini. 2014. Traditional uses of plants in a rural community of Mozambique and possible links with miombo degradation and harvesting sustainability. *Journal of Ethnobiology anf Ethnomedicine* 10:1-22.

Burkill HM. 1985. The useful plants of west tropical Africa. Vol 5. http://plants.jstor.org/compilation/tacca.leontopetaloides [diunduh 7 April 2017].

Dianawati M, S Ilyas, GA Wattimena, AD Susila. 2013. Minitube rproduction of potato aeroponically by determining optimum rate of nitrogen foliar fertilizer. *Jurnal Hortikultura* 23(1):47-55.

Evanita E, E Widaryanto, YBS Heddy. 2014. The influence of cattle dropping fertilizer on growth and yield of the eggplant (*Solanum melongena* L.) in intercropping pattern with napier grass (*Penisetum purpureum*) of the firt crop. *Jurnal produksi Tanaman* 2(7):533-541.

Fitter, A.H & R.K.M. Hay. 1981. *Environmental Physiology of Plants*. University of York England. Diterjemahkan oleh Andani, S. & E.D. Purbayanti, tahun 1992. ISBN 979-420-181-2. Gadjah Mada university Press. 421 hal.

Gonggo BM, B Hermawan, dan D Anggraeni. 2005.pengaruh jenis tanaman penutup dan pengolakan tanah terhadap sifat fisika tanah pada lahan alang-alang. *Jurnal Ilmu-ilmu Pertanian Indonesia*. 7(1):44-55

Habila JD, LA Bello, AA Dzikwe, Ladan, and M Sabiu. 2011. Comparative evaluation of phytochemicals. Antioxidant, and antimicrobial activity of four medicinal plants native to NorthernNigeria. *Australian J. Basic and Appl Sci.* 5(5): 537-543. ISSN 1991-8178

Mastur. 2015. Source and sink syncronize to improve seed productivity of physic nut. *Buletin Tanaman Tembakau, Serat, dan Minyak Industri* 7(1):52-68.

Misra S and MK Misra. 2014. Ethno-botanical study of plants with edible underground parts of south odisha, india. *International Journal of Agricultural and Food Science* 4:51-58.

Mulyani ME dan Sukesi. 2011. Analisis proksimat beras merah (*Oryza sativa*) varietas slegreng dan aek sibundong. *Prosiding Kimia FMIPA ITS*. Hal 1-8. http://digilib.its.ac.id/public/ITS-Undergraduate-20154-Paper-352945.pdf [diunduh 5 April 2017].

Ndouyang CJ, RM Nguimbou, YN Njintang, J Scher, B Facho, and CMF Mbofung. 2014. In vivo assessment of the nutritional and subchronic toxicity of Tacca leontopetaloides (L.) tubers. *Scholarly Journal of Agricultural Science* 4:5-13.

Neltriana N. 2015. Pengaruh dosis pupuk kandang kotoran sapi terhadap pertumbuhan dan hasil ubi jalar (*Ipomoea batatas* L.). Skripsi. Fakultas Pertanian. Universitas Andalas. Padang.

Nusifera S dan A Karuniawan. 2009. Respon tanaman bengkuang budidaya (*Pachyhizus erosus* (L.) urban) terhadap pemangkasan reproduktif untuk karakter hasil dan kualitas ubi. *Jurnal Bionatura* 11(1):1-11.

Panggabean FDM, L Mawarni, TC Nissa. 2014. Respon pertumbuhan dan produksi bengkuang (*Pachyhizus erosus* (L.) urban) terhadap waktu pemangkasan dan jarak tanam. *Jurnal Online Agroekoteknologi* 2(2):702-711.

Pate HS, SH Badi, HD Dikwahal, GG Jibung, and MD Molchen. 2014. Response of african arrowroot *Tacca leontopetaloides* to fertilizer and intra-row spacing at Garkawa. *Journal of Biology, Agriculture and Healthcare* 4(11): 97-101. www.iiste.org [diunduh 4 April 2017]

Perdana F dan Hardinsyah. 2013. Analisis jenis, jumlah, dan mutu gizi konsumsi sarapan anak Indonesia. *Jurnal Gizi dan Pangan* 8(1):39-46.

Pertamawati. 2010. Pengaruh fotosintesis terhadap pertumbuhan tanaman kentang (*Solanum tuberosum* L.) dalam lingkungan fotoautotrof secara invitro. *Jurnal Sains dan Teknologi Indonesia* 12(1):31-37.

Purnamasari, R.T. 2016. Pengaruh waktu tanam dan tingkat kepadatan tanaman jagung (*Zea mays* L.) pada pertumbuhan dan hasil tanaman talas *Colocasia esculenta* (L.) Schott var. *Antiquorum* yang ditanam secara tumpangsari. *Gontor Agrotech Science Journal* 2(2): 37-52.

Setiawan E. 2013. The exploration of tacca leontopetaloides L: distribution pattern and ecology in bangkalan district. Seminar Nasional Universitas Trunojoyo. Hal 570-574.

Sitohang VBL. 2013. The utilization of kecondang (*T.leontopetaloides*) in karimunjawa island as alternative food. Proceeding International Conference on Global Conservation. Brawijaya University, February 7-8th 2013.p44-48.

Suminarti, NE. 2010. Pengaruh pemupukan N dan K pada pertumbuhan dan hasil tanaman talas yang ditanam di lahan kering. *Akta Agrosia*. 13(1):1-7.

Susiarti S dan D Sulistiarini. 2015. Keanekaragaman umbi-umbian di beberapa lokasi di Provinsi Bangka Belitung dan pemanfaatanya. Prosiding Seminar Nasional masyarakat biodiversitas indonesia 1(5):1088-1092.

Susila AD. 2006. *Panduan Budidaya Tanaman*. Departemen Agronomi dan Hortikultura. Fakultas Pertanian. Institut Pertanian Bogor. 131p.

Syarif F, P Lestari, dan AH Wawo. 2014. Growth characteristics variation of Tacca leontopetaloides (L.) Kuntze (Taccaceae) in java and surrounding island. *Berita Biologi* 13(2):161-171.

Syarif F. 2015. Pengaruh jenis media tanam dan pupuk kalium terhadap pertumbuhan dan produksi umbi african arrowroot. Prosiding Seminar Nasional Hasil Unggulan Bidang Pangan Nabati. Bogor, 25 September 2014. Hal 315-322.

Tuasikal MA. 2009. Faedah surat Al Mulk, hikmah Allah menciptakan bintang di langit. https://rumaysho.com/674-faedah-surat-mulk-hikmah-allah-mencipafrican arrowrootn-bintang-di-langit.html [diunduh 7 April 2017].

Ubwa ST, BA Anhwange and JT Chia. 2011. Chemical analysis of Tacca leontopetaloides peels. *American Journal of Food technology* 6:932-938. DOI: 10.3923/ajft.2011.

Ukpabi UJ, E Ukenye and AO Olodeje. 2009. Raw-material potentials of Nigerian wild African arrowroot (Tacca leontopetaloides) tubers and starch. *Journal of Food Technology* 7:135-138.

Wawo AH, P Lestari, dan NW Utami. 2015. Studi perbanyakan vegetatif tanaman taka (*Tacca leontopetaloides* (L.) Kuntze) dan pola pertumbuhannya. *Jurnal Berita Biologi* 14(1):1-9.

Wijaya MK, SDY Wiwin, dan L Setyobudi. 2015. Study of shoot pruning towards growth and crop production of baby cucumber (*Cucumis sativus* L). *Jurnal Produksi Tanaman* 3(4):345-352.

ISBN 978-979-8539-82-4