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Seed Exploration for Seed Banking Purpose in Cibodas Botanical Garden

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Abstract

Due to contribution of Target 8 of the Global Strategy for Plant Conservation GSPC Botanic Gardens, Cibodas Seed Bank committed to increase its *ex situ* plant collection through seed banking. This research aimed to assess population and collect seeds from Cibodas remnant forests and its neighboring, Mt. Gede Pangrango National Park forest, for seed banking purpose. Thirty-two numbers of seeds belonging to fifteen families were collected. Two species were included in IUCN red list i.e. *Saurauia cauliflora* (vulnerable) and *Magnolia blumei* (least concern) and one species classified as endangered species by World Conservation Monitoring Unit (WCMC) i.e. *Pinanga javana*. Sixty-five percent population were possible to collect without affecting the availability of the seeds in the nature. Around a half of seeds were collected at natural dispersal stage to ensure the seed maturity. Ninety-one percent collected seeds were expected as orthodox seeds that can be stored in Cibodas Seed Bank and three species were recalcitrant and cannot be stored in Cibodas Seed Bank i.e. *Calamus ciliaris, C. reinwardtii* and *Daemonorops rubra*. Cut-test results showed 78% seed collected were full seeds, that were not infested, empty or immature seeds. This study provides information regarding the readiness of population for seed collection and species were collected and stored in Cibodas Seed Bank.

How to Cite

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INTRODUCTION

Tropical forests in Java play a critical role as biodiversity refuge because of land conversion into human settlement (Irawan et al., 2012). Conservation areas are as the last defense for biodiversity loss (Bille et al., 2012). Botanic gardens are one of the main institutions involved in *ex situ* conservation of wild plant species through its plant collections and seed banking (Hurka & Neuffer, 2004; Nadhifah et al*.,* 2018). Seed banking involves collecting seeds from wild plants, drying, and storing them in cool conditions (Hay & Probert, 2013). Seed banks offer resistance from several threats to plants *in situ* including habitat loss and degradation, introduction of alien species, overexploitation, pollution, disease and climate change (Heywood, 2017; O'Donnell & Sharrock, 2017).

Seed banking is a vital component of plant conservation. Botanic gardens are the main institutions involved in such seed banking and many botanic gardens around the world maintain effective seed banks for wild plant species, contributing to the long term and efficient storage of plant diversity (Oldfield, 2015; O'Donnell & Sharrock, 2015). Seeds should be collected from a large number of individuals within a population to capture the most genetic diversity (Peres, 2016; Richards et al*.,* 2007). In order to collect seeds with have high viability, efforts must be made at the time of collection to gather mature and viable seeds (O'Donnell & Sharrock, 2015).

Due to integrated approach in conservation and contribution to Target 8 of the Global Strategy for Plant Conservation (GSPC), "at least 75% of threatened plant species in *ex situ* collections, preferably in the country of origin, and at least 20% available for recovery and restoration programmes", Botanic Gardens - Indonesian Institute of Sciences (LIPI) particularly Cibodas Seed Bank committed to increase its *ex situ* plant collection through seed banking. To support this *ex situ* conservation program, LIPI joined the Millenium Seed Bank Partnership in 2016 which focused on the seed collecting and banking of Indonesian native plants (Hardwick et al., 2017). Collaboration is essential at local, national and global level for seed banking to be the most effective efforts (Oldfield, 2015).

This research aimed to assess population and collect seeds from Cibodas Botanical Garden (Cibodas BG) remnant forest and neighboring area, Mt. Gede Pangrango National Park (Mt. Gede Pangrango NP) forest, for seed banking purpose. The seeds collected from this study were stored in Cibodas Seed Bank and will duplicate in Bogor Seed Bank for research and restoration purposes as well as regular viability testing.

METHODS

In this study, seed exploration consisted of pre-collection assessment and seed collecting in the field. Pre-collection assessment aimed to gather information of seed avaliablity and possibility to collect healthy seeds without interfere the nature. Field data and herbarium speciments also collected for gathered additional information and verify the indentification of species respectively.

Study Site and Materials

Seeds were collected weekly from Mt. Gede Pangrango NP and several areas of remnant natural forest in Cibodas BG on September to December 2017 which located in West Java, Indonesia with annual rainfall of 2,082 mm and monthly precipitation as shown at Figure 1. Climatic data were obtained from Cibodas BG Field Station. The seed collection focused on native Indonesian trees and shrubs species predicted to have orthodox seeds, which is not presented on the Millennium Seed Bank's Data Warehouse Base List. Moreover, this study was prioritizing species that endangered, endemic or useful.

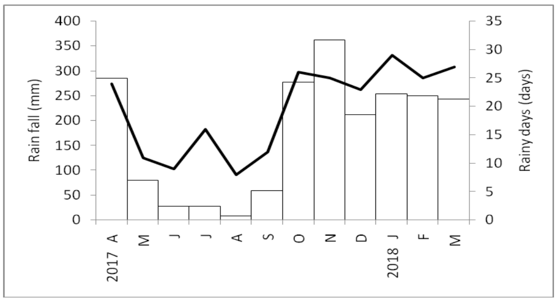

Figure 1. Monthly precipitation in Cibodas

Pre-collection Assessment

Seed pre-collection assessment covered plant identification, population assessment and readiness of population for seed collection, physical quality of seeds and availability of seeds. Cut-test was held to assess seed quality. Ten seeds were cut-tested from random individuals in the population and make a record of the number of full, empty, infested and immature seeds. Number of viable seeds for collection were estimated using the following formula (Way and Gold, 2014):
Estimated number of viable seeds for collection = Maximum number of seed that can be collected

× Percentage of full seeds
Maximum number to seed that can be collected = Available population × 20%
Available population = Total population × Percentage of full seeds
Total population = Estimated number of plants at natural dispersal × Average number of fruits or dispersal units per individual plants × Average number of seeds per fruits or natural dispersal units

Seed Collecting

Fully ripe fruits were collected from wild plants. Seeds were collected using different collecting techniques such as hand picking, using pole over a tarpaulin, or by climbing depending on the species and type of dispersal units. Seeds were harvested using pole (tree pruner) and secateurs at around natural dispersal stage to maximize longevity in long-term storage. Seeds were removed from its fleshy fruits immediately after the field trip to prevent fermented seeds. Collected seeds were stored in cloth bags and immediately transferred to Cibodas Seed Bank for processing and drying.

Number of seeds collected was large enough to be held in Cibodas Seed Bank and a duplicate to be stored at Bogor Seed Bank. All seed collections accompanied by a fertile herbarium specimen and linked field data form with a unique collection number. Herbarium specimens were sent to the Herbarium Bogoriense of Research Centre for Biology - LIPI and Kew's Herbarium for plant identification and botanically verified purposes.

RESULTS AND DISCUSSION

Seed pre-collection assessment was done in nine locations covering Mt. Gede Pangrango NP (1,421 to 1,802 m asl) and remnant forest areas inside Cibodas BG (1,313 to 1,411 m asl) (Table 1) with 32 seed species found at natural dispersal. The floristic composition of the study area was a typical sub-montane tropical rain forest and dominated by *Villebrunea rubescens, Ostodes paniculata, Strobilanthes hamiltoniana, Cyrtandra picta, Diplazium pallidum,* and *Calamus reinwardtii* (Mutaqien & Zuhri, 2011).

Seed Pre-collection Assessment

Thirty-two number of seeds belonging to 15 families were found and assessed from forests (Table 2), particularly form Rubiaceae (seven species), Arecaceae (five species) and Moraceae (five species). Two species were included in IUCN red list i.e. *Saurauia cauliflora* (vulnerable) and *Magnolia blumei* (least concern) and one species classified as endangered species by World Conservation Monitoring Unit (WCMC) i.e. *Pinanga javana*.

Figure 2. Some seeds collected during the exploration (a) *Saurauia cauliflora,* (b) *Magnolia blumei,* (c) *Pinanga javana.*

Sixty-five percents seeds were possible to collect without affecting to seed availability in nature, but in this study, seeds with a small number (< 500 seeds) were also collected and then the estimated date to return and re-collect seeds were recorded. Multi-year collecting and propagation may be necessary to achieve a good-sized seed collection for such species (Hay & Probert, 2013;

Table 1. Locations of seed pre-collection assessment

Location	Latitude	Longitude	Altitude (asl)
Mt. Gede Pangrango NP			
Cibodas resort	06.74311 S	107.00598 E	1,421
Mandalawangi	06.73105 S	106.99256 E	1,683
Gunung Putri	06.76642 S	107.00295 E	1,802
Cibeureum	06.74837 S	106.99345 E	1,592
Lumut forest	06.74480 S	107.00320 E	1,385
Cibodas BG			
Wornojiwo forest	06.74176 S	107.01044 E	1,378
Kompos forest	06.74251 S	107.00648 E	1,356
Jalan akar forest	06.74074 S	107.00563 E	1,411
Cibogo forest	06.74201 S	107.00604 E	1,313

Table 2. Seed pre-collection assessment

Species	Population area (m^2)	Number of plants	Full seeds (%)	Seeds per fruit	Fruits per plant	Estimated number of viable seeds to collect	Collection possible*
Antidesama tetrandum	10,000	51-100	100	1	18,480	40,656	Yes
Ardisia fuliginosa	20,000	51-100	100	1	30,360	303,600	Yes
Ardisia villosa	40,000	51-100	100	1	32	384	No
Boehmeria diversifolia	40,000	11-50	100	50	50	5,000	Yes
Calamus ciliaris	10,000	51-100	90	1	246	3,587	No
Calamus reinwardtii	10,000	11-50	90	1	197	1,596	No
Daemonorops rubra	40,000	11-50	100	1	188	1,880	No
Dichroa febrifuga	20,000	1-10	100	50	198	9,900	Yes
Ficus ampelas	10,000	11-50	100	46	3,848	221,260	Yes
Ficus deltoidea	20,000	11-50	100	4	11	132	No
Ficus fistulosa	40,000	11-50	100	696	6,480,000	13,530,240	Yes
Ficus ribes	40,000	51-100	100	244	38,016	185,518,080	Yes
Ficus variegata	20,000	11-50	100	1.388	1,000	6,940,000	Yes
Homalanthus giganteus	40,000	11-50	60	2	19,320	41,731	Yes
Lasianthus laevigatus	20,000	11-50	100	1	1,920	7,680	Yes
Lasianthus rigidus	20,000	51-100	100	4	160	8,960	Yes
Lasianthus sp.	20,000	11-50	70	5	700	10,290	Yes
Magnolia blumei	5,000	11-50	100	27	1,200	2,400	No
Medinilla speciosa	40,000	11-50	100	195	8,000	7,800,000	Yes
Montanoa grandiflora	40,000	11-50	100	5	100	2,000	No
Morinda sarmentosa	10,00 0	11-50	100	14	2,240	188,160	Yes
Mycetia cauliflora	20,000	11-50	100	200	20	9,600	Yes
Pinanga coronata	10,000	101-1000	90	1	1,200	48,600	Yes
Pinanga javana	40,000	1-10	100	1	6,600	10,560	Yes
Psychotria angulata	20,000	11-50	100	2	100	1,600	No
Psychotria montana	10,000	51-100	80	2	162	4,147	No
Saurauia cauliflora	40,000	1-10	100	100	238	47,600	Yes
Saurauia nudiflora	100,000	1-10	100	758	2,576	3,905,216	Yes
Saurauia pendula	200,000	51-100	80	240	50	115,200	Yes
Schefflera scandens	50	11-50	100	5	65,000	2,600,000	Yes
Tetrastigma sp.	10,000	1-10	100	2	160	320	No
Vaccinium korthalsii	50	11-50	100	4	100	3,200	No

* Possibility to collect 5,000-10,000 healthy seeds around natural dispersal without taking more than 20% of available seeds

Way & Gold, 2014). Ideally, 5,000-10,000 healthy seeds were collected around natural dispersal without taking more than 20% of available seeds (Way and Gold, 2014). It can cover seeds for several purposes i.e. maintaining base collection (long term storage in case of the wild population is threatened by extinction), developing an effective germination protocol and viability monitoring, viability monitoring over the anticipated 200 year lifespan of the collection, duplication at another bank for safety, distribution for users, future propagation, restoration and reintroduction projects (Hay & Whitehouse, 2017).

Population assessment was known by estimated area of population, number of accessible individual plants, and evidence of damage or disturbance. Population areas were estimated using Google Maps Area Calculator Tool. It was easily to calculate area using a Google maps interface and found the latitude and longitude of a specific point. Population area in this study ranged from 50 to 200,000 m^2. *Vaccinium korthalsii* and *Schefflera scandens* were present only in small area with limited number of individual. Number of plants were estimated after scanning the study area and counting the population member. Number of seeds collected were sampled randomly and evenly across the extent of the population to ensure the representative population (Way & Gold, 2014). Some of study areas had damage which were caused by the invasion of invasive alien species, especially *Chimonobambusa quadrangularis* and *Strobilanthes laevigatus* in adjacent forest area.

Physical quality of seeds were showed by cut-test of ten seeds randomly from the examined samples and then the result was referred as the most frequently occurring state, i.e. full seeds, infested seeds, empty seeds, and immature seeds. Generally, a full seed was firmed and white inside, not shrivelled or overly dry. Empty seeds contained little or no seed tissue inside and may even have evidence of insect damage or an aborted seed. Twenty five species were 100% full seeds and the others had empty and immature seeds. There were no mouldy and infested seeds in the cut-test results. Mouldy seeds were indicated by dead seeds which had necrotic and often brown tissue. Infested seeds showed evidence of insect predation. Cut-testing is used to assess the quality of seed collections after cleaning and it provides an indication of the proportion of the empty, poorly developed or insect-infested seeds (Terry & Sutcliffe, 2014). Furthermore, cut-tests is subjective and although most people will obtain similar results, interpretations may vary greatly (Kolotelo, 1997).

The readiness of the population for seed collection was shown by the most frequently occurring stages. Seeds must be collected at the optimum stage of the development to maximize their longevity in long-term storage (Way, 2000; Whitehouse et al., 2015) and it showed by the changes in the colour of fruit and seed coat colour, fruits splitting or breaking open, seeds rattling, seed hardening and drying, and dispersal of some seeds (Way & Gold, 2014). Four species were found in fully natural dispersal stages i.e. *Boehmeria diversifolia, Dichroa febrifuga, Ficus deltoidea, F. fistulosa,* and *Saurauia cauliflora* (Figure 3). An estimated suitable return date for collecting *F. ribes* and *Pinanga javana* was recorded as in the immature seeds stage.

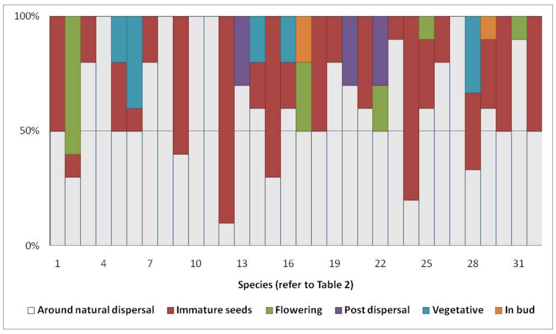

Figure 3. Readiness of the population for seed collection. Each bar represented species in Table 2.

Seed Collection

All seed species in the list of seed pre-collection assessment (Table 2) were collected and stored in Cibodas Seed Bank. Eventhough 11 species did not possible to collect at an ideal number of seeds, the seeds were still collected and the date to return and re-collect of the ripened seeds later was estimated. Three species that have recalcitrant seeds based on the Seed Information Database of Kew Royal Botanic Gardens (http://data.kew.org/sid/) and cannot be stored in Cibodas Seed Bank i.e. *Calamus ciliaris, C. reinwardtii,* and *Daemonorops rubra*. Recalcitrant seeds need to be used immediately after collection or they may die. Moreover, the recalcitrant seeds are shed at high water content, desiccation sensitive and cannot be stored under conditions conventionally employed for desiccation tolerant orthodox seeds (Baskin & Baskin, 2001; Pammenter & Berjak, 2013).

Meanwhile, 91% collected seeds were expected as orthodox seeds and can be stored in Cibodas Seed Bank after dried. Orthodox seeds can be dried to 2-5% without loss of viability and tolerate relatively long storage periods, and hence have inherent primary seed dormancy (Pammenter & Berjak, 2013). Moreover, orthodox seeds are seeds that acquire desiccation tolerance during the development, they can dry to low water contents (generally less than 5%) and retain viability in the dry state for predictable periods (Baskin & Baskin, 2001).

Most of collected seed species (37.5%) were tree life form and consist of 12 species, i.e. *Antidesma tetrandum, Ardisia fuliginosa, Ficus am-*

pelas, F. fistulosa, F. ribes, F. variegata, Homalanthus giganteus, Lasianthus sp., *Magnolia blumei, Saurauia cauliflora, S. nudiflora,* and *S. pendula* (Table 3). It was appropriate with this study which focused on collection of native Indonesian trees and shrubs. Besides that, the other life forms occured in study area, i.e. epiphytic treelet, liana, tree palm, and woody climber. In vegetation studies, trees are commonly used to understand ecological correlation of composition, richness and density. Other plant life forms, such as lianas, herbs, and ferns, are less studied, although they contribute substantially to plant richness and density (Pasion et al.*,* 2018).

Table 3. Seed species collected based on life form

Life form	Number of seed species	%
Tree	12	37.50
Shrub	10	31.25
Herb	0	0
Epiphytic treelet	4	12.50
Liana	1	3.12
Tree palm	2	6.25
Woody climber	3	9.37
Total	32	

Five species of figs (*Ficus*), i.e. *F. ampelas, F. deltoidea, F. fistulosa, F. ribes* and *F. variegata,* were collected from forest patch areas of Cibodas BG and interior forest of Mt. Gede Pangrango NP. Seeds of subgenus *Ficus* have a sticky mucilaginous seed coat and when this material dries, seeds become attached to the surface of tree branches, leaves, soil, rocks and so on. Fig seeds are orthodox in large number inside the syconium. Moreover, fig seeds can remain dormant for long periods, at least several years, in dry and cool (artificial) conditions (Baskin & Baskin, 2001). *Ficus variegata,* an edible fig, had the greatest number of seeds per fruit, i.e. more than 1,000-minute seeds per fruit.

Three species of *Lasianthus* obtained were *L. laevigatus, L. rigidus,* and *Lasianthus* sp. *Lasianthus* plants were encountered frequently along the line track with lots of number fruits per plant, for example, *L. laevigatus* with around 1,900 fruits. *Lasianthus* genus is present with a 4-9 locular ovary and a single, erect, basal ovule in each locule, which normally develops into a drupe with 4-9 pyrenes (Zhu, 2015). Moreover, *Lasianthus* species are usually present in large numbers in the tropical forests of Asia and may therefore represent an ecologically important element and also show interesting distribution patterns (Zhu et al., 2012).

Estimated number of viable seeds to collect may represent the possibility to collect healthy seeds around natural dispersal (Ruxton & Schaefer, 2012; Way, 2000). Seven species of seeds collected under minimun number required, i.e. *Ardisia villosa, Ficus deltoidea, Psychotria angulata, P. montana, Saurauia pendula, Tetrastigma sp.,* and *Vaccinium korthalsii.* The number of estimated viable seeds to collect should be higher than the number of collected seeds (Figure 4). However, some species showed number of collected seeds that was higher than estimated number of viable seeds to collect, i.e. *Boehmeria diversifolia, Dichroa febrifuga, Lasianthus rigidus,* and *Mycetia cauliflora.* It might be the failure to extrapolate population and estimate number of plants at natural dispersal stage.

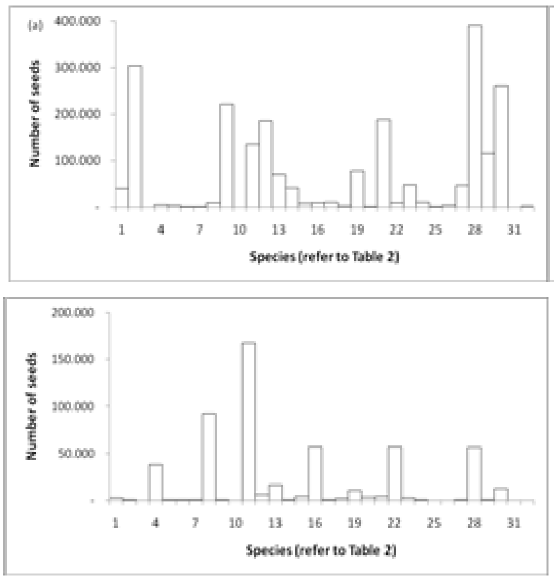

Figure 4. (a) Estimated number of viable seeds to collect; (b) Number of collected seeds. Each bar represented species in Table 2.

Sampling data in this study was represented by the number of sample plants, number of plants found, sampling areas, and % plants producing seeds (Morellato et al., 2009; Figure 5). The number of sample plants ranged from one to twenty-three plants and the most abundant sample plant was *L. rigidus* in Kompos Forest with four seeds per fruit. The highest number of plants found was 50 plants from three species, i.e. *Pinanga coronata, L. rigidus,* and *Psychotria angulata.* It may represent the dominance of these species in the study areas. The sampling areas were varied depending on the size of the study areas. Due to areas of Mt. Gede Pangrango NP which are larger than forest areas inside Cibodas BG, the estimated number of viable seeds ready to collect in Mt. Gede Pangrango NP was higher than forest areas of Cibodas BG. Nevertheless, forest areas inside Cibodas BG had uniqueness in terms of species diversity and its abundance. Furthermore, 41% species produced 100% seeds and indicated the peak of fruiting time.

An average of two seed species per day was collected by weekly seed exploration. In this study, number of seed collected was influenced by rainfall (Table 4). At the late dry season, on September 2017, thirteen seed species were harvested and plants producing seeds were 56% at average. During the early rainy season, on October 2017, number of seed species was constant, i.e. thirteen species, and plants producing seeds increased slightly than previous month. At the peak of rainy season, number of seed species and plants producing seeds decreased sharply. It may be caused by some fruits that might be fallen and failed to form fruit during rainy season. Natural dispersal of each species in this study was presented on Figure 6. Based on the forest ecological study in the tropical montane forest near Cibodas, the highest peak of fruit season was on September. Moreover, the fruiting period was much greater in the transition of rainy season to dry period (Yamada, 1976).

Table 4. Number of seed species collected monthly

	Sept				Oct				Nov			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Average number of plants producing seeds (%)	76	88	60	0	97	70	80	55	0	78	55	0
Number of seed species	5	5	3	0	6	2	3	2	0	4	2	0
Rainfall (mm)	7.8	21.4	1	28	103	35	74.2	64.4	41.6	38.8	90.2	191.4

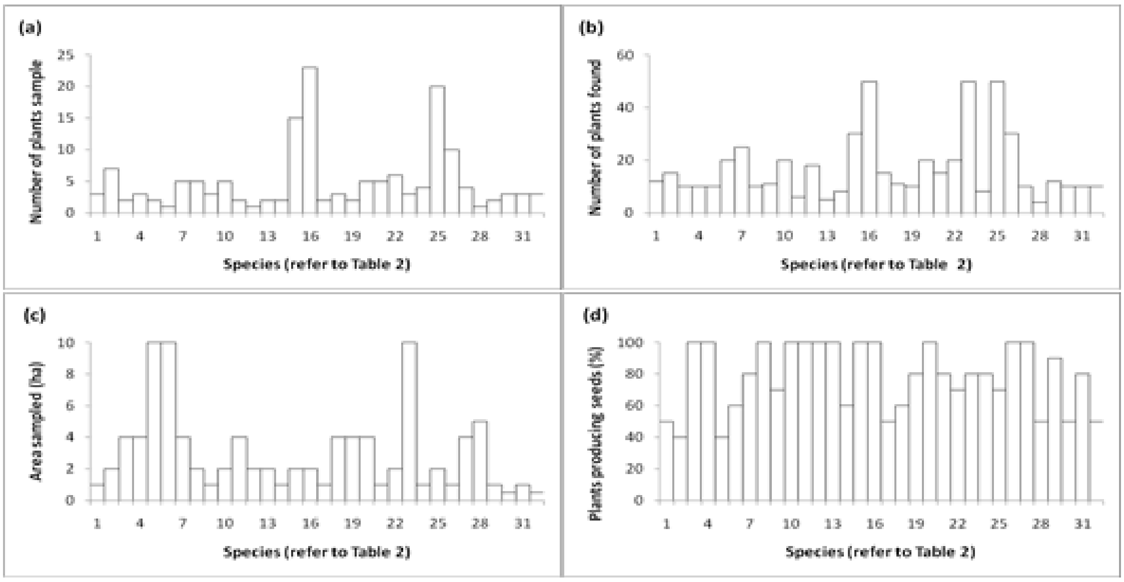

Figure 5. Sampling data of (a) number of plants sampled; (b) number of plants found; (c) sampling area; and (d) plants producing seeds (%). Each bar represented species in Table 2.

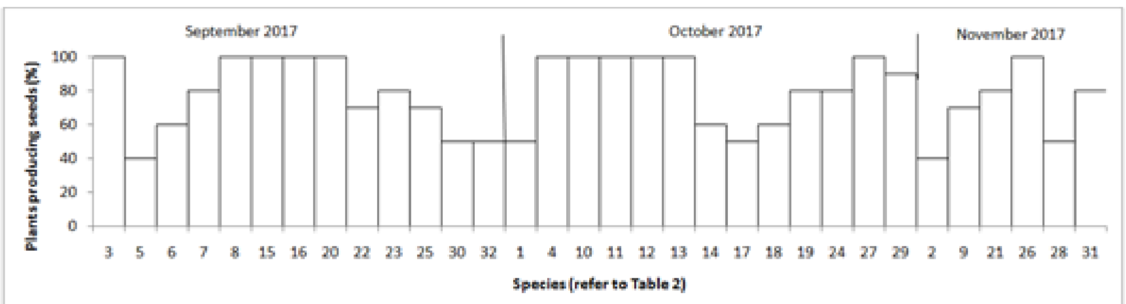

Figure 6. Natural dispersal of species in this study. Each bar represented species in Table 2.

Seed pre-collection assessment could describe the phenological pattern of each species. It is useful to learn about forest regeneration in the tropics. The information obtained from this study can be used as a basis in conducting conservation effort in Cibodas Botanical Garden remnant forest and neighboring area, Mt. Gede Pangrango National Park forest. Furthermore, the seed collected can also be used for research, restoration and reintroduction purpose.

CONCLUSION

Thirty-two number of seeds belonging to 15 families were assessed and collected from Cibodas area of Mt. Gede Pangrango NP and remnant forests inside Cibodas BG. *Saurauia cauliflora* and *Pinanga javana* were identified and categorized as vulnerable species by IUCN and endangered species by WCMC. Sixty-five percent of population was possible to collect without affecting the seed availability in the nature. The cut-tests resulted 25 seed species with 100% full seeds and the remaining seeds had empty and immature seeds. Moreover, number of seed was influenced by rainfall.

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