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Short Communications

Seed Conservation of *Anaxagorea luzonensis* A. Gray (Annonaceae) Through Storage Behaviour and Morphology

Dewi Ayu Lestari[1]*, Ananda Kautsar Nadia Faya[2]
1)Research Center for Applied Botany, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta– Bogor Km. 46, Cibinong, Bogor 16911, West Java
2)Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Jl. Veteran 65145, Malang, East Java
* Corresponding author, email: chunyang.dee@gmail.com

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ABSTRACT
Fruits and seeds morphological traits and seed storage behaviour of *Anaxagorea luzonensis* are important for seed and plant conservation. Fruits and seeds characterization using a digital microscope and morphometry method. Seed storage behaviour was determined using 100-seed test method. The findings revealed that the colour of *A. luzonensis* fruit did not change and *A. luzonensis* seed was glossy, brittle, black in colour, 9.05 x 5.89 x 2.93 mm sized and oval. The seeds are desiccation-tolerant and are categorized as orthodox seeds. These results can become the findings of *A. luzonensis* seed storage behaviour, because information about it never existed before. Knowing the seed storage behaviour and seed morphology of *A. luzonensis* is essential so that the recommendation of conservation efforts at PBG in the future are covering the fruit, modifying the environment, breaking dormant seeds, vegetative propagation, and preserving seeds.

Anaxagorea is one of the genus from Annonaceae family (the soursop group), which has 43 species that are generally found in Indo-China, Borneo, Java, Sumatra, Philippines, Thailand, Burma, Ceylon, and tropical America (Husain et al. 2012). *Anaxagorea luzonensis* A. Gray is one of the plant collections from Annonaceae family where conserved in Purwodadi Botanic Garden (PBG), East Java, and become a new plant collection since 2014 (Lestari 2014). This species has shrub habitus, brown cylindrical stems with single and alternate leaves. Based on the information from PBG Registration Unit (2022), there is one specimen of *A. luzonensis* in PBG and categorized as critical plant collection. According to the IUCN Red List, *A. luzonensis* is categorized as Least Concern (LC) for conservation status (Verspagen & Erkens 2020). Barks of *A. luzonensis* have been used as blood tonic, stomachic, antipyretic, and treat of muscle pain. Stems of this species also contain xanthones and flavonoids, both of which have antioxidant properties (Gonda et al. 2000). The leaves can be used as medicine to treat articular rheumatism and can be used for postpartum care (Aziz et al. 2016).

Considering its conservation status and the utilization of *A. lu-*

zonensis, ex-situ conservation activities are required through a study of seed morphology and storage behaviour which is included in reproductive biology research. Reproductive biology research can be used to propagate plants for conservation purposes. Monitoring and research about reproductive traits of living collection in botanic garden is one of the factors that encourage prolific breeding and long-term maintenance. Fruit, seed, and germination traits are connected to plant reproduction which are crucial for plant dispersal (Xu & Zang 2023). Fruit and seed characteristics may be used as a potential tool to forecast dispersal mechanisms for conservation and restoration of biodiversity. In addition, other plant characteristics such as size of fruits and seeds, might influence seed dispersal. Size variation can have an impact on dispersal distance, because small seeds are often dispersed farther by water or wind but large seeds are dispersed by animals (Leslie et al. 2017). de Jager et al. (2019) reveal that in warm lowlands, large seeds generally spread more widely than small seeds when carried by flowing water. Besides that, large seeds that come from open-growth environments will be more adaptable than small seeds (Smith et al. 2022). Small seeds are preferred by rodents as dispersal agent in the zoochory mechanism (Lang & Wang 2016). Seed dispersal techniques are positively associated with size, mass, and form of seed (Liu et al. 2014). So far, research or study about fruit and seed characteristics, seed storage character and germination of *A. luzonensis* has never been reported, especially in PBG.

Proper seed storage is essential and will be able to protect germplasm in the form of seeds. The seeds storage condition also relies on several variables, including oxygen pressure, moisture content, storage temperature, and storage period. However, the most important factor when storing seeds is the tolerance level of seed towards to desiccation or drying. This factor aims to know the seed storage character. Based on the drying tolerance, seeds are categorized into three groups, i.e., orthodox seeds, recalcitrant seeds, and intermediate seeds. Orthodox seeds are tolerant-drying seeds, can be stored for a long time at -10°C and can endure drying to a moisture content below 7%. In contrast to orthodox seeds, recalcitrant seeds are extremely sensitive to drying and freezing because they can quickly lose their viability when kept in conventional method. Metabolic functions of recalcitrant seeds will be active during fruit development until harvesting. But intermediate seeds lose their viability rather quickly than orthodox seeds (Kijak & Ratajczak 2020). Recalcitrant seeds do not experience water content loss during the ripening process, so that its very sensitive to the seed desiccation. Therefore, the distribution of recalcitrant seeds is limited to the tropical forests. The differences of desiccation tolerance in orthodox, intermediate and recalcitrant seeds are crucial for long-term seed storage, biodiversity protection, and seed conservation (Smolikova et al. 2021).

Aims of this study are to know the morphological traits of *A. luzonensis* fruit and seeds, to determine the storage behaviour of *A. luzonensis* seeds, and to recommend the conservation efforts in PBG. This is required to ensure the long-term viability of seeds and plant collections in PBG. The findings of this study are recommended as a fundamental guide for managing plant collection, especially for *A. luzonensis*, in supporting seed and plant conservation at PBG.

Fruit of *A. luzonensis* was harvested from plant collection in block XVIII.E.26 at PBG, East Java (Figure 1). Fruit of *A. luzonensis* is categorized as dehiscent fruit, when fruits that open up at maturity in order to release their content (Figure 2). This is because *A. luzonensis* seeds are dispersed by explosion (ballistically), at a maximum distance of 5-7 meters from the mother plant (Gottsberger 2016).

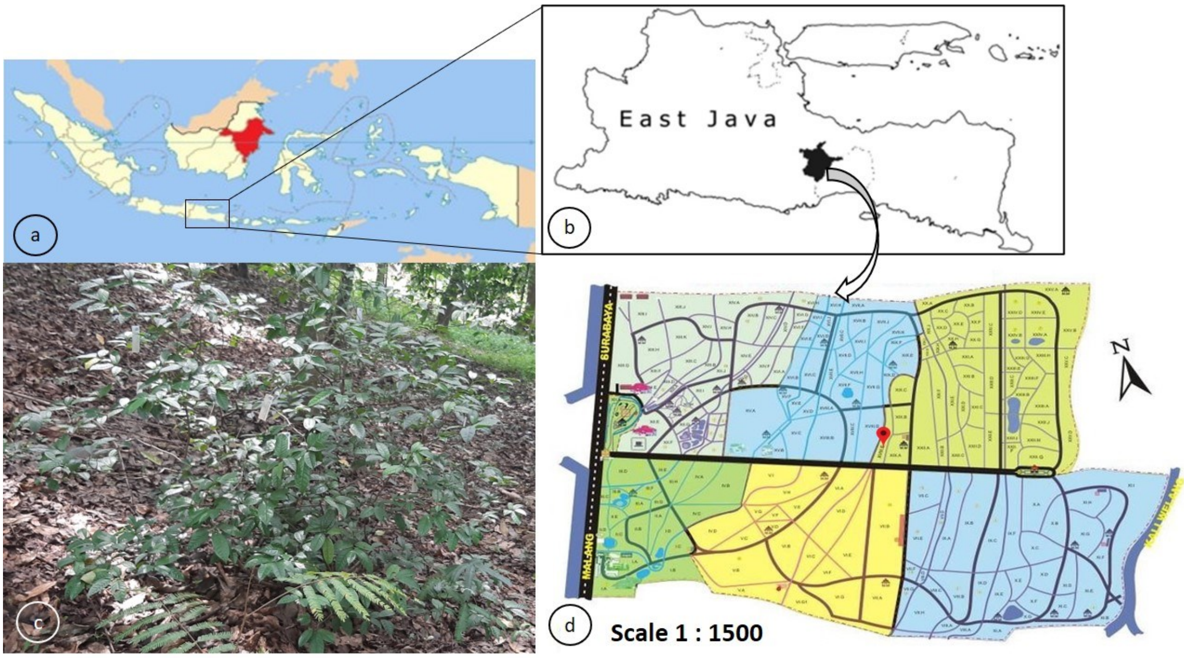

Figure 1. Study site of *Anaxagorea luzonensis* in Purwodadi Botanic Garden; a. map of Indonesia, b. map of East Java, c. habitus of *Anaxagorea luzonensis* in block XVIII.E.26, and d. map of Purwodadi Botanic Garden (location symbol).

Figure 2. Peels of *Anaxagorea luzonensis* fruit at maturity and ready to harvest.

Morphological characterization of *A. luzonensis* fruits and seeds was done in laboratory of PBG in March 2022. The morphological characteristics of fifty fruits were determined, including size (length, width, and thickness of fruit), colour, texture and shape of fruit, and the quantity of seeds in each fruit. Seed extraction is done manually with hand, by separating seeds from peel and allowing them to air-dry at ambient temperature. According to Lestari (2013), 100 seeds are taken randomly and characterized using morphometry method. The morphological characteristics were observed include size (length, width, and thickness), colour, texture, shape, and condition of the seed being observed (the whole or part of the seed). The morphology of internal seeds was examined using the Dino lite AM-311 digital microscope, by cutting cross-sections of the seeds using scalpel. The detail of internal seed was described in the figure from seed coat to the deepest part of *A. luzonensis.*

Seed storage behaviour study of *A. luzonensis* was done in laboratory and greenhouse in PBG from March until October 2022. The defined *A. luzonensis* seeds were sown using the 100-seed test method to determine seed storage behaviour (Pritchard et al. 2004). A total of 100 seeds are divided into four categories, i.e., 10 seeds for initial moisture content, 26 seeds (13 seeds per replication) for initial germination, 32 seeds for desiccation with silica gel (6 seeds for moisture content after desiccation and 26 seeds for germination after desiccation), and 32 seeds for controlled humidity storage treatment. Desiccated *A. luzonensis* seeds were packed in a calico bag and placed in a desiccator with silica gel, with the weight of the silica gel being equal to the weight of the seeds to be inserted into the desiccator. The seeds were weighed once every three days, and the process was ended when the weight was steady. The *A. luzonensis* seeds, on the other hand, were enclosed in black fabric bags and placed in a desiccator filled with vermiculite with storage treatment in controlled humidity. The percentage of humidity is kept at 8-10%. The desiccator was opened once every three days to maintain air aeration in the desiccator. After assessing the moisture content of the seeds destructively using the oven method at 108 °C for 18 hours, *A. luzonensis* seeds for initial germination were sowed (ISTA 2015) (Formula 1). *A. luzonensis* seeds with desiccation and controlled humidity treatment are sowed simultaneously whereas after weight of seeds in desiccation treatment are stable. Seeds were sown in straw paper medium. Straw paper is the medium testing for seed germination. When the paper straw medium appears dry during germination period, the seeds are watered with aquadest. The germination parameters observed include the initial seed count for germination and the total number of seeds germinating for each treatment. Calculation of seed germination percentage using (Sutopo 2010) (Formula 2). All data is processed and analysed using Microsoft Excel 2017 with mean and standard deviation analysis. The resultant curve from seed germination result is adjusted the graph according to (Pritchard et al. 2004).

$$seed\ water\ content\ (\%) = \frac{\mathrm{M2} - \mathrm{M3}}{\mathrm{M2} - \mathrm{M1}} x\ 100\%$$

…………………….. Formula 1

Where M1 = the weight of petridish, M2 = the weight of seed and petridish before the oven and M3 = the weight of seed and petridish after the oven

$$germination\ percentage = \frac{\text{number of germinated seeds}}{\text{number of seeds sown}} x\ 100\%$$

…. Formula 2

Plant collection of an *A. luzonensis* in PBG produce fruit twice a year in 2022, at the beginning of the year (February–March) and at the end of the year (August–September), resulting in a twice-yearly fruiting period. This is consistent with Lestari (2019) who reported that *A. luzonensis* fruiting twice a year throughout the year in a sub-annual cycle. A pattern in the phenological period of blooming and fruiting that happens more than once a year is known as a sub-annual pattern (Newstrom et al. 1994; Barbosa et al. 2018). When *A. luzonensis* reaches fruiting stage, the colour of fruit does not change until maturity (dehiscent fruit) and the fruit is yellowish-green in colour (Table 1; Figure 3a). Seed of *A. luzonensis* is forced out by the fruit through a ballistic process, ripe fruit is distinguished by the shattering of peel. Seed morphology of *A. luzonensis* is characterized by seed coat colour is shiny black, ovate, which has mean seed size of 9.05 x 5.89 x 2.93 mm (Table 1; Figure 3b). According to Lestari (2014), ripe fruit of *A. luzonensis* is dark green and ranges in size from 2.5 to 4.4 cm, seeds are shiny black with a little white hilum at the tip of the seed. Scharaschkin and Doyle (2006) claimed that seeds from the *Anaxagorea* group have two seeds linked by having seeds that are

Table 1. Morphological character of *Anaxagorea luzonensis* fruits and seeds.

Morphological character	Fruits	Seeds
Quantitative character		
Length (mm)	22.44 ± 2.76	9.05 ± 0.52
Width (mm)	7.27 ± 0.66	5.89 ± 0.41
Thickness (mm)	6.83 ± 0.75	2.93 ± 0.51
Sum of seeds in one fruit	1.72 ± 0.45	-
Qualitative character		
Colour	green yellowish	black
Surface	glabrous	glabrous, shiny
Shape	spatulate	ovate
Condition	rind is broken	a flat surface facing one other in one fruit

Figure 3. Fruits and seeds of *Anaxagorea luzonensis*; a. ripe fruit, b. seeds, and c. seed cross section with Dinolite (scale bar = 50 mm).

asymmetrical and have a flat surface facing one other in one fruit. As with plants species from *Hura crepitans* or *Hevea* spp., which feature an active burst mechanism in which the fruit will release its seeds at a substantial distance from the parent plant, *A. luzonensis* seeds are categorized as an active autochory dispersal type (Parolin et al. 2013).

Cross-sectional images of *A. luzonensis* seeds were taken with Dino-lite digital microscope revealed ruminate endosperm, which is a feature of seeds from the Annonaceae family (Figure 3c-ii). Ruminate endosperm is a distinguishing feature of Annonaceae seeds and can be used to identify them (Gottsberger 2016; Johnson & Murray 2018; Lestari & Pratiwi 2022). The seed coat is fragile, thin and shiny black colour (Figure 3c-i), and an embryo and a microphyllar plug are also present (Figure 3c-iv). An embryo of *A. luzonensis* has white colour (Figure 3c-iii).

The amount of water in a seed that determines its viability and ability to be stored for a certain period is known as seed water content. Initial water content of *A. luzonensis* seeds is 7.91% and decline to 5.65% after desiccation treatment. However, after being stored in controlled humidity treatment, seed water content of *A. luzonensis* seeds increased to 8.88% (Table 2). This happens as a result of the seeds absorbing water from the substrate. The water content of *Mauritia flexuosa* seeds reduced by up to 25% after desiccation (de Almeida et al. 2018). The amount of *Coccoloba gigantifolia* seeds decreased after 6 days of desiccation (Ferreira et al. 2021). Seed water content value declined and will be followed by a decrease in seed germination percentage, both seeds were treated with desiccation or at controlled humidity up to range of 38-46% (Table 2). When seeds are dried in a desiccator using silica gel, their water content will decline, and therefore their chance of germinating will decrease (Asomaning & Sacande 2019).

Table 2. Water content and germination percentage of *Anaxagorea luzonensis* seeds based on 100-seed test method.

Treatment	Seed water content (%)	Seed germination percentage (%)
Initial	7.91 ± 0.08	50 ± 5.44
Desiccation	5.65 ± 0.29	12 ± 5.40
Storage with controlled	8.88 ± 0.53	3.85 ± 5.44

The 100-seed test method can be used as an initial screening method for seeds storage behaviour or characteristics of various species of seeds. It is used to determine the seed storage characteristics with a small number of seed lots i.e., minimum of 100 seeds (Pritchard et al. 2004; Wardani & Mimin 2020). Figure 4 demonstrates that *A. luzonensis* seeds are categorized as orthodox seeds. This is demonstrated by the curve of the percentage of germination during desiccation treatment, which can still germinate because the seeds are desiccation-tolerant. The seed is categorized as a recalcitrant seed if the desiccation treatment's germination curve is unable to demonstrate germination. Seed storage behaviour of *A. luzonensis* seeds is likewise not accessible, according to Seed Information Database from Royal Botanic Gardens, Kew.

After being desiccated and stored in controlled humidity treatment, the seed germination of *A. luzonensis* seeds decreased. The percentage of seeds that germinate will decline when kept in vermiculite at ambient temperature with controlled humidity (Yuan et al. 2019). González-Morales et al. (2016) reported that following desiccation and storage for a week, seeds will lose their viability and decline by at least 5%.

Based on Figure 4, initial germination of *A. luzonensis* seed occurs in 8 weeks after sown. This shows that this seed has slow germination rate and has physical dormancy. Germination can be accelerated through seed breaking dormancy with physical dormancy treatment using physical or mechanical scarification and immersion in the chemical solution. Seed germination of *A. luzonensis* categorized as–epigeal type germination (Figure 5), the cotyledons are raised above the soil surface for the first time during germination. Some species from Annonaceae family have epigeal germination type, such as *Annona montana*, *Artabotrys hexapetalus*, *Cananga odorata*, *Fissistigma latifolium*, *Uvaria purpurea*, *U. micranthum* etc. (Handayani 2019; Pratiwi et al. 2022).

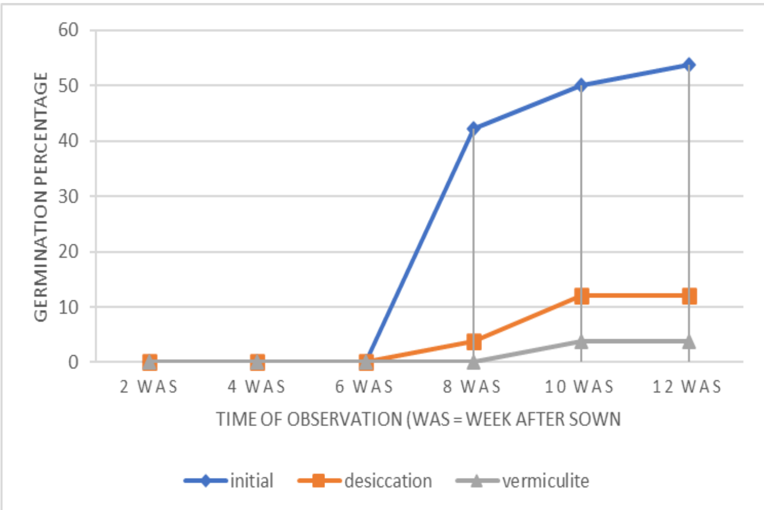

Figure 4. Curve of seed germination percentage of *Anaxagorea luzonensis* with 100-seed test method.

Figure 5. Epigeal germination type of *Anaxagorea luzonensis*

Based on this study, some of conservation efforts can be carried out with point of view from seed dispersal mechanism, fruit and seed morphology, and seed storage behaviour. These conservation efforts can support plant collection management scientifically in PBG, especially for *A. luzonensis*. Some of conservation efforts that can be taken are covering the fruit, modifying area around *A. luzonensis* plant collection, further research about breaking dormant seeds, vegetative propagation, and preserving seeds for long-term conservation.

Fruit of *A. luzonensis* is covered when fruit is in immature phase. This endeavour was made because *A. luzonensis* seed has the ballistic (explosive) type in seed dispersal, so that seeds would be accommodated in the fruit cover if the fruit had been shattered and ripe. The mesh fruit cover is one of the options for use to cover fruit, which aims to improve plant growth in the form of fruit quality and to reduce pests and other environmental factors that could lead to fruit abortion before the fruit is dispersed (Setiawan 2018).

Besides covering the fruit, other conservation efforts that can be done are modifing an environmental area around plant collections through reducing the shade plants around *A. luzonensis* plant collection, fertilizing with growth hormones to promote flowers and fruits development, and pruning to rejuvenate plant collection thus stimulating the growth of shoots. Reducing amount of shade plants around *A. luzonensis* plant collection is required because the canopy cover around *A. luzonensis* is lush and require full sun to support plant growth and development.

Manure or organic fertilizers and growth regulators including auxins, gibberellins, and cytokinin, which can be sprayed on plants frequently, can all be used to fertilize plants. To improve the quality and quantity of flower and fruit production, natural growth regulator such as such as coconut water can also be employed as a natural plant hormone (Njoku & Okorie 2021). Fruits are abundant with high quality will produce more seeds, which can be saved for future use in seed conservation.

Considering the low seed production of *A. luzonensis* (<500 seeds per harvest) in PBG and the fact that there is only one plant specimen of *A. luzonensis* in PBG, it is necessary to propagate this plant through vegetative propagation. Several methods can be used to propagate are stem cuttings or tissue culture. Tissue culture will increase the number of seedlings that can be reproduced and ensure that they inherit their parents' genetic traits (Tefera 2019; Rather et al. 2022).

A. luzonensis seeds had very slow germination rate and required very long time to germinate. This suggests that further study of *A. luzonensis* seeds is required to break seed dormancy so that seeds can germinate more quickly and simultaneously. The findings of this study indicate that *A. luzonensis* seeds are orthodox seeds, which typically have dormant phase. Therefore, further research can be conducted in breaking seed dormancy research with a variety of scarification techniques such as mechanical, chemical, and physical treatment. Orthodox seeds can be scarified using a variety of techniques (Side et al. 2021), likewise with *A. luzonensis* seeds.

AUTHORS CONTRIBUTION

D.A.L. designed and conceptualization the research, conducted field observation, collected and analysed data, wrote and review the original manuscript. A.K.N.F. collected data, wrote and review the original manuscript.

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CONFLICT OF INTEREST

The authors state no conflict of interest from this manuscript.

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