

Flowering and fruiting phenology of *Anaxagorea luzonensis* A. Gray (Annonaceae)

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Manuscript received: 10 December 2022. Revision accepted: 3 February 2023.

Abstract. Lestari DA, Ningrum LW, Nada FMH, Pradipta NN, Harsono DR. 2023. Flowering and fruiting phenology of *Anaxagorea luzonensis* A. Gray (Annonaceae). *Biodiversitas* 24: 784-792. *Anaxagorea luzonensis* is one of the critical plant collections from the Annonaceae family, with only one specimen in Purwodadi Botanic Garden (PBG). In protecting these plants, it is necessary to know the process of flowering and fruiting through the phenological process. This research is expected to observe plant propagation, physiology, environmental response, and increased reproductive success. The aim of this research is to know the details of flowering and fruiting phenology stages, determine reproductive success, and explain the floral biology of *A. luzonensis*. Research on the flowering and fruiting phenology of *A. luzonensis* was carried out from December 2021 - November 2022 in one season at PBG. The method is observative, including changes in color, shape, and size of flowers and fruit in each phenological stage. In addition, the method includes the count of reproductive success and observation of the *A. luzonensis* flower. This study's results stated that the flowering and fruiting phenology of *A. luzonensis* experienced a shorter flowering time than fruiting time and a low percentage (<30%) of reproductive success. Flowering stages are divided into five stages, i.e. initiation, flower bud, before bloom, bloom, and anthesis; fruiting stages are divided into three stages, i.e. immature-sized, mature-sized, and ripe fruit, which occurred in period 73-121 ± 85 days. Environmental and genetic factors cause the low value of reproductive success. This research can be useful for the conservation strategy of *A. luzonensis*, especially for reproduction biology and seed conservation.

Keywords: Conservation, morphology, pollination, Purwodadi Botanic Garden, reproduction

INTRODUCTION

Purwodadi Botanic Garden (PBG) is an 85 hectares garden located in Pasuruan, East Java Province, Indonesia. It is an ex-situ plant conservation area in the dry lowlands with various plant habitus collected, from herbs to trees. This botanic garden functions as a conservation spot, tourism, and environmental education area and has an important role in preserving biodiversity, especially endangered (threatened) species. (Indrawardani et al. 2019). Based on registration data from 2022, PBG has plant collections from 191 families, 935 genera, 11,023 specimens, and 6,088 collection numbers. From this plant collection, 590 collection numbers are included in the critical plant collection, which is a plant collection that only has one plant specimen in PBG. One of the critical plant collections is *Anaxagorea luzonensis* A. Gray. Unfortunately, information about this species has not been widely published.

Anaxagorea luzonensis is a species from the Annonaceae plant family, which is ex-situ conserved in PBG. In 2018, *A. luzonensis* was added to the IUCN Red List of Threatened Species, with its conservation status

listed as a Least Concern species (reference). The species is distributed along Andaman, Bangladesh, Borneo, Java, Sulawesi, Maluku, Laos, Myanmar, Philippines, Sri Lanka, Thailand, Vietnam, Cambodia, to Southeast China (Powo 2022). *Anaxagorea luzonensis* is also found in Wawonii Island, Southeast Sulawesi, based on exploration activities by Rugayah (2014). Annonaceae is a tropical plant family, i.e. it is found in countries with tropical climates, including Indonesia (Johnson et al. 2021). Species of the Annonaceae family have many advantages, i.e. as a source of wood, edible fruit, biopesticides, ornamental plants and producers of spices, roadside plants, cosmetic and perfume ingredients, and traditional medicines (Moghadamtsousi et al. 2015; Al Kazman et al. 2022). In addition, it contains an anti-oxidant as a blood tonic, stomachic, antipyretic, and for treatment of muscular pain (Gonda 2000).

Many species of Annonaceae have an abundance of flowers and certain time of fertilization processes. In terms of flower abundance, members of the Annonaceae family have had little changes in flowering peak and frequency of flowering and fruiting plants for the past eight years observations by Lestari (2019). One of the main factors affecting the reproduction of Annonaceae is the

asynchronous flowering and the anthesis period, especially between male and female flowers (Handayani 2016). However, collections of Annonaceae originating from East Java in PBG have several problems, such as the generative character not fully appearing and becoming an obstacle to identifying species based on morphological characters (Lestari et al. 2018). Based on Lestari (2019), *A. luzonensis* experiences flowering and fruiting two times (twice a year) with irregular classes, namely annual frequency (for the flowering pattern) and sub-annual frequency (for the fruiting pattern).

Information about flowering and fruiting phenology is very important to increase productivity and understanding plant physiology and environmental response (Handayani 2016; Lestari 2019). Climate change affecting the environmental conditions has impacted less-than-optimal plant growth and development. In less-than-optimal conditions, plant growth will be disrupted, reducing the production and yield quality of flowers and fruits. Other important factors triggered by climate and environmental changes are shifts in plant response, plant morphology, and plant phenology (Pulatov et al. 2015; Sarvina 2019). Plant phenology is important for predicting reproductive success in plant growth and development. Phenology is strongly influenced by temperature; then, it can be used to study the effects of climate change on plant growth, such as shifts in flowering and fruiting times (Wang et al. 2017; Zhu et al. 2019). Besides having a negative impact on the fruit quality, some of the negative effects of the increase in temperature (Qu and Zhou 2016) is the increase of plant-

disturbing organisms. High air temperatures greatly affect the life cycle of plant pests; high temperatures will accelerate the regeneration of their life cycle so that plants will be easily disturbed (Forrest 2016; Nopsa et al. 2014).

Based on this background, this research aims to know the details of the flowering and fruiting phenology stages of *A. luzonensis*, to determine the reproductive success of *A. luzonensis*; and to explain the floral biology of *A. luzonensis*. Furthermore, this research can be useful for the conservation strategy of *A. luzonensis*, especially for reproduction mechanism biologically and seed conservation.

MATERIALS AND METHODS

Study area

Research on the flowering and fruiting phenology of *Anaxagorea luzonensis* was carried out from December 2021 to November 2022 in one season. This research was done at Purwodadi Botanic Garden, Research Center for Plant Conservation, Botanic Garden and Forestry, National Research and Innovation Agency for Republic Indonesia (BRIN), Purwodadi (Figure 1), East Java, Indonesia. The materials used were *A. luzonensis* plant collection in collection number XVIII.E.26, with small treelets or small tree habitus, about 1,5 m high, 26 years old, and originated from exploration results in Dumoga Bone Mongondow National Park, Bolaang Mongondow, North Sulawesi, Indonesia.

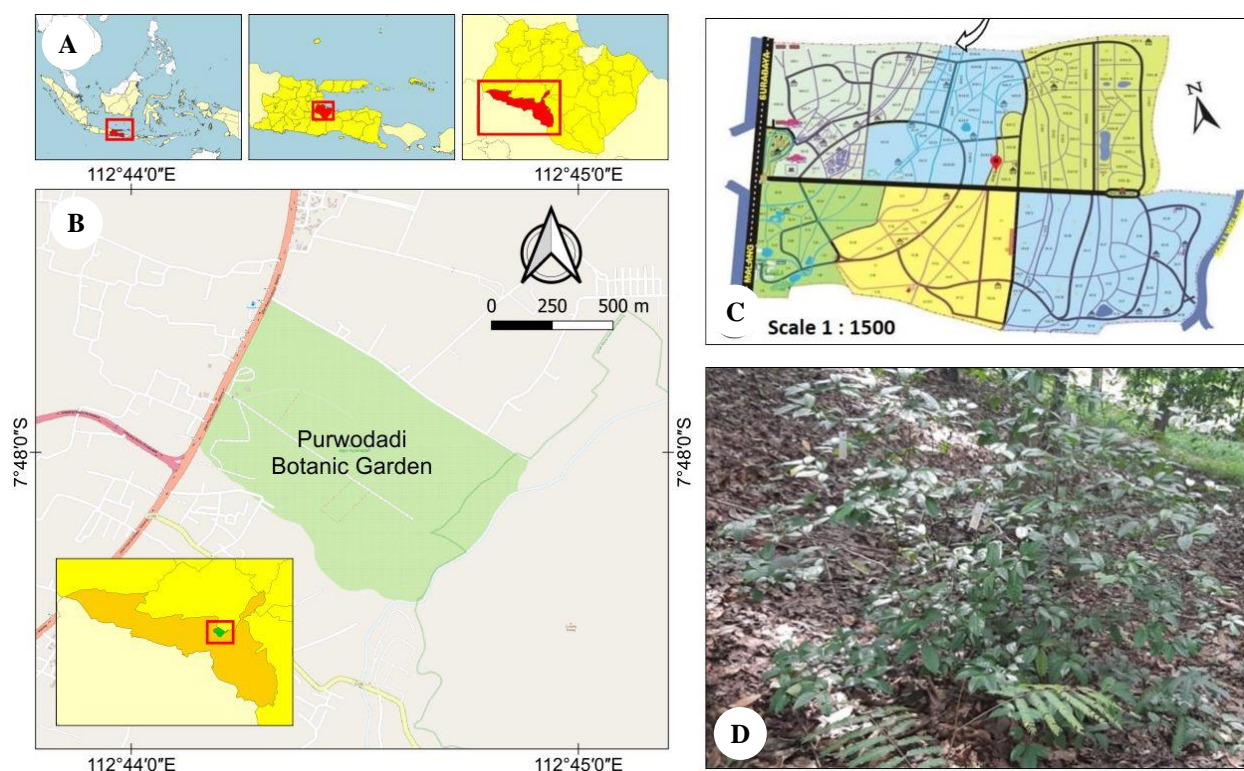


Figure 1. Observation location of flowering and fruiting phenology of *Anaxagorea luzonensis* in Purwodadi Botanic Garden East Java; A. Map of Indonesia, East Java, and Pasuruan District, B. Map of Purwodadi Botanic Garden, C. Study site in Purwodadi Botanic Garden (location symbol), and D. Habitus of *Anaxagorea luzonensis* in the site of XVIII.E.26

Procedures

Flowering and fruiting phenology

The plant material observed was one specimen, with observation plots determined based on four cardinal directions (north, south, east, and west) with three replications for each direction. One specimen was observed in each cardinal direction; therefore, there are 12 flowers in total. The method was observative, including the flowers' color, shape, size, and fruit changes in each phenological stage. In addition, the time required for each flowering and fruiting stage was also calculated. Those development start from the flower bud until blooms and anthesis occurs. The stages of fruit development start from ovary formation, immature, and mature until ripe fruit. Each stage was recorded, measured using a digital caliper, and documented using a digital camera. Observations were done every day. Each stage from flowering and fruiting phenology was described based on the BBCH scale (Meier et al. 2009).

Reproductive success

The determination of reproductive success is calculated by the ratio of fruits and flowers and the ratio of seeds and ovules (Syamsuwida and Aminah 2020). Fruit set calculation refers to Kusumayati et al. (2015), which is based on the ratio of the total fruit formation and the total of flowers.

Floral biology

The floral biology observations require three flower samples as replicates. These samples differ from those for flower phenological stages; the flowers' stadia are observed when they bloom. The parameters observed are the size, shape, surface, and color of flower stalks, sepals, petals, stamens, carpels, and torus (floral receptacle). Each replicate was recorded and documented using a digital microscope Dino-lite AM3113T. Besides, the flower formula and diagram are also drawn and documented.

Data analysis

Using Microsoft Excel, data were analyzed using descriptive analyses and averages with standard deviation (SD). In addition, the photo files documented data of flowering and fruiting stages as supporting data.

RESULTS AND DISCUSSION

Plant flowering and fruiting phenology can be used and interrelated for species and seed conservation. In this research, observations of *A. luzonensis* were carried out through several stages, starting from flowering and fruiting phenology, the value of reproductive success, flower morphology, and the conservation strategies in PBG. Flowering and fruiting phenology

The flowering and fruiting phenologies of *A. luzonensis* were determined by the period of the flowering stage and the period of the fruiting stage based on cardinal directions (Figure 2). Flowering stages are divided into five stages, i.e. initiation, flower bud, 50% of flower, bloom, and anthesis. Fruiting stages are divided into three stages, i.e. immature-sized, mature-sized, and ripe fruit. Each stage from each cardinal direction has different periods. The time required for flower initiation is 5-9 days, with the longest time in the northern cardinal direction and the fastest time in the western cardinal direction. Flower buds require 12-16 days until bloom and anthesis (each stage is 1-3 days). The size and shape changes of young fruit distinguished the growth stage from immature to mature-sized fruit. These stages are long (average of 7-67 days). The ripe fruit required 2-7 days until the seeds were ready to harvest. The flowers in the southern cardinal directions could not produce mature and ripe fruits.

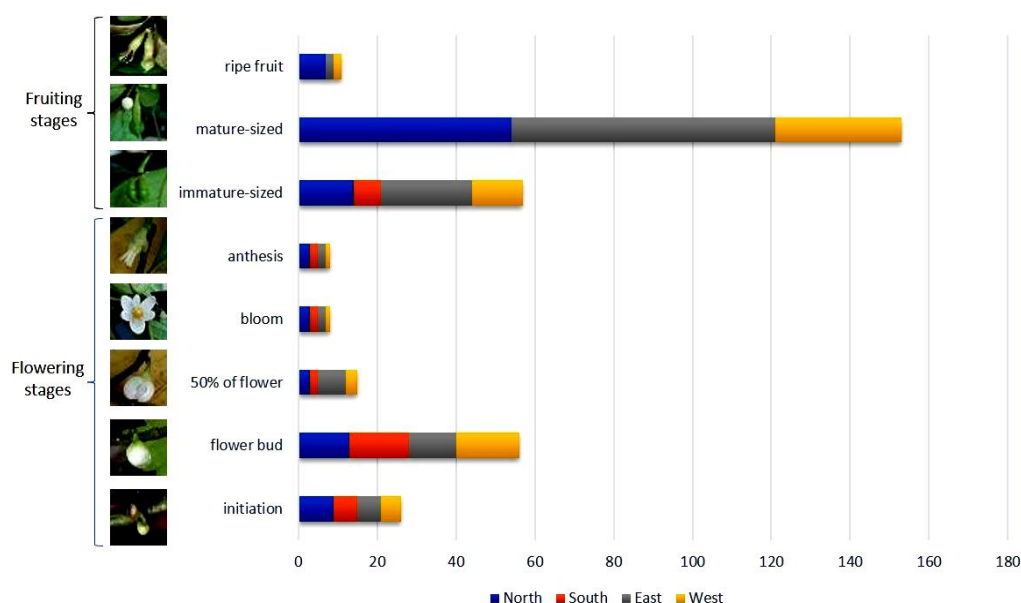


Figure 2. Period of *Anaxagorea luzonensis* flowering and fruiting stages

The detailed descriptions from each flowering and fruiting stage of *A. luzonensis* based on the BBCH scale are shown in Table 1 and Figure 3. The inflorescence emergence stage (code 5) until the flowering stage (code 6) took 29 days. The flower of *A. luzonensis* is protogynous anthesis. Development of fruit from immature-sized to mature-sized took 53 days. Finally, three days were required for fruit ripening or maturity until the seed was ready to be dispersed.

Reproductive success

The value of reproductive success in the eastern cardinal has the highest percentage (3,17%; Figure 4), and

the lowest value was in the southern cardinal (0%; Figure 4). That is due to the micro-environment in eastern and northern cardinals receiving sunlight and are not blocked by other tree canopy cover. The value of reproductive success affects the percentage of fruit and flower set. If the reproductive success percentage is high, the fruit and seed set yields are also high, and vice versa (Figure 4). On the other hand, the fruit and the seed set in the southern and the western cardinal are lower because the flowers fell and pollination did not occur, and immature-sized fruits were dropped due to heavy rain.

Table 1. Detail description of *Anaxagorea luzonensis* flowering and fruiting stages based on BBCH scale

| Code | Stage | Description | Period |
|------|--|---|---------------------|
| 5 | Inflorescence emergence | Stage from the appearance of a flower bud (initiation stage) until before the flowers bloom | 25 ± 6.68 days |
| 6 | Flowering | From blooming to the end of flowering (anthesis begin) | 4 ± 1.64 days |
| 7 | Development of fruit | From immature-sized fruit until mature-sized fruit (size increased) | 53 ± 50.31 days |
| 8 | Ripening or maturity of fruit and seed | Starting from the distal part of the carpel has a thickened wall until it breaks (opening at the ventral) | 3 ± 3.89 days |

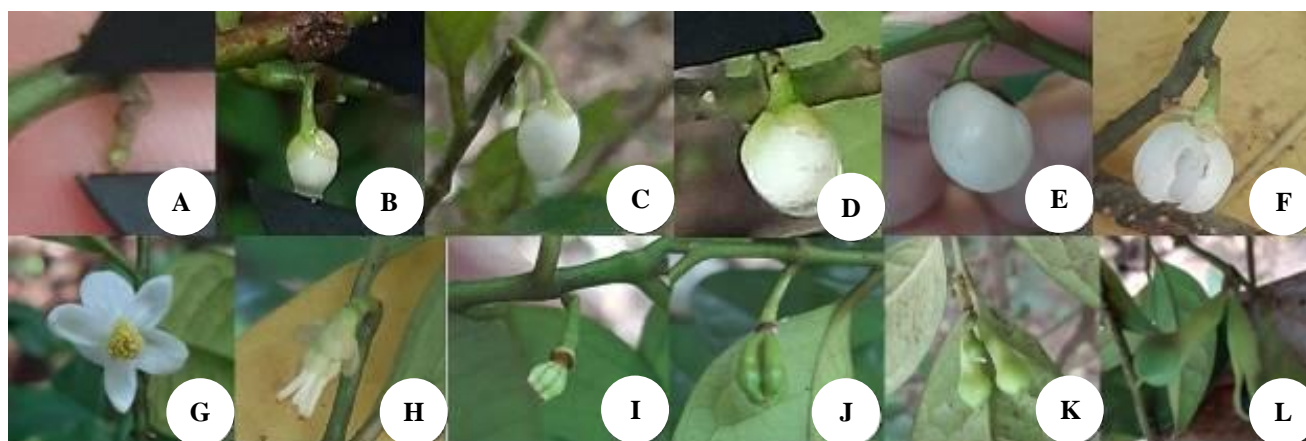


Figure 3. Flowering and fruiting stages of *Anaxagorea luzonensis*; A-G. flowering stages, H. anthesis, I-L. fruiting stages

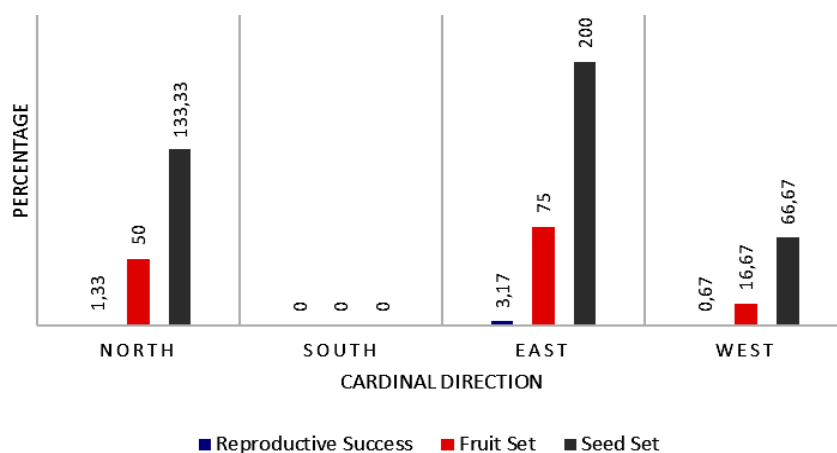


Figure 4. Reproductive success, fruit set, and seed set percentage of *Anaxagorea luzonensis* based on cardinal direction

Flower biology

The morphology of the flowers of *A. luzonensis* is shown in Figure 5. Inflorescence terminal, flowering pedicels 0.9-0.95 cm long, ca. 1.37-1.39 mm in diameter, green, glabrous. Sepals 3, light green, ovate, not fleshy, free, simple hairs with brown color, ca. 0.25-0.4 cm long, ca. 0.2-0.4 cm wide. Petals 6, in 2 whorls of 3 inner and 3 outer petals. Outer petals 3, ca. 1-1.45 cm long, 0.4-0.75 cm wide, more width than inner petal, white, free, fleshy, ovate. Inner petals 3, elliptic, ca. 0.8-1.2 cm long, 0.3-0.5 cm wide (Figure 5A-B). These observation results are relevant to Lestari (2014). Torus is deeply concave (Figure 5C). This torus type is almost the same as *Fusaea* and *Ophrypetalum* species studied by Van Heusden (1992). Stamens fleshy, ca. 46-52 per flower, filament white, anther yellow, tongue-shaped, ca. 0.22-0.45 mm long

(Figure 5D). The tongue-shaped stamen is similar to those found on *Fissistigma fulgens* (Bangkomnate et al. 2021). Carpels spatulate, ca. 5-7 per flower, apocarpous, glabrous, fleshy, stigma light brown, style white, ovule yellow (Figure 5E). The carpel is apocarpous with a long and conspicuous style (Li et al. 2021). Each carpel from *Anaxagorea* spp. could become one of this genus's important characteristics (Li et al. 2020).

The Flower morphology of *A. luzonensis* is then drawn to get a flower diagram and formulated. Based on the observations, the floral formula of *A. luzonensis* is $K_{(3)}, C_{(3)+(3)}, A_{\infty}, G_{(6)}$. The flower is zygomorphic and hermaphroditic, has three sepals, six petals in 2 whorls (3+3) with many stamens, and six apocarpous carpels. The flower diagram is shown in Figure 6.

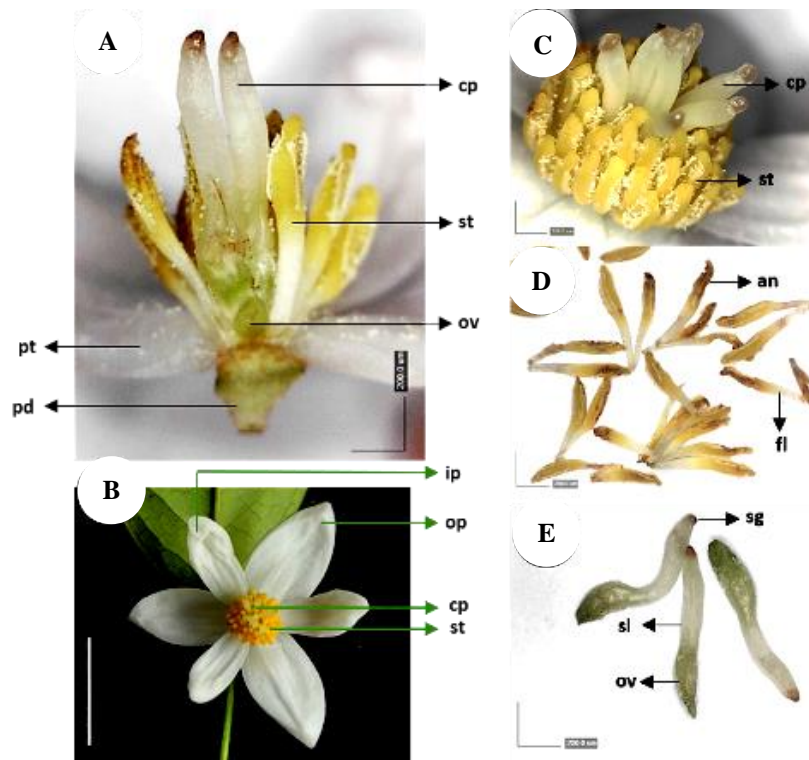


Figure 5. Morphological flower of *Anaxagorea luzonensis*; A. cross-section of flower (one outer petal removed), B. flower view from above, C. torus of flower, D. stamen, and E. carpel. an: anther, cp: carpel, ip: inner petal, fl: filament, op: outer petal, ov: ovule, pd: peduncle, pt: petal, sg: stigma, sl: style, and st: stamen. Scale bar = 200 µm (Figure 4A, C-E) and 1 cm (Figure 4B)

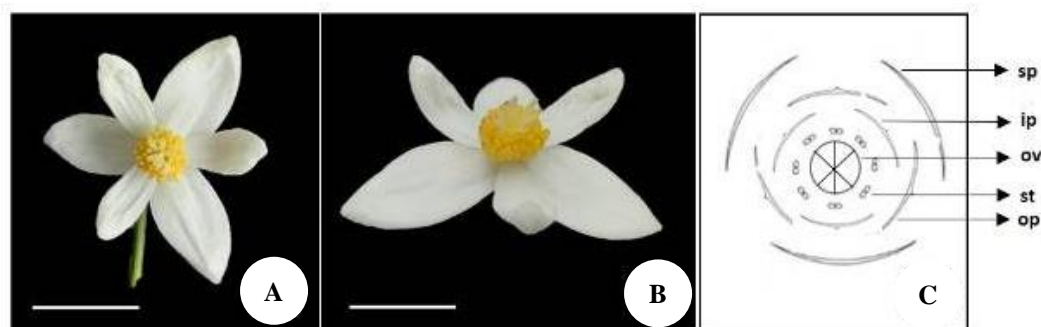


Figure 6. Flower diagram of *Anaxagorea luzonensis*; A. flower (top view), B. flower (side view), and C. flower diagram. ip: inner petal, op: outer petal, ov: ovule, sp: sepal and st: stamen. Scale bar = 1 cm

Discussion

Phenology is one of the plant physiological processes related to biological event timing and environment. The phenology of each plant species is different, including in the Annonaceae family. Based on these results, the flowering and fruiting phenology of *A. luzonensis* is taken a shorter flowering time than fruiting time, short anthesis time, and a low percentage of reproductive success. Species from the *Anaxagorea* plants group have different anthesis rhythm variations among individuals (Gottsberger 2016). The low percentage of reproductive success is largely due to fruit formation failure, reducing seed production. In addition, the mechanism of autochorous seed dispersal makes it difficult to monitor the regeneration process in nature. The seed dispersal in the *Anaxagorea* is ballistic (explosion seed dispersal); this autochorous mechanism covers a larger distance from the mother trees (Braun and Gottsberger 2011; Gottsberger 2016). That causes nature plant regeneration of *A. luzonensis* in PBG is critical.

The flowering time of *A. luzonensis* in PBG from this results study is at the end of the year (December) to the beginning of the year (January) and continues with fruiting time until May. The next flowering time will start at the end of the dry season, so it blooms twice a year and ripens throughout the year. This phenomenon is called a sub-annual phenology pattern (Lestari 2019). The phenology observation based on the detailed BBCH scale can facilitate and identify standard phenological stages. Besides, it can be used as basic information for studies related to climate change and will assist and plan the agronomic management protocols development (Liu et al. 2015; Varban et al. 2021; Ferrer-Blanco et al. 2022). The phenological cycle of *A. luzonensis* in PBG from the bud's emergence until the fruits' complete physiological maturity occurred in periods $73-121 \pm 85$ days. At the same time, the phenological cycle of the atemoya tree is 217 days (Mendes et al. 2019). The interaction of environmental and genetic factors causes differences in periods of plant flowering and fruiting stages. That causes differences in flowering period length and fruit development (Ariza et al. 2015; Cho et al. 2016; Sulistiawati et al. 2017). The fruit production of *A. dolichocarpa* and other Annonaceae species is a slow process and takes a long period (Braun and Gottsberger 2011). The flowering of the species follows either the annual pattern, have the cornucopia mode of flowering, with a two- or three-month flower-producing period in the second half of the year. *Anaxagorea*, which exhibits the annual flowering mode and its flowering period from September to October, has fruit ripened mainly during October of the following year. The floral scent of *Anaxagorea* species contains fruit-like components, and the pollinators, primarily Nitidulidae, are attracted by deceit (Gottsberger 2016).

Plant reproductive success is the outcome of the antagonistic or mutualistic of plants with animals and plants with their environment. Increased plant reproductive success is affected by sufficient pollination by pollinators and supported by a suitable environment (Grass et al. 2018). The reproductive success value of *A. luzonensis* in PBG is categorized as low because the percentage is below

30% (Owens 1993). The reason for low reproductive success value is caused by high rainfall during the observation process so that flowers fail, unsuccessful pollination, flower abortion occurs before and after anthesis, and fruit failure to ripen due to genetic factors. Flowers in the southern cardinal direction fall because the micro-environmental factors do not support anthesis stages. That is due to micro-environmental factors (such as light intensity) in the southern cardinal direction, i.e. immature-sized fruit drop. Some Annonaceae species' flowering and fruiting periods are affected by micro-climate in mother trees (Lestari and Fiqa 2020). Based on Gottsberger (2016), the *Anaxagorea* plants' group required luminous gaps for growth. The low fruit set can affect plant species' reproductive success. They are affected by the small population, weather activities, climate, pollination mechanism, the morphology of flowers, abundance, and pollinator behavior (Racsko et al. 2007; Cao et al. 2015; Singh et al. 2018; Graves and Gimondo 2021; Zhang and Gao 2021).

A. luzonensis is the smallest tree in the *Anaxagorea* group, with 0.3-1.5 m in height. The lowest numbers of secondary veins (5-10) and leaf-opposed to the terminal inflorescence, outer petals are mostly up to 10 mm long, no staminodes and stipitate stigmata (carpels attenuate towards the stigma), long-beaked monocarps (1.5 mm long or more) and have shorter pedicel than *A. javanica*. Flower buds globose to broadly ovoid, brownish or shades of green to orange, or reddish at the base (Maas and Westra 1984; Maas and Westra 1985). The fruits of *A. luzonensis* are greenish colored, and fruits mature with explosive mode (Lestari 2014). The flower of *A. luzonensis* is trimerous with a whorl of sepals, have numerous stamens, and usually exhibits 2-4 completely separate carpels (Li et al. 2021).

Based on this research result, plant collection conservation and seed conservation strategy efforts of *A. luzonensis* are (i) Control the sunlight exposure because *A. luzonensis* plant collection requires partial to full sunlight. If the environmental conditions are too dark or low sunlight, pruning the surrounding trees that shade the *A. luzonensis* plants is necessary. Solar radiation is an important factor in the metabolism of plants with green leaves because sunlight influences plant production, thereby increasing the rate of photosynthesis, and increased sunlight usually accelerates flowering and fertilization (Amthor 2010; Yang et al. 2021; Osnato et al. 2022). (ii) Cleaning weeds around the plants because weeds are competitors for soil nutrients, growing space, and sunlight. A good growing environment is needed for a plant's growth and development. This plant growth environment is a vital requirement for plant growth to have normal growth, vitality, and production capacity. The requirement for the growing environment for each plant is different, but the elements of activity for growth and development needed for each plant are the same. These elements are light, temperature, water, soil, air, and plant nutrients (Khalil et al. 2015; Gavrilescu 2021). Weeds control is managing weeds by suppressing the presence or population of weeds to a level that is not economically detrimental (MacLaren

et al. 2020). (iii) Propagate the plant using seeds (generative propagation) or vegetative through cutting, grafting, or tissue culture. Seeds and natural pollination with insects, wind, or other pollinators can usually be done for generative plant propagation (Tanawani and Lengkong 2020). Vegetative plant propagation is the multiplication of plants from their parent plant parts, such as stems, branches, tubers, shoots, twigs, and roots, to produce new plants (Chauhan et al. 2021). Another propagation technique, by tissue culture or in vitro, is an alternative to produce superior seeds in a short time, under aseptic conditions quickly, and genetically the characteristics of the produced plant will be the same as their parents. Tissue culture can avoid the loss of the conserved heritage due to environmental changes and keep plants' genetic background (Tefera 2019; Rather et al. 2022). The benefits of tissue culture are easier production of healthy plants with superior properties, faster production with identical plants, elimination of pathogens, conservation of germplasm, facilitating breeding programs and an extremely efficient and cost-effective technique (Hasnain et al. 2022). (iv) Give additional hormones to these plants because additional hormones can usually increase flowering and add hormones already present in plants. Some of the hormones given include gibberellin, ethylene, and kaolin. Gibberellin is a hormone that makes plants grow quickly because it promotes the formation of seeds, fruits and flowers, as well as the elongation of stems. Ethylene is a growth hormone in the form of a gas that can accelerate fruit ripening, found in fruits and leaves that are aging. Kalin is a growth hormone that affects the formation of plant organs such as roots, stems, leaves, and flowers. (v) Monitoring pests and plant diseases. Pest and disease attacks can disrupt the plant productivity of flowers and fruit, along with the growth of these plants (Demolin-Leite 2024). (vi) Regular application of manure or compost. This application will increase nutrition on land and plant fertility, increasing the productivity of flowers and fruit. (vii) Monitoring environmental factors of temperature, air, and weather that affect flowering and fruiting processes. Air temperature is a climatic factor that greatly affects plant phenological processes. Fruit formation is influenced by various factors, including pollinator effectiveness (Abrol et al. 2019; Tanda 2022), endogenous factors (Yuan and Yang 2018; Yan et al. 2019; Bu et al. 2020), the number and synchronization of male and female flower maturity (Handayani 2016; Oliveira et al. 2019; Fathin et al. 2021), and environmental factors (Lestari and Fiqi 2020; Zhang et al. 2022). Endogenous factors influence, for example, morphological and anatomical chemical construction, such as the content of carbohydrates, water, minerals, nutritional status, and growth hormone. Environmental factors influence include air temperature, air humidity, rainfall intensity, light intensity, photoperiod, and canopy position (Zanne et al. 2015; Yan et al. 2019; Lopez et al. 2021). (viii) Further research related to the simulation model of *A. luzonensis* plants on the influence of climate factors on plant phenology. The simulation model could show production projections and future crop cultivation planning. This method can simulate the

processes of each plant phase development, biomass production from each organ in roots, stems, leaves, and tubers, leaf area index (LAI), and soil water content. Furthermore, the production projections and future crop cultivation planning could be examined. Each phase's longevity under the changing climate, such as temperature (Kumar et al. 2019; Afifah et al. 2022).

ACKNOWLEDGEMENTS

The National Research and Innovation Agency, Republic of Indonesia, funds this study. Sincere thanks are also dedicated to the botanic garden technician in the Annonaceae family who assisted in the fieldwork and data collection activities.

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