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Short communication: Germination monitoring of selected Annonaceae seeds: Seed bank collections of Purwodadi Botanic Garden, East Java, Indonesia

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Abstract. *Pratiwi A, Lestari DA, Romdhonah Y. 2022. Short communication: Germination monitoring of selected Annonaceae seeds: Seed bank collections of Purwodadi Botanic Garden, East Java, Indonesia. Biodiversitas 23: 3567-3572.* Monitoring activities are an essential aspect to determine the viability and germination of seeds after storage for a certain period. The seed bank of Purwodadi Botanic Garden collected 20 seeds of 11 different species of Annonaceae. Therefore, this study aimed to examine the seed viability of 11 selected species of Annonaceae after being stored for a certain time. Twenty seeds with three replications, i.e., 60 seeds in total were sown. Meanwhile, for genera of *Uvaria* consisted of *U. purpurea, U. rufa,* and *Uvaria* sp. were sown at 30 seeds in total, which means 10 seeds with three replications. The observed parameters were percentage of germination, seed viability, initial germination, and germination type. The results showed only 4 out of 11 selected species of Annonaceae were germinated, namely *A. montana, F. latifolium, U. micranthum,* and *U. purpurea.* These four species have epigeal germination types, and monitoring activities included stock rejuvenation, and evaluation of storage procedures for *Alphonsea javanica, Annona glabra, Orophea enneandra, Polyalthia littoralis, U. rufa,* and *Uvaria* sp. Furthermore, *P. longifolia* requires collection regeneration due to its recalcitrant character, and the number of empty seeds from the cut test was >75%. We suggested that *P. longifolia* seeds might be better to be stored in the refrigerator.

Keywords: Annonaceae, ex-situ conservation, germination, seed storage, viability

Abbreviations: BBG: Bogor Botanic Garden; ISTA: International Seed Testing Association; PBG: Purwodadi Botanic Garden

INTRODUCTION

Ex-situ and in-situ conservation could be solutions to save biodiversity at the genetic, species, and ecosystem levels. One of the efforts to save plant germplasm is through a seed bank (Lestari and Asih 2015) and it is one of the effective ex-situ plant conservation methods that play an essential role in conserving endangered wild plant species (Hay and Probert 2013). The Global Strategy for Plant Conservation (GSPC) program also saves wild plant seeds for long-term storage. Moreover, those stored in seed banks can be returned to their natural habitat through reintroduction, increasing species genetic diversity, and conserving rare species or earmarked for education programs (Merritt 2014; Helmanto et al. 2017; Dau et al. 2018).

Monitoring of seed germination aims to measure the viability and germination rates of seeds after storage. Subsequently, the initial round of germination tests after storage may indicate the eligible ones by physiological conditions of viability and germination can reach above 85% (Liu et al. 2020; Perez et al. 2021). Seed viability and germination will be reduced after storage for a certain period and these conditions affect the decline in the viability of seeds (Cheyed 2020; Wawrzyniak et al. 2020). Period of storage becomes the most crucial factor in maintaining seed viability. De Vitis et al. (2020) stated that the seed storage character of a species should be known to maintain viability after storage.

Seed germination is a test on seed viability monitoring which becomes an essential aspect of seed bank management. Economically, the main purpose of seed storage is to preserve the seed stocks so that they are available for the next season. Seed viability monitoring is an essential quality control aspect for the seed bank. Besides the potential resources of the seed bank, information about the relative time of seed storage can be obtained (Justice and Bass 1978; Hay and Whitehouse 2017).

Purwodadi Botanic Gardens (PBG) is one of the ex-situ conservation institutions with a collection of seed banks. Some of the seeds conserved and stored in the seed bank of PBG are from the Annonaceae family. Based on the PBG database, there are 20 collection numbers of Annonaceae seeds stored, including 11 species with a total of 12,888 seeds. These seeds were stored for 2-5 years, and according to Ferreira et al. (2016; 2019), most from the Annonaceae family have orthodox storage characters, and some species have mechanical to physiological dormancy mechanisms. Orthodox seeds will require monitoring of viability after five years of storage (De Vitis et al. 2020). Therefore, monitoring the germination of selected Annonaceae species from the seed bank of PBG is necessary. This study aims to observe the germination of selected Annonaceae seeds stored in the seed bank of PBG. The results are expected to determine the survival rate of selected Annonaceae seeds, and the evaluation of the storage sustainability can be determined.

MATERIALS AND METHODS

Study area

The study was carried out from October 2021 to March 2022 at the Seed Bank Laboratory and the research greenhouse of the Purwodadi Botanic Gardens, East Java, Indonesia. Furthermore, 11 selected species from the Annonaceae family, including *Alphonsea javanica* Scheff., *Annona glabra* L., *Annona montana* Macfad., *Fissistigma latifolium* (Dunal) Merr., *Orophea enneandra* Blume, *Polyalthia littoralis* (Blume) Boerl., *Polyalthia longifolia* (Sonn.) Thwaites, *Uvaria micranthum* (A.DC.) Hook.f. & Thomson, *Uvaria purpurea* Blume, *Uvaria rufa* Blume, and *Uvaria* sp. were used as seed materials. The 11 selected Annonaceae species have been stored for 2-5 years in a freezer at a temperature of -20°C. These were obtained from harvesting seeds in living plants of PBG's collection and plant exploration in the national park (Table 1).

Procedures

Monitoring of seed germination

Each seed was 20 in number, and with three replications, it means 60 seeds were sown. Except for those from the genera *Uvaria* (*U. purpurea*, *U. rufa*, and *Uvaria* sp.), 10 species were taken with three replications for a total of 30 sowed due to the low number of seeds of those species in PBG (≤ 250 seeds). Subsequently, each seed was planted in a seedling tank filled with sand and watered every day or once the planting medium started to dry out. Germination was observed from the beginning of the seedling until there was no increase in the number of germinating species. Meanwhile, parameters observed were initial seed germination (Garwood 1995), percentage of seed germination (Formula 1; Sutopo 2010), and percentage of seed viability (Formula 2; Davies et al. 2015), germination type (Amarullah et al. 2021), and results of the cut test.

$$Percentage\ of\ seed\ germination = \frac{G}{X}\ x\ 100\%$$

$$Percentage\ of\ seed\ viability = \frac{G + F + A}{X - (E - I)}\ x\ 100\%$$

Notes: G = sum of normal seedlings, X = sum of seeds sown, F = sum of fresh seeds, A = sum of abnormal seedlings, E = sum of empty seeds, I = sum of infested seeds

Seed cut-test analysis

A cut test was performed as an evaluation material for germination monitoring, assuming the seeds cannot grow. The cut test findings were divided into four categories, i.e., fresh seeds, empty seeds, infected seeds, and abnormal germination.

Data analysis

The data obtained were analyzed descriptively, and analysis of variance was done using Microsoft Excel 2016. The results of seed observations and descriptions were explained in the figures.

RESULTS AND DISCUSSION

Monitoring of seed germination

This study showed that only four out of 11 plant species (36%) were capable of germination, namely seeds of *A. montana*, *F. latifolium*, *U. micranthum*, and *U. purpurea* (Table 2, Figure 1). Furthermore, seeds of *A. montana* could germinate up to 91.67%, while the other three species were only able to germinate at 10%, 26.67%, and 13.33%, respectively. Seeds that can germinate with a percentage above 85% indicate good seed viability and hence could be stored in the freezer. Meanwhile, seeds with germination below 85% showed less viability, and storage procedures need to be evaluated. The same applies to seeds that cannot germinate, considering that some seeds from the Annonaceae family are orthodox (Ferreira et al. 2019).

Table 1. Seed materials of 11 selected Annonaceae species of PBG seed bank collections

Species	Collection date	Storage date	Location of collection	Weight (g)	Sum of seeds
Alphonsea javanica Scheff.	16/01/2017	31/01/2017	PBG (XVIII.E.6)	77	498
Annona glabra L.	01/02/2017	07/02/2017	PBG (XVIII.C.27)	250	836
Annona montana Macfad.	15/02/2019	15/04/2019	PBG (II.B.3)	139	595
Fissistigma latifolium (Dunal) Merr.	09/03/2018	01/04/2018	PBG (XVIII.D.3)	135	180
Orophea enneandra Blume	21/04/2019	01/08/2019	Alas Purwo National Park	75.4	470
Polyalthia littoralis (Blume) Boerl.	21/04/2019	01/08/2019	Alas Purwo National Park	210	950
Polyalthia longifolia (Sonn.) Thwaites	20/02/2017	09/06/2017	PBG (XVIV.E. (NC))	1050	764
Uvaria micranthum (A.DC.) Hook.f. & Thomson	11/05/2018	26/07/2018	PBG (XVIII.C.34)	24.6	250
Uvaria purpurea Blume	21/04/2019	01/08/2019	Alas Purwo National Park	19.8	98
Uvaria rufa Blume	25/05/2016	27/07/2016	PBG (XVIII.C.47)	12.4	116
Uvaria sp.	03/04/2018	10/06/2018	PBG (XVIII.C.50)	17.4	80

Therefore, seeds that cannot grow require evaluation to assess the suitability of planting media, seeds quality (germination vigor, physical purity, seed moisture content, and insect/microorganism free), decrease in physical or physiological dormancy, or storage errors due to the lack of information on the seeds' storage characteristics. According to Ferreira et al. (2019), seeds of *Polyalthia longifolia* have a recalcitrant character. This indicates an error in the storage, considering that recalcitrant seeds will experience a decrease in quality once stored at low temperatures. This result indicated that *P. longifolia* seeds could not germinate due to decreased seed quality. Based on Taini et al. (2019), seeds with storage error will experience decline or quality deterioration with signs of decreased quality, low viability, abnormal seedlings, and decreased yields.

The seed viability of the 11 selected Annonaceae plant species from the seed bank of PBG showed a value of 100-111.11% for germinated seeds (Table 2, Figure 1), indicating that the seeds were viable. However, a low percentage was caused by fungi or insect infection during sowing.

Initial germination of the four seed plant species started from 31 to 40 days after planting with the epigeal germination type (Table 2, Figure 2). According to Garwood (1995), the initial criteria of seed germination are divided into fast, slow, and delayed. Seeds with initial germination that occurred within 2-4 weeks are categorized as fast germination, 5-16 weeks classified as slow germination, and for more than 16 weeks classified as delayed germination, while the results of this study were classified as slow germination. According to Handayani (2019), the seedlings of *A. montana* and *P. littoralis* in the Bogor Botanic Gardens (BBG) started growing at 25 and 49 days, respectively, after planting. These differences may be due to environmental conditions during germination. Ecological factors in PBG is drier than in BBG, also BBG is wetter. According to Siahaan et al. (2018), PBG is a dry lowland with an altitude of 300 m above sea level, a rainfall of more than 2000 mm occurs during the rainy season between November and March, and a C-organic content of 1-4%.

Table 2. Seed germination results from 11 selected Annonaceae species of seed bank collections of PBG

Species	Percentage of seed germination	Percentage of seed viability	Initial germination (DAP)	Germination type
Alphonsea javanica Scheff.	0	0	*	*
Annona glabra L.	0	0	*	*
Annona montana Macfad.	91.67 ± 7.64	100 ± 0	31 ± 2.08	Epigeal
Fissistigma latifolium (Dunal) Merr.	10 ± 5	100 ± 0	40 ± 1	Epigeal
Orophea enneandra Blume	0	0	*	*
Polyalthia littoralis (Blume) Boerl.	0	0	*	*
Polyalthia longifolia (Sonn.) Thwaites	0	0	*	*
Uvaria micranthum (A.DC.) Hook.f. & Thomson	26.67 ± 15.28	100 ± 0	37 ± 0.58	Epigeal
Uvaria purpurea Blume	13.33 ± 5.77	111.11 ± 19.25	31 ± 1.73	Epigeal
Uvaria rufa Blume	0	0	*	*
Uvaria sp.	0	0	*	*

Note: Rows with * marked indicate seeds are unable to germinate, ± indicate the standard of deviation

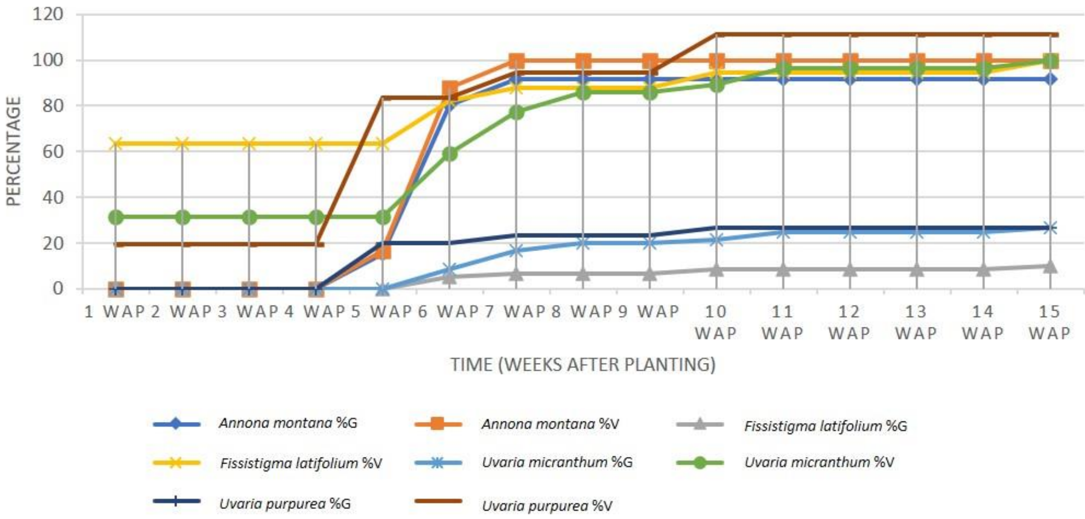

Figure 1. Seed germination percentage (%G) and seed viability percentage (%V) of 11 selected Annonaceae plant species from the seed bank of PBG

Meanwhile, Rachmadiyanto et al. (2020) stated that BBG is a wet lowland with an altitude of 257 m asl, a rainfall of 3161.76 mm/year, and a C-organic content of 3.1-5%. Epigeal germination is categorized as the first-time position of the cotyledons raised above the soil surface. The cotyledons are lifted above the soil surface and detached from the seed coat. This is caused by the enlargement of the cotyledons so that they push the seed coat. This study showed the seed coat was separated from the cotyledons. Epigeal type with seed coat detached from cotyledons is called phanerocotylar (Handayani 2021). According to Handayani (2017, 2019), the type of seed germination from the Annonaceae family is generally epigeal.

Seed cut-test analysis

The results of the cut test on seeds from seven plant species of Annonaceae that we are unable to germinate are shown in Figure 2. The seeds failed to grow due to fungi infections resulting in seed embryos turning brown to black in color (Figure 4, blue circle). Moreover, some of the seeds were also empty (Figure 4, red circle), consisting of few or no embryos. Only the seeds of *F. latifolium* and *U. micranthum* were abnormal due to wilting and drying of germination (Figure 3). In general, a seed viability test was used to determine seed quality, specifically in those that have deep dormancy through the germination ability (Ma et al. 2016).

Overall, the seeds that we are unable to germinate until the end of the observation (5 months after sowing) might be due to insect infection and the empty seeds. Seed infection may occur when post-harvest handling management is not conducted properly. So that, the insect or fungi in an infected seed may enter the seed storage, and it is possible to transmit to the healthy seeds. Some of the reasons for this include inappropriate germination conditions, experiencing dormancy, dead seeds, and not viable seeds. Viable species have the ability or potential to germinate, while dead seeds are viable but useless. Meanwhile, empty and infected seeds are non-viable but not dead seeds (ISTA 2015).

Discussion

Based on the results of this study, various recommendations can be implemented to monitor the germination of selected Annonaceae plant species, especially from the seed bank of PBG. Germination monitoring is one of the methods to evaluate the quality of the seeds stored in the seed bank, and quality evaluation is vital in conservation. Monitoring of seed quality through germination is aimed at long-term seed conservation (Efendi et al. 2019; Puspitaningtyas and Handini 2021). Seed quality can be affected by internal and external factors, i.e., seed drying procedure, insects and microorganisms' infection, slow germination, or damage due to inappropriate humidity before storage (Socolowski et al. 2012). Furthermore, the seeds should have high viability and can be reused for research, reintroduction, complementary, and supplementary. According to Kolo and Tefa (2016), good storage techniques determine high-quality seed availability and guarantee the success of farming.

Several recommendations for monitoring the germination have been conducted, these included: 1) Seeds of *A. montana* can still be stored in the seed bank since the germination percentage value was >85%, and the seeds were still viable, 2) The results of the cut test showed that the seeds of *O. enneandra* need to be tested for breaking dormancy because the number of fresh seeds was >75%, 3) Seeds of *P. longifolia* require collection regeneration, as they are classified as recalcitrant since the number of empty seeds from the result of the cut test was >75%. Also, refrigerator storage becomes essential, 4) *Fissistigma latifolium*, *U. micranthum*, and *U. purpurea* require a planting medium treatment test, considering that the seeds can germinate, even though the percentage of germination is low. The use of a planting medium may be not suitable, then many seeds are infected by insects or microorganisms, 5) Other Annonaceae plant species that were not able to germinate require a new seed collection, 6) It is necessary to handle seeds properly so that seeds have good viability, vigor, purity, and healthy quality. Seed health status can be analyzed through several tests, i.e., visual inspection on dry seeds, incubation test (blotter test and agar plate), and tetrazolium test, to detect the presence of pathogens, insects, or microorganisms in a seed lot. The conventional seeds health test such as visual inspection on dry seeds, incubation test (blotter test and agar plate), and tetrazolium test. and 7) Seed health testing is a method to obtain information about the possibility of a risk of infectious disease.

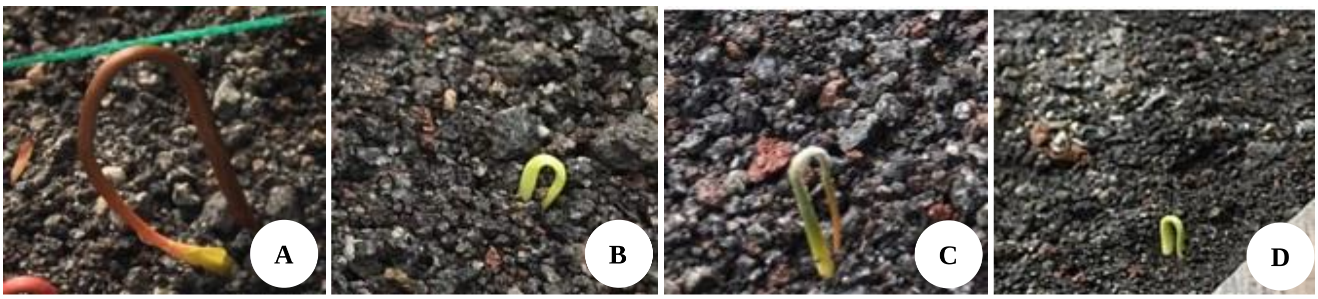

Figure 2. Epigeal seed germination from selected Annonaceae plant species; A *Annona montana,* B. *Fissistigma latifolium,* C. *Uvaria micranthum,* D. *Uvaria purpurea*

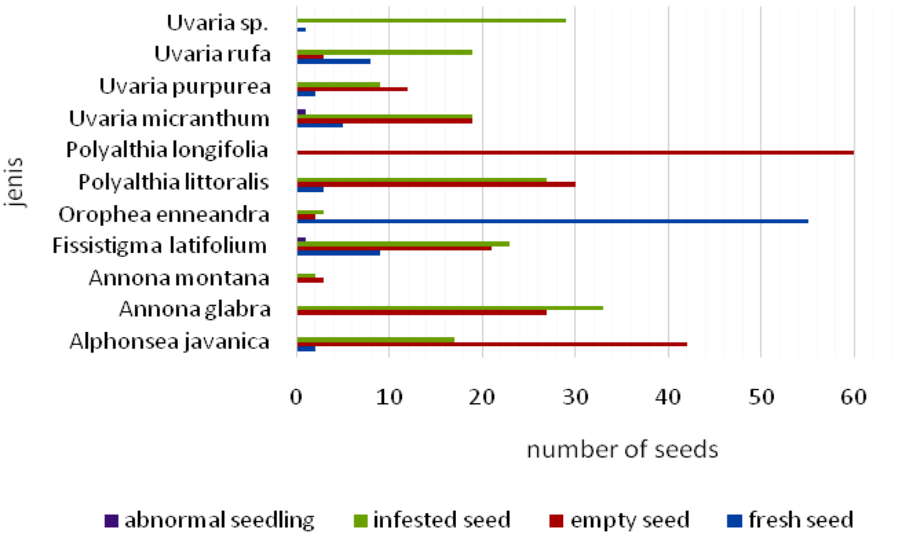

Figure 3. Number of seeds based on cut-test analysis (abnormal seedling, infested seed, empty seed dan fresh seed) of 11 selected Annonaceae plant species

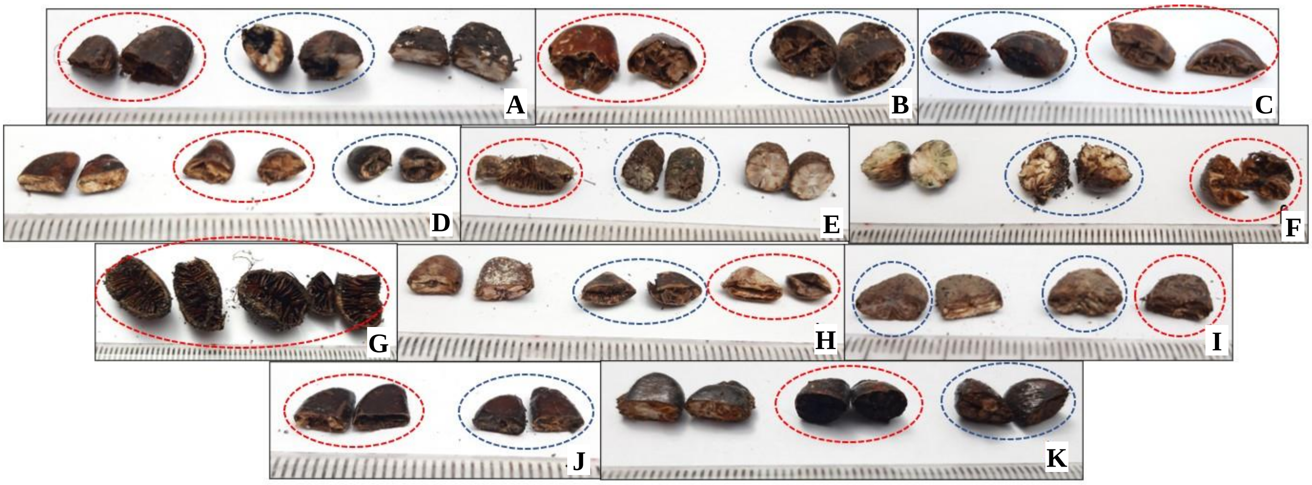

Figure 4. Cut-test results of 11 selected Annonaceae plant species; A. *Alphonsea javanica,* B. *Annona glabra,* C. *Annona montana,* D. *Fissistigma latifolium,* E. *Orophea enneandra,* F. *Polyalthia littoralis,* G. *Polyalthia longifolia,* H. *Uvaria micranthum,* I. *Uvaria purpurea,* J. *Uvaria rufa,* and K. *Uvaria* sp. Infested seeds are in blue circles, empty seeds are in red circles, and fresh seeds are not circled

Based on the storage period, each plant species has a different seed storage behavior. If seed viability has decreased from 85%, so collecting the new one from the same plant species needs to be carried out. Seed stock collections can be used as material for research on the seed storage period (Hay et al. 2021). Seed viability in ex-situ storage will decrease in the sigmoid curve. It is important to estimate the age of the seeds during storage before the seeds lose their viability (Chau et al. 2019).

High seed viability means high seed quality and a high probability of germination. According to Mangena and Mokwala (2019), a high seed germination rate is highly dependent on seed viability and storage time. Monitoring the seed bank collection is a method to maintain seed viability in PBG. Therefore, periodic germination tests are performed to determine the viability of seeds stored for a certain period. Monitoring stored-seed viability is one of the routine activities to support seed bank management (Hay et al. 2021). The monitoring is distinguished based on seed storage, where recalcitrant species is every 3-6 months, intermediate is every 6 months to 1 year, and orthodox is every 5-10 years (Ellis et al. 1990; ISTA 2015; De Vitis et al. 2020). The viability monitoring of seeds from the Annonaceae family in PBG was the first time, it was performed after storage for 2-5 years, and this activity will be repeated periodically in the following years. Therefore, seed storage has become an essential and decisive element in various plant conservation programs

(De Vitis et al. 2020). Further monitoring activities include stock rejuvenation and storage procedures evaluation for the seeds of *A. javanica, A. glabra, O. enneandra, P. littoralis, P. longifolia, U. rufa,* and *Uvaria* sp. necessary.

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