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Ex-situ Conservation Efforts to the Seedlings Collected from Botanical Exploration in Mount Gede Pangrango National Park, Indonesia

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Abstract: A botanical exploration and collection were already conducted in March 2019 in Pasir Banteng and Pasir Pogor, Mount Gede Pangrango National Park, Indonesia, by a staff team from Cibodas Botanic Gardens (CBG). The following steps are to ensure these plants will be vigorous and survive in the nursery, which will later be ready to be planted and displayed as a garden collection. This study aimed to describe a series of treatments and management of these collections, analyze the survivorship level in the nursery, and record which seedlings were successfully planted in the garden. We explained the plant maintenance used a qualitatively-descriptively method; the survivorship level was measured based on the proportional numbers of the survived individuals; the planted seedlings in the garden were recorded from the field observation. The findings showed that in the first three months of maintenance, seedlings occurred a large amount of mortality. Next, from June 2019 to August 2020, the seedlings performed good development with no mortality reported. In September 2020, as many as 13 specimens experienced mortality. At the end of December 2020, the total crude mortality was 41 specimens, and the survivorship level was 75.15%. All the orchid specimens have been planted as the garden collections.

Keywords: Cibodas Botanic Gardens; Ex-situ Conservation; Living Plant Collection; Seedling; Survivorship.

Introduction

Human disturbances have caused severe damage to the ecosystems and human survival, including habitat loss, spreading pathogens, waste and pollution, climate changes, and many other disadvantages. Around 40% of the world's plants are threatened and face extinction risk (Antonelli et al., 2020; Mounce et al., 2017; Murphy & Romanuk, 2014). Even though various preventive measures have been adopted by nations worldwide during the last decade, the aim of halting biodiversity loss has not been achieved (Secretariat of the Convention on Biological Diversity, 2020). The plant diversity loss might lead to destruction for biological types, including humans, so more powerful maintenance of threatened plants is urgently needed (Brummitt et al., 2015). Two key strategies for conserving plant diversity are in-situ conservation and ex-situ conservation. Ex-situ conservation is a crucial complementary purpose of in-situ conservation and may cover up the limitations of in-situ conservation (Braverman, 2014).

Ex-situ conservation means displacing living plants or seeds from their natural habitat to a man-made environment for protection and maintenance, to avoid plants from being harmed by natural causes or human disturbances (Abeli et al., 2020). Ex-situ plant collections are becoming more and more crucial for maintaining biodiversity in the face of mounting human stresses (Smith & Pence, 2017), including excessive harvesting, natural environment conversion, and climate change. Furthermore, ex-situ stocks resulting from its propaga-

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tion can provide seedlings required for species reintroduction, redeployment, or supplementation (Thomas et al., 2022). The Global Strategy for Plant Conservation also trusts to enhance the value of ex-situ conservation by setting a goal of a minimum of 75% of the threatened species to preserve such as ex-situ, and 20% prepared for restoration and recovery (Sharrock et al., 2018).

Botanic gardens hold a crucial role in ex-situ conservation and a properly positioned to organize improving numbers of plant species related to conservation notice (Westwood et al., 2021). In Indonesia, one government institution obligated to establish and conduct ex-situ conservation efforts is the botanical gardens. Based on Presidential Regulation Number 93 of 2011, botanical gardens have some duties to carry out ex-situ plant conservation efforts and manage the site that contains plant collection resulting from these efforts, and the data must be well-documented.

Cibodas Botanic Gardens (CBG) is an Indonesian government institution as one of the botanic gardens under the National Research and Innovation Agency (*Badan Riset dan Inovasi Nasional - BRIN*) of the Republic of Indonesia. CBG is concerned with conducting ex-situ conservation of wet-tropic montane plants, particularly from western Indonesia. Tropical montane plants are urgent to be conserved due to the rate of degradation in these regions also tends to increase (Boehmer, 2011). Worldwide climate changes are also a potential warning in the conservation efforts regarding montane plants (Rathore et al., 2019; Thang et al., 2020). Therefore, CBG needs to conduct simultaneous efforts concerning ex-situ montane plant conservation.

In March 2019, a CBG team conducted a botanical exploration and collection in Pasir Banteng and Pasir Pogor, Mount Gede Pangrango National Park, Indonesia. Within those efforts, hundreds of specimens from 36 plant families were successfully collected and transferred to the nursery of CBG (Hidayat & Kurniawati, 2021). The next critical steps are to ensure the survivorship of these collections to keep thriving and vigor, so the latter are ready to be planted in the garden and become plant collections.

Ensuring the seedlings keep healthy and flourishing needs extra attention and maintenance. The level of seedling mortality may reach 90% or even more during their early development. Therefore, this period is a critical life phase of the tree seedlings (Conlisk et al., 2017). So, this study aimed to describe a series of treatments of these collections in the nursery, analyze the survivorship of the seedlings, and record the seedlings that were successfully planted as the garden living plant collection. The findings will hopefully increase the understanding of the value of ex-situ plant conservation efforts, benefiting future research, educational endeavors, and public awareness.

Method

A botanical exploration and collection were already conducted in Pasir Banteng and Pasir Pogor, Mount Gede Pangrango National Park, Indonesia, in March 2019. The activities successfully collected as many as 165 specimens (53 species from 36 families) in the form of seedlings (Hidayat & Kurniawati, 2021). Then, all the seedlings collected were delivered to the nursery unit of CBG to receive acclimatization and maintenance treatments.

The process and survivorship observation were conducted from the end of March 2019 to December 2020. The intervals were considered to be trustworthy and capable of enhancing seedling survival. After these periods, the maintenance in the nursery keeps on going until the seedlings are ready to be planted in the garden. However, it will not be further explained. Then, the recording of which seedlings were planted as a garden collection was observed until December 2020.

Preparation Stage

The nursery unit of CBG has been prepared with a greenhouse to accommodate the arrived seedlings from the field. To avoid direct exposure to the sunlight, keep the moisture, and reduce the transpiration, then at the top of the inner room was installed a paranet shade with a density of 65% (Irawan et al., 2020).

Each seedling will be planted in a polybag of diameter (Ø) of 15 cm in size. A growth medium of the tree seedlings and terrestrial orchids was a mix of forest compost and paddy husks with a ratio of 1:1 (Irawan et al., 2020; Prasetyonohadi & Kuswanto, 2016). Epiphyte orchids, such as *Bulbophyllum* spp., were grown in the medium of chopped ferns. The different mediums used to adapt the seedlings to be compatible with their natural habitat in the wild. The mediums were also given an insecticide (*Furadan*®) at a dose of 7.5 to 10 gr. each polybag to prevent interference from worms, termites, and other insects.

Maintenance Stage

Soon the seedlings arrived at the nursery, each plant displaced to the polybag added with medium according to their types (tree, terrestrial orchid, or epiphyte orchid). Only *Bulbophyllum* sp. was grown in the medium of chopped ferns, and the rest were planted in a mix of forest compost and paddy husks medium. Each specimen was already moved to a polybag, then equipped with a plant identity (i.e., species name, plant family name, the collector's name code, date of collection, and elevation).

All polybags were well-arranged on the greenhouse floor which allows the staff's movement during the treatment. Then, it was conducted by watering once to twice a day, depending on the room's temperature and humidity. Weed and pest control once a week, growth hormone supplies once in two weeks, and fertilizing once a month. Growth hormone was added to restore the plant condition and stimulate shoots and root development (Irawan et al., 2020). These serial processes are conducted to the seedlings at the nursery unit until they are ready and vigor planted in the garden. However, in this study, the maintenance observation was only conducted from the end of March 2019 to December 2020.

To calculate the survivorship level of the seedlings during these periods, then be analyzed some parameters, namely: crude mortality rate (d), which is the ratio of the number of deaths during a specific period (in this study was twenty-one months) to the number of the previous population; proportional mortality rate (q), which is the proportion of death individuals to the number of the early population; and, survivorship level (l), which is the proportion of the survived individuals to the number of the early population at the end of the observation period (Alvarez-Aquino & Williams-Linera, 2012). The survivorship observation was conducted once a week and accumulated monthly. Next, all the parameters are presented in tables and graphics.

Garden Plant Collection Record

After the seedlings were maintained and morphologically thrive and vigorous, they were ready to be planted in the garden. The planting location of new plants is usually based on the closeness of kinship or taxon with other plants. It also considers the adequacy of sunlight, soil type, topography soil, and water availability. Then, after three months in the garden field, if the plant performs good development, it is registered as a new plant seedling collection. Next, this new data is recorded in the garden collections database.

Result and Discussion

In the early three months of maintenance, it occurred a significant loss of the seedlings. As many as 28 specimens were dead that 13 specimens in May and 15 specimens in June 2019 (Table 1). Even *Lindera polyantha* (Lauraceae) and *Lindsaea cultrata* (Lindsaeaceae) were unable to survive in their first three months. The species of *L. polyantha* has a high ability in water absorption (Lailati, 2021). Despite its tolerance to wood decay being moderately resistant Djarwanto et al. (2019), the CBG's microclimates which are characterized by high to very high humidity (> 85%), caused the failure of the early development stage of these seedlings. For *L. cultrata*, it was assumed that the growth medium and the microclimates were unsuitable likely their natural habitat in the deep forest (Abas, 2017).

It was 17 species (from 15 families) that experienced mortality in their population (d). In this first critical period of plant seedling development, a plant frequently has some difficulties to well-grow or even often dies (Conlisk et al., 2017). At this early stage of the seedling development, the survivorship level (l) reached 83.03% accumulatively, and the proportional mortality rate (q) reached 16.97%, or there occurred ~17 specimens' mortality in every 100 populations.

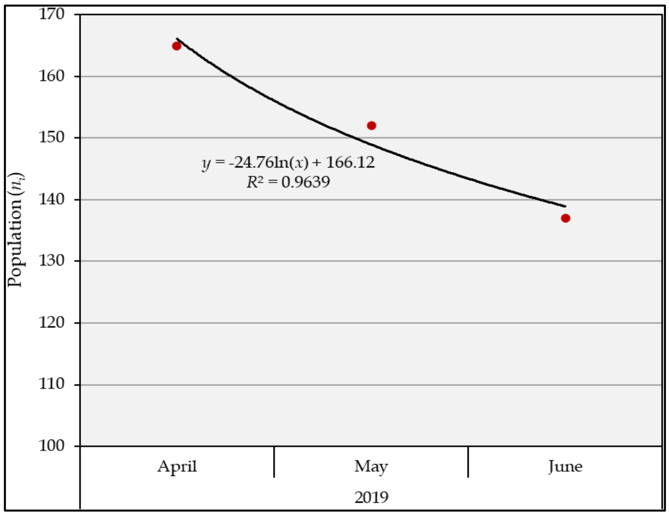

Figure 1. Survivorship Curve of the Total Seedlings in their First Three Months of Maintenance

Figure 1 also describes the survivorship condition of the seedling in these first three months of maintenance. The coefficient was negative, and the value of R^2 was 96.39%. It means that the number of mortalities is intensely significant in affecting the survivorship level of the seedling population. At this critical period, seedlings are highly sensitive to drought and desiccation. Nursery conditions also roles a significant part in emerging resilience (Palma & Laurance, 2015). For example, the facility of the watering system and microclimate control, especially temperature and air humidity, should fulfill the seedling's necessity to grow.

The other species occurred mortality even though not the entire specimen died, at least leaving one or two specimens to live. Some of these reasons were a new condition at the CBG's nursery is a shocking environment for these new seedlings. A recent circumstance, such as microclimates and a new growth medium, are unable to support its early development or vice versa, and these new seedlings failed to adapt to these new conditions. Microclimates supported by other biophysical factors have a linear influence on plant growth and sustainability (Maclean et al., 2015).

The growth medium in composition between forest compost and paddy husk with a ratio of 1:1 still needs further research. An accurate and suitable growth medium may be different among plant seedlings because each plant requires a specific growth medium and composition, such as soil pH (Offord et al., 2014).

The different quality of each seedling from its natural habitat is also assumed to be a driving factor of the failure. For certain species, seedlings of stressed resistant grandparents or parents generated a faster regeneration, more developed root system, and growing faster than seedlings from the same genetic lineages whose grandparents or parents did not receive stress conditions (Herman et al., 2012; Palma & Laurance, 2015).

Table 1. The Seedlings Experienced Mortality in Their First Three Months of Maintenance

Species name	Family	Early population (n_0)	Survived population (n_i) 2019 April	May	June	Crude mortality (d_i)
Acronychia trifoliolata Zoll. & Moritzi	Rutaceae	3	3	2	1	2
Begonia muricata Blume	Begoniaceae	5	5	4	3	2
Cryptocarya sp.	Lauraceae	2	2	1	1	1
Cryptostylis arachnites (Blume) Hassk.	Orchidaceae	4	4	3	3	1
Daphniphyllum glaucescens Blume	Daphniphyllaceae	3	3	3	1	2
Eurya acuminata DC.	Pentaphylacaceae	4	4	4	2	2
Fagraea elliptica Roxb.	Gentianaceae	3	3	2	2	1
Lindera polyantha Boerl.	Lauraceae	1	1	1	0	1
Lindsaea cultrata (Willd.) Sw.	Lindsaeaceae	5	5	3	0	5
Polyalthia subcordata (Blume) Blume	Annonaceae	5	5	4	3	2
Schima wallichii (DC.) Korth.	Theaceae	5	5	5	4	1
Strobilanthes paniculata (Nees) Miq.	Acanthaceae	6	6	5	4	2
Turpinia montana (Blume) Kurz	Staphyleaceae	4	4	3	3	1
Urophyllum sp.	Rubiaceae	4	4	3	3	1
Strobilanthes involucrata Blume	Acanthaceae	5	5	5	3	2
Strobocalyx arborea (Buch.-Ham.) Sch.Bip.	Compositae	2	2	1	1	1
Viburnum sambucinum Reinw. ex Blume	Adoxaceae	2	2	1	1	1

Furthermore, from June 2019 to August 2020, the seedlings started to perform good adaptation and development in the new environment. There was no death of the seedlings reported in that period. It indicated that the treatments and maintenance efforts performed by CBG's staff were already suited to the plant growth needs. Moreover, in July 2020, all the orchid species have been displaced to the orchid house and planted as garden collections (Table 2).

Table 2. Orchid Species Planted as Garden Collections

Orchid species	Genus	Number of individuals
Apostasia wallichii R.Br.	Apostasia	3
Bulbophyllum sp.	Bulbophyllum	5
Calanthe sp.	Calanthe	5
Cryptostylis arachnites (Blume) Hassk.	Cryptostylis	2
Cymbidium lancifolium Hook.	Cymbidium	4
Plocoglottis javanica Blume	Plocoglottis	5

Environment degradations have several impacts on the species variety, including orchids. Due to overharvesting, orchids are one of the most endangered flowering plants. However, orchid species are going extinct in the wild caused of their lost habitat. Humans' intrusions and activities, such as agricultural practices, urbanization, extending settlements, deforestation, and illegal harvesting for economic purposes, are threatened the existence of orchid species in their natural habitat (Phillips et al., 2020; Seaton et al., 2013). Therefore, orchids are crucial to be preserved and as one of the main targeted plants in every botanical exploration and conservation activity conducted by CBG.

Next, in September 2020, several mortalities occurred in the seedlings. As many as 13 specimens from nine species occurred mortality in their population (Table 3). *Calophyllum* sp., *Rubus alceifolius*, and *Strobocalyx arborea* experienced complete death and left no surviving seedling. It is a bad fortune, even though this species has potential uses as an ornamental and medicinal plant (Slik, 2022; Valkute et al., 2018).

The most common matter assumed for these failures was stem and root decay. Highly humidity surrounding the nursery has implications for humidity conditions in the greenhouse. During the seedling development, it should be a good humidity circulation in the greenhouse. Where moisture losses from ventilation and the stability between plant transpiration and soil evapotranspiration (Shamshiri et al., 2018). Microclimate conditions around the greenhouse, such as low temperatures combined with high humidity at night and in the morning, and non-optimal air circulation in the greenhouse, are assumed to result in water vapor excess that causes the decayed of the seedlings.

Table 3. The Seedlings Experienced Mortality Between August to September 2020

Species name	Family	Survived population (n_i) 2020		Crude mortality (d_i)
		August	September	
Ardisia villosa Roxb.	Primulaceae	5	4	1
Calophyllum sp.	Clusiaceae	2	0	2
Eriosolena composita (L.f.) Tiegh.	Thymelaeaceae	2	1	1
Medinilla speciosa Blume	Melastomataceae	3	1	2
Strobilanthes involucrata Blume	Acanthaceae	3	2	1
Myrsine avenis (Blume) A.DC.	Primulaceae	2	1	1
Rubus alceifolius Poir.	Rosaceae	2	0	2
Tacca chantrieri André	Dioscoreaceae	3	1	2
Strobocalyx arborea (Buch.-Ham.) Sch.Bip.	Compositae	1	0	1

Furthermore, from September to the end of December 2020, the number of seedlings remain stable, with no mortality experience. Until this period, no additional seedlings have been planted in the garden for collection. These seedlings remain maintained in the nursery, awaiting the upcoming planting schedule.

Overall, during the maintenance periods (from April 2019 to December 2020), the seedling population experienced mortality or crude mortality (d) of as many as 41 specimens. Survivorship level (l) reached 75.15%, where R^2 was 69% (Figure 2). It means that the population decreases are significantly influenced by the amount of mortality. The proportional mortality rate (q) was 24.85%, or there occurred ~25 specimens' mortality in every 100 populations. However, this level was considered a moderate success in managing the seedlings survivorship. Some important notes that need attention are how the best manage or modify the microclimate in the greenhouse so that it remains to fulfill the requirements for optimal seedling growth.

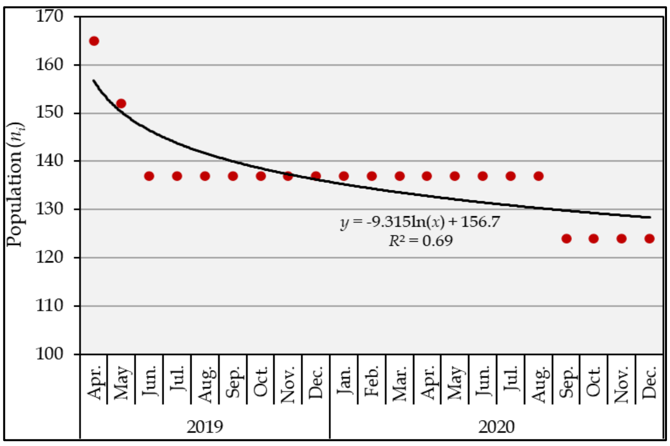

Figure 2. Survivorship Curve of The Total Seedlings After Twenty-One Months of Maintenance

Based on the type of the curve, the survivorship characteristic of this study included the type of III. This type describes how the number of mortalities at the early stage of development tends to be high, but then the life expectancy gradually improves for the individuals who survived at their first stage (Molles & Sher, 2019). This description corresponds with this study. In the first three months, plant seedlings suffered high mortality, but then in the following periods, the mortality level decreased, even to zero. It was only experienced another mortality with a lower amount than the previous. At this stage, the plants tried to adapt to a new environment after being displaced from their natural habitat. The differences in the microclimate and other biophysical conditions influence the survival rate of the seedlings.

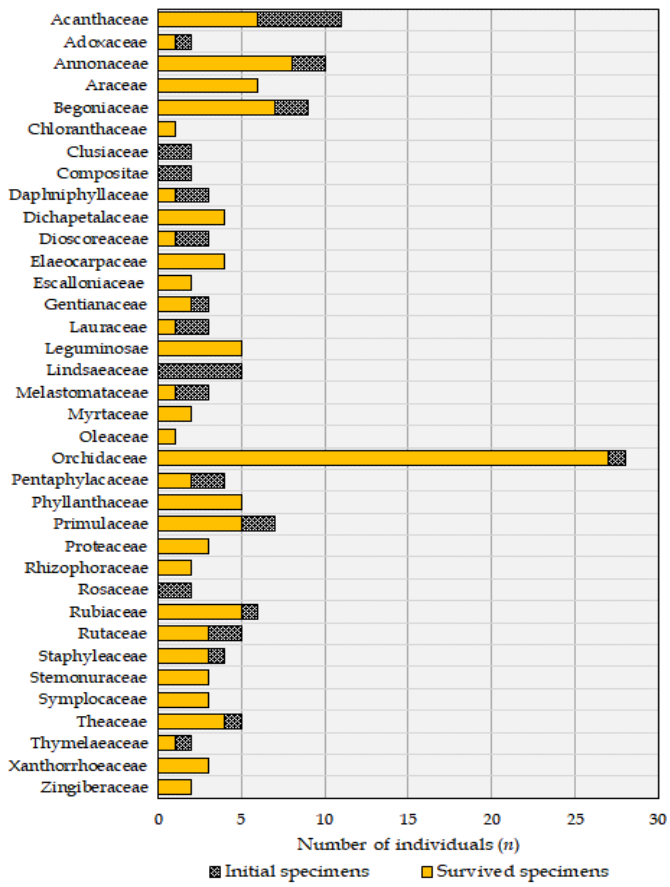

Figure 3. Survivorship Proportion Between the Number of Initial and Survived Seedlings Based on Their Families

This study also showed that specimens from families of Clusiaceae, Compositae, Lindsaeaceae, and Rosaceae failed to survive during the maintenance period (Figure 3). They contributed as much as 26.83% of the total mortality. The remaining families were left with at least a specimen to be maintained in the nursery, such as Adoxaceae, Chloranthaceae, Daphniphyllaceae, Dioscoreaceae, Lauraceae, Melastomataceae, Oleaceae, and Thymelaeaceae. As many as 15 families that showed 100% successfully survived during these periods, such as Araceae, Chloranthaceae, Dichapetalaceae, Elaeocarpaceae, Escalloniaceae, etc.

The optimization efforts to maintain the remaining surviving seedlings still be continued. Watering, fertilizing, providing the supplement, and weed and pest control are routinely performed, either daily or weekly. Hopefully, with these routine maintenances along with plant maturation, the survival rate might be increased or at least be maintained. The seedlings will keep placed at the nursery until later planting activities are ready to be established and the seedlings confirmed planted in the garden.

Based on the above description, ex-situ plant conservation conducted on those seedlings gives a crucial role in the plant stock availability of CBG to enrich its collections. Plant enrichment is not only significant to the existence of CBG but also enhances the value of plant conservation. When those seedlings are eventually planted in the garden, they may trigger further research and educational purposes that are beneficiaries to the community. These efforts are also a form of plant species preservation and rescue when natural habitats continuedly experiencing degradation and destruction problems.

Conclusion

At the early stage of development, the seedlings experienced significant losses. But then, the maintenance efforts gradually succeeded in reducing the losses and keeping the survivorship relatively stable. The nursery staffs of CBG were capable of maintaining the survivorship of three-quarters of the total seedlings. All the orchid seedlings were planted as garden collections and displaced to the orchid's house. However, this study pointed out that the greenhouse facilities and the knowledge of the proper growing media need to be developed immediately to improve the survivorship level of the maintained seedlings. The survived seedlings were a crucial plant stock to enrich CBG's plant collections and enhance the value of ex-situ plant conservation, particularly for the tropical montane plants.

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