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Seed Exploration and Conservation in Alas Purwo National Park, Banyuwangi, East Java, Indonesia

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Seeds storage through Seed Bank can be viewed as a potential alternative vehicle for conservation of biological resources in general and ex situ conservation in particular in Botanical Gardens. However, there is not much that describes one by one the yield of collected seed, the morphological and storage properties, as well as the specific method of extraction to maintain optimal seed viability. The seed exploration activity aimed to increase the variety of seeds collection for the Seed Bank storage function in Purwodadi BG and to provide complete information about the collected seed. This research was conducted on April 15-27, 2019 in Alas Purwo National Park, Banyuwangi, East Java. The method used was an explorative method consisting of fruits and seeds collection in the field, plant identification, seed extraction and temporary storage of seeds before being transferred to the Seed Bank of the Purwodadi Botanical Gardens. Fruit and seed characterization was described as descriptively based on direct observation. The Seed characters observed were included the number of weight, length, width, the thickness of seeds. The method of extracting each seed is documented and recorded, especially those that require special handling. Among 45 collected seed most of them were shrub, which are dominated by Annonaceae, Moraceae, and Rubiaceae, families. Based on the seed storage behavior, most of the seed were orthodox, this was possible in 14 days of trip for the seeds to maintain their viability, as evidenced by the germination rate above 75% at most of collected seeds. Some seeds had special characteristics in their extraction because of the different morphological properties of the seeds and fruits. Most of the seeds obtained from nearshore ecosystem were easy to extract because the seeds were quite dry.

Keywords: Seed, Seed Bank, Alas Purwo, Purwodadi Botanic Garden

INTRODUCTION

The Java Island, as part of Sundaland, has a high plant diversity (Kusmana and Hikmat, 2015). However in the last few decades there has been damage to the ecosystem, causing a lot decrease to this diversity. To prevent it from continues decreasing, plant diversity should be maintained through ex situ and in situ conservation efforts in the ecosystem, species and genetic level (Samedi, 2015; Zegeye, 2017). In general, ex situ conservation is additional supplement for *in situ* conservation, which refers to conservation of plants diversity in its natural habitats (Kjaer et al. 2001). Ex situ conservation includes a variety of activities, from managing captive populations, education and raising awareness, supporting research initiatives and collaborating with in situ efforts (Melfi, 2012). Ex situ conservation for plants can be conducted in the form of botanical gardens, arboretum, genetic bank (pollen, seeds, DNA) and cryopreservation techniques (Borokini, 2013). Seeds exploration as a part of seeds conservation is also supporting activity for ex situ plants diversity conservation (Dau et al. 2018),

where the results are used as collections in the form of seed banks.

Purwodadi Botanical Garden (PBG)-LIPI in East Java is one of the ex situ plant conservation institutions that has a storing function in the form of Seed Banks. Seeds storage through Seed Bank can be viewed as a potential alternative vehicle for conservation of biological resources in general and ex situ conservation in particular in Botanical Gardens (Linington, 1997; 2003; Wanda et al. 2020) as it is a vital component in plants conservation (Zuhri et al. 2019). Seeds are generally relatively small, so only a small storage space is required. In addition, seed storage technology has developed rapidly to allow seeds storage for years without losing their viability (Schimdt, 2000). Seeds storage through the Seed Bank at PBG aims to contribute to 8th target of the Global Strategy for Plant Conservation GSPC Botanic Garden. One of the locations for seed collection with vegetation similar to PBG for storage in Seed Bank is Alas Purwo National Park (Alas Purwo NP).

Alas Purwo NP is one of the in situ conservation areas with dry lowland forest vegetation in East Java. Plants living in Alas Purwo NP have habitat similar to that of Purwodadi BG with dry lowland climates. According to Tisnawati (2012), more than 700 plant species of 123 families were identified in Alas Purwo NP with additional formation of mangrove and coastal vegetation besides lowland forest vegetation. The available fruits and seeds in Alas Purwo NP are used as food for animals and several plants species are endemic to Alas Purwo NP so they need to be conserved ex situ.

The seed exploration activity at Alas Purwo NP aimed to increase the variety of seeds collection for the Seed Bank storage function in Purwodadi BG and to provide complete information about the biological properties and proper handling of seeds so that they can be stored appropriately and their viability in the Seed Bank is maintained.

MATERIALS AND METHODS

Time and Site of Study

The, which is under the Natural Resources Conservation (NRC) Region III, Jember, East Java. TNAP is located in Tegaldlimo District and Purwoharjo District, Banyuwangi Regency, East Java, Indonesia. Geographically, Alas Purwo NP is located at the southeastern tip of Java Island in the south coast area between 8°26'45"–8°47'00"S and 114°20'16"–114°36'00"E.

Method

The method used was an explorative method consisting of fruits and seeds collection in the field, plant identification, seed extraction and temporary storage of seeds before being transferred to the Seed Bank of the Purwodadi Botanical Gardens. Seed collection in the field was focused on native Indonesian species, particularly those that are endangered, endemic, potential, having conservation value or as complement to the collection of botanical gardens.

Ripe fruits were collected using different collection techniques such as hand picking, using sticks or pruning poles, or by climbing depending on the species. Some fruits were given special treatment before extraction and some were extracted immediately after to prevent the beans from fermenting. The stages of seed extraction for each species were different. The collected seeds were then dried, collected in cloth bags and stored in a portable desiccator so that the moisture content were reduced before being transferred to the Purwodadi Seed Bank for further processing. Plant identification was performed by taking the herbarium.

The method of fruit extraction, morphological characterization of seeds, determination of moisture content using the oven method (ISTA, 2010) and testing of seed viability to determine the storage character of the collected seed specimens were established. The character or behavior of seed storage was supported by available information about the taxa in the Seed Compendium and the Seed Information Database of Kew Millennium Bank (Hong et al. 1988; Liu et al. 2008). The fruits obtained were directly extracted or seeded for stored in conditions according to the characteristics of the seeds. After being stored for several days, the seeds were sown in the greenhouse of the Purwodadi Botanical Garden for viability testing. The seedling medium used was porous sand in a cement tub.

Fruit and seed characterization was described as descriptively based on direct observation. The Seed characters observed were included the number of weight, length, width, the thickness of seeds. The method of extracting each seed is documented and recorded, especially those that require special handling

Materials and tools needed in this study were sacks, plastics, filters, knives, harvesters and portable desiccators. To measure the agro-

ecological conditions of the environment, several tools including GPS, thermo-hygrometer, pH meter, lux meter, altimeter, and camera were used.

RESULTS AND DISCUSSION

Alas Purwo National Park and its surroundings is generally flat although at one location it has steep cliffs, it is located at an altitude of 17-81 m asl and has a quite high level of forest vegetation. The temperature is 26-38^{o}C with air humidity of 61-82%. Soil pH is 5.8-7 and the light intensity of 83.2-3165 lux. The composition of the flora of the Alas Purwo NP and its surroundings is very heterogeneous. The trees species include *Manilkara kauki* (Sawo kecik), *Terminalia catappa* (ketapang), *Calophyllum inophyllum* (nyamplung), *Sterculia foetida* (kepuh), *Barringtonia asiatica* (keben), and many species of bamboos.

In this study, there were forty five (45) species of seeds collection for 12 days of exploration in ten (10) locations at the Alas Purwo NP. The seed collection consisted of 29 families and 32 genera. Some collections were stored in the Purwodadi and Bogor Botanical Garden Seed Bank and other collections were germinated to determine their seeds viability. The moisture content of seeds having a small amount cannot be measured and cannot be stored in the seed bank of the Bogor Botanical Gardens.

Many seed plants were found along the exploration path, but only a few of them met the criteria for seedling. Seeds collected were those in large numbers on the tree or in its habitat so that there would be no scarcity of the seeds. Seeds were taken from tree species bringing benefits as conservation plants and botanical garden collections.

Not all trees produce seeds every year and some may only do so occasionally i.e. they have their own timetable; therefore not all fruits and seeds in the target list can be found. Based on the exploration, there found 45 species of seed plants with 4 categories of habitus, namely large and tall trees, small and not too tall trees (poles), shrubs and herbs or ground cover. The most seeds collected seeds came from shrubs (18 species), followed by trees (15 species), poles (11 species) and ground cover (1 species) (Figure 1). This method of categorization was based on the diameter and height of the plant. In Figure 1, the distribution of plants in which the seeds collected is presented based on their habitus.

It can be seen in Figure 1 that most of the collected seeds came from shrubs. As represented in Figure 2, the exploration results mostly consisted of Moraceae, Annonaceae, and Rubiaceae. Annonaceae and Rubiaceae are dominated by shrubs (Couvreur et al. 2012), while Moraceae provide a comfortable place for the regeneration of other plants beneath them, as studied by Cottee-Jones et al. (2016) where the density of saplings growing below *Ficus* trees were twice compared to ones growing under other non *Ficus* trees thus there are lots of bushes under them. Shrubs usually produce many small and light seeds hence there lies a great opportunity to be blown by the wind and distributed by water or seed-eating animals. Plants frequently found in almost every plot, such as *Polyalthia littoralis*, *Orophea enneandra*, and *Tetracera scandens* were shrubs.

The collected seeds in this study came from 29 families where most of them were Moraceae and Annonaceae as shown in Figure 2. Annonaceae is always present and even dominant in tropical and subtropical rainforests, where it is present in the form of trees, saplings, shrubs, ground covers and lianas (Couvreur et al. 2012). It occupies a large number of plant groups and plays an important ecological role in species diversity, especially in tropical rainforest ecosystems (Nagel 2011; Couvreur et al. 2012). It is also a good source of food and shelter for fauna and even other flora (Handayani, 2018). Moraceae is also a family that often occurs in tropical rain forests and is an important component. *Ficus*, which is one of the genera of Moraceae, plays an important role in ecological restoration (Cottee-Jones et al. 2016). Its fruit and seeds are important food for frugivores (Shanahan et al. 2001; Kinnaird & O 'Brien 2005). The seeds dispersal from frugivores on critical land shows that *Ficus* is an effective restoration agent (Slocum 2001; Cotte-Jones et al. 2016). Study conducted by Cotte-Jones et al. (2016) showing that there was a large number of saplings with high diversity under *Ficus* trees suggests it supports regeneration for plant communities (Corbin & Holl, 2016).

Generally all plots in the exploration area have almost the same environmental conditions as the Purwodadi Botanical Gardens which leads successful nurseries to large extent of seeds collected. In Figure 3, it can be seen that most of the seed had the viability of above 75%. It is because most of the seeds collected were orthodox.

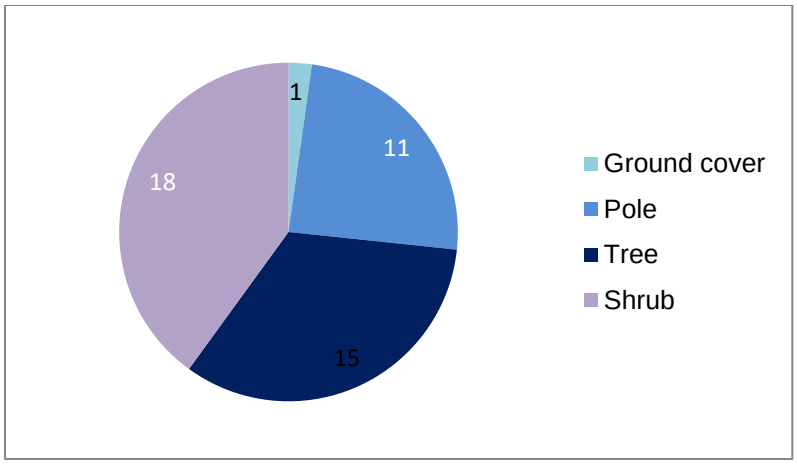

Figure 1: Distribution of Plants where the Seeds Collected Based on Their Habitus

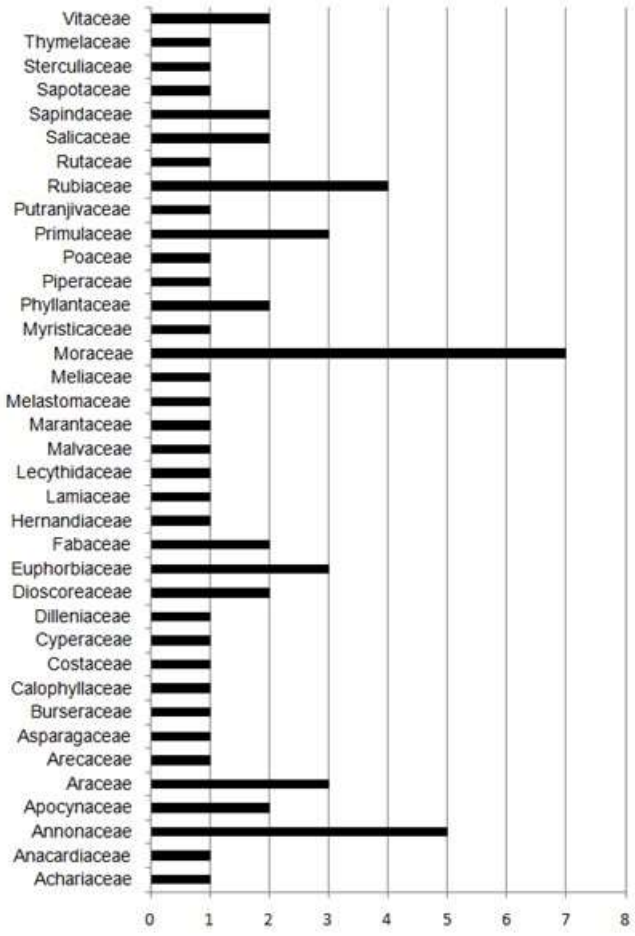

Figure 2: Seeds Collected Based on Their Plant Families

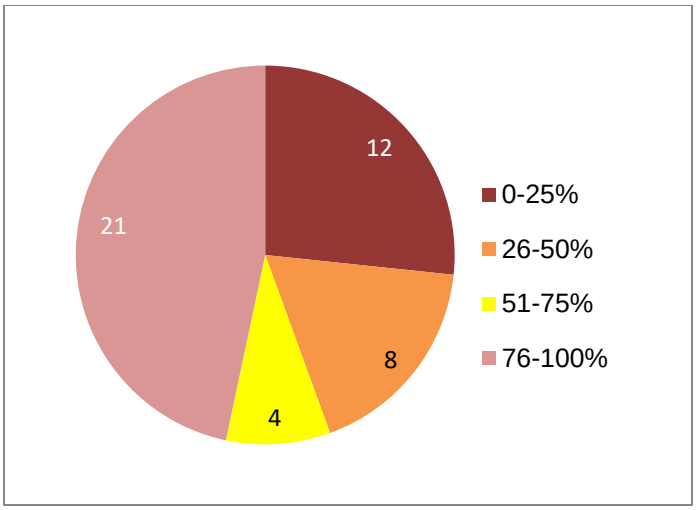

Figure 3: Seeds Composition Based on Viability Percentage

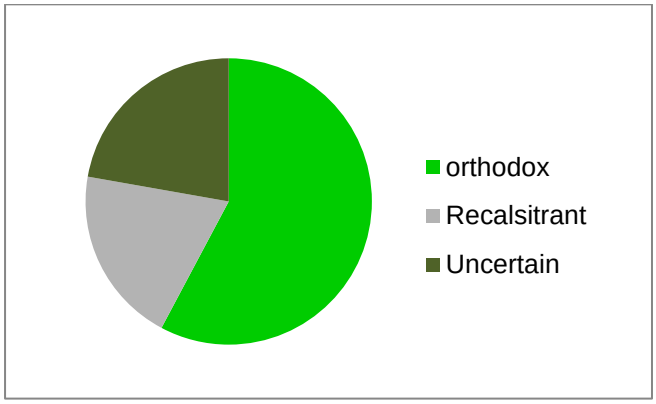

Figure 4: Seeds Composition Based on Storage Behaviour

Orthodox seeds are generally more resistant to low moisture content and have a longer shelf life than recalcitrant ones. Meanwhile, the viability of recalcitrant seeds decreases rapidly if its moisture content declines beyond the critical water content limit (Roberts, 1973). Whereas the handling in field plus a 14-day trip, we could only use fairly simple methods such as using tissue, cotton, or moss to maintain moisture or water content of recalcitrant seeds thence some recalcitrant seeds could not maintain their viability. The composition of orthodox and recalcitrant seeds is presented in Figure 4.

Only a few of the recalcitrant seeds such as *Spondias pinnata*, *Uvaria grandiflora*, *Polyalthia littoralis*, *Corypha utan*, *Ixora smeruensis*, and *Phaleria capitata* had a viability of up to 75%, and some did not even germinate after being sown. The same also counted for some orthodox seeds since they need some pretreatment such as the requirement of a certain storage period or scarification to break seed dormancy. Dormant seeds are unable to germinate in a certain time despite their favorable environmental factors (temperature, humidity, light, etc.). This dormant condition becomes advantageous when the seeds become inactive when stored. According to Baskin and Baskin (2014), seed dormancy is divided into 5 classes, namely physiological, morphological, morphophysiological, physical, and combination of physical and physiological. Seeds undergoing physiological dormancy are still permeable for water but there is an inhibitory mechanism in the embryo whereby the radicles cannot appear. This kind of dormancy can be broken by the cold damp stratification method (Baskin and Baskin, 1991; Walck, et al., 2002). Morphological dormancy caused by immature or immature embryos can be broken by storing seeds until maturity; whereas physical dormancy caused by water blockage from entering the seed because one or more layers of palisade cells in the seed or fruit coat are difficult to penetrate,

causing the failure of seed germination, can be broken by immersion in water or chemicals (Baskin el al. 2000). The combination of physiological and morphological dormancy is called morphophysiological dormancy.

Collected orthodox seeds, such as *Pangium edule*, and *Ochrosia ackeringae, Corypha Utan* have hard seeds and may require pretreatment. Each fruit of *Pangium edule* contains 13-40 hard stony, reticulated, ribbed, triangular-ovoid seeds (3.5-6.0 cm), embedded in a pungent mesocarp (Van Heel, 1974; Hore et al. 1985; Faridah-Hanum, 1996); while *Ochrosia ackeringae* is the only species of *Ochrosia* with hemisyncarpous fruit, its endocarps are not split into diverging fibres, hard, thick, massive, surrounding two lateral cavities (Hendrian, 2004). Hanson (1983) stated that thick endocarp contains two lateral cavities filled with spongy tissue. This seed structure will certainly inhibit the imbibition process to stimulate embryo germination. Such nature of the testa/endocarp causes a low skin permeability to oxygen and a low rate of respiration thus the seeds remain dormant. A study conducted by Naiola and Nurhidayaf (2009) on *Corypha utan* seeds from Timor Island, East Nusa Tenggara showed that only 20% or slightly above 20% of *Corypha utan* seeds contain embryos. This value of 20% is obtained from a preliminary study (Naiola et al. 2006). The phenomenon of the embryolessness in seeds is explained by Gomez-Cadenas et al. (2001) as a result of the phytohormones interaction. Physiologically ripe *Corypha utan* seeds have a very hard testa/endocarp covering the entire endosperm which is composed of a very hard lignocellulose. In the process of seed germination in nature, *Fusarium* is believed to be a microbial agent to break down their endocarp and endosperm (Naiola & Nurhidayaf, 2009).

Among the 45 seeds-producing species found, some of them were abundant and available in almost every site of exploration, including: *Piper retrofractum* Vahl, *Allophylus cobbe* (L.) Raeusch, *Tetracera scandens* Linn. Merr., *Orophea enneandra* Blume, and *Polyalthia littoralis* (Blume) Boerl. Their small seeds are easily dispersed by water and wind. In addition, their interesting colors of ripe fruits attract many animals such as squirrels, birds, beetles, and so on and help the process of spreading the seeds. Moreover, these species are suitable for growing in tropical rainforest ecosystem and are easy to germinate.

Based on desiccation sensitivity, seeds of all those five species are classified as orthodox, except *Polyalthia littoralis*; and three of them, namely *T. scandens, O. enneandra, P. retrofractum* are shrub. In line with result, a study conducted by Kay et al. (1988) showed that 115 shrub species of 29 families native to the Mojave Desert had orthodox seeds. This storage behavior classification is also based on association of taxonomic classification and seed storage behavior which state that most of the Piperaceae, Sapindaceae, and Dilleniaceae are orthodox, while Annonaceae can be classified to orthodox and recalcitrant (Hong et al. 1996). Seed moisture content at maturity or shedding also affects seed storage behavior in which species with recalcitrant seed has moisture contents between 36 and 90%, moisture contents of harvested seed of around 20% or below are very likely in to the orthodox group, although there are several species with moisture content <35% are unlikely to show recalcitrant seed storage (Hong & Ellis, 1995). A study demonstrated that the two categories of seeds have different water content, i.e. the water content of orthodox seeds was below 20%, while the recalcitrant was 38%. The orthodox seeds showed a gradual decrease in moisture content after being desiccated showed, not like recalcitrant ones which showed a sharp decrease (Hong et al. 1996), in other words the low initial of moisture content is sensitive to drying while the high initial moisture content is not sensitive to drying (Lan et al. 2014). As recalcitrant seeds are shed at high moisture content, they are highly sensitive to desiccation (Chaitanya et al., 2000 a, b; Greggains et al., 2001). In this study, orthodox seeds had long life span in dry storage for 14 days of trip and more than 75% of them could germinate except for *O. enneandra* which may require pretreatment to break dormancy or break the thick seed coat. While *P. littoralis*, recalcitrant seeds, could easily germinate 2 weeks after being sown. Recalcitrant seeds cannot be stored too long in the desiccators since the moisture should always be maintained and they must be prevented from drying out. Three other orthodox seeds species, *T. scandens, A. cobbe, P. retrofractum*, have thin seed coat and it is usually associated with rapid germination since it may be an advantageous for rapid water uptake and germination (Pritchard et al. 2004; Daws et al. 2005)

Piper retrofractum Vahl

P. retrofractum Vahl is a dicotyledon flower plant belonging to the Piperaceae. In Indonesia, it is known as Javanese chili. It is easily found in

Indonesia and contains about 2.03 to 3.65% piperine, a compound causing spicy taste (Wood, *et al.,* 1988; Zuchri, 2008; Suwijiyo, 2008). Piperine and piplartin also serve as an antioxidant which protect body cells from free radical damage thus acting as natural oral anticancer (Khajuria, et al. 1998*;* Vogel, 2002;Bidarisugma et al. 2011). In addition, *P. retrofractum* Vahl contains bioactive compounds against pathogenic microorganisms (Panphut et al. 2020). In East Java, *P. retrofractum* in widely cultivated in Lamongan and Madura (Zuchri, 2008) in which the products are utilized domestically and exported to various countries such as Singapore, Hongkong, Malaysia, and India (Soleh, 2003). *P. retrofractum* produces fruits throughout the year (Januwati and Effendi, 1992)

P. retrofractum can grow well at below 600 m asl, temperature 20 – 30^{o}C, 1.200- 3.000 mm/year rainfall and 40-80% humidity (Djauhariya and Rosman 2008; Evizal, 2013). It has high adaptability in soil with a range of acidic to alkaline pH (4-8), infertile soil, rocky, and dry climate as well as on a variety of climbing host with sandy, porous, and well-drained clay texture (Evizal, 2013).

P. retrofractum is a climbing plant, the fruits are finger shaped with a length of 4.5–5.2 cm and a 0.3–0.5 cm diameter. The young fruit is of green color and a small size and the ripe fruits become of a red-orange color. It is a multiple fruit development and has multiple seed orientation surrounded the same receptacle. The fruit cross section structure is surrounded by a number of ovules (± 3-8 seeds). The seeds can reach 80-135 fruits and not all ovaries will become seeds. The round seeds are ivory yellow and ± 1 mm in diameter. Fruit flesh and especially seeds (inside) are spicy (Panphut, 2020;Haryudian and Rostiana, 2009; Zuchri, 2008: Suwijiyo, 2008).

***Allophylus cobbe* (L.) Raeusch**

A.Cobbe is a member of Sapindaceae. *A. cobbe* has a highly variable habitus, but mostly shrubs, some are climbing plants with stem up to 4 cm in diameter, and few of them are tree can be up 25 meters tall. Commonly found on sandy beaches and coastal rocks, in and along the Barringtonia formation, in brackish as well as freshwater swamps, in open places, shrubberies, and in secondary as well as primary forests of all kinds, from sea level up to 1500-2000 m altitude (Leenhouts, 1994).

Fruit globose to obovoid, coriaceous, at most 0.4-1.3 by 0.3-0.8 cm. Fruits mostly with only 1 mericarp developed, globular to obovoid and narrowed at the base (larger ones), smooth to slightly wrinkled, red (black to brown when dry), somewhat fleshy and almost glabrous when ripe (Leenhouts, 1994). Annual flowering and fruiting: July-November. Bees visit their flowers and the birds eat their fruits (Sasidharan, 2004).

Wild plants are harvested for use as a medicine, food and source of wood. The wood is hard but not very durable; it is mainly used as a timber for temporary structures and indoors. The pulped leaves, or an extraction or decoction of them, as well as a decoction of the roots and the bark, are used in native medicine against stomachache and fever. The berries, though a bit sour, are edible. In New Guinea, fruits are used as fish poison (Leenhouts, 1994).

3. *Tetracera scandens* Linn. Merr.

T. Scandens belongs to Dilleniaceae. It is liana (up to 30 m) or small shrub (up to 2 m). The glabrous-fruited form. Carpels with 0.4-0.7 mm long rigid hairs; ovules ca 10. Capsule ovoid, ca 10 by 6 mm, (l-2)-seeded. Seeds 4 by 3 mm. Aril 2-3 mm long, creeper or climber in thickets and secondary forests, especially on riverbanks and near the seacoast; in more open, it forms small shrubs vegetation. Its habitat is from sea-level up to 1000 m,rarely above 500 m (Hoogland. 1951). The leaves are used for polishing wood and metal. The stems may be used as cordage, traditional medicine to treat diarrhea symptoms and showed high inhibitory activity (Muliyah, et al. 2017)

***Orophea enneandra* Blume**

O. enneandra is a member of Annonaceae. *O. enneandra* produces flowers and fruits throughout the year in PBG. Several types of *O. enneandra* collection in Purwodadi Botanic Garden came from National Parks Banyuwangi (Lestari,2011). *O. enneandra* is one of 30 species of *Orophea* which characterized by the presence of an indument on the young shoots and carpels, six ovules per carpel, and cylindrical fruits and seeds (Mols et al. 2004).

***Polyalthia littoralis* (Blume) Boerl**

Polyalthia littoralis is a member of Annonaceae. Usually it is evergreen shrub or small tree to 5 m tall, simple rough leaves and small, white flowers, the fruits are bright red when ripe. It is native to rainforest margins in southern China and Indochina to 800 m and best suited to tropical climates hence it is mostly found in

Southeast Asia. It is a fast growing species and suitable for plantation crop (Heyne 1987).

Based on some preliminary tests, it is known that *P. littoralis* seeds are recalcitrant (Handayani 2004). As all recalcitrant seeds, its seeds have some limitations such as rapid decline in their seed viability along with the decrease of moisture content and cell damage due to drying and low temperatures (Walters et al. 2013). Based on the observation of germination type, its seeds are epigeal i.e. the cotyledons are raised above the surface of the growing medium. Its seeds are polyembryonic thus the seedlings have 2-4 roots. It takes 5-7 weeks for the radicle to appear until the opening of the first leaf (Handayani 2004).

Common uses of the *Polyalthia* are traditional medicine, house building, furniture, veneer, and plywood. Some of its leaf extracts are known to have insecticidal effects (Lemmens and Bunyapraphatsara 2003). The results of the study proved that extract solution of *P. littoralis*'s seed is toxic to the *Coptotermes curvignathus*, a subterranean termite, thus it can be used as an alternative to environmentally friendly preservatives both for use under roofs and in open spaces (Sari, 2016)

CONCLUSION

There were 45 species of seeds collected from Alas Purwo National Park, most of which were shrub, which dominated by Annonaceae, Moraceae, Rubiaceae, and Phyllantaceae. Based on the seed storage behavior, most of the seed were orthodox, hence it was possible for the seeds to maintain their viability after 14 days of trip, as evidenced by the germination rate above 75% at most of collected seeds. Some seeds had special characteristics in their extraction because of the different morphological properties of the seeds and fruits. Most of the seeds obtained from nearshore ecosystem were easy to extract because the seeds were quite dry.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Darmayanti, A.S. is a main author who explorated the data, drafted the manuscript, analized and interpretated of the data. Lestari, D.A is a second author who explorated the data, designed the method and processing data. Firdiana , E.R is a third author who analized the data and revised English. All authors read and approved the final version

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