

Study of the Germination of Six *Begonia* Species as an Effort to Preserve Genetic Resources

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Abstract. *Begonia* is an ornamental plant that has a high level of morphological diversity. *Begonia* propagation is mostly done vegetatively and very rarely to propagate sexually. Therefore, this study was conducted to determine the ability of six types of *Begonia* seeds to germinate. Six types of *Begonia*, namely *B. x albopicta* W. Bull 'Coral Rubra', *B. gambutensis* Ardi & D.C.Thomas, *B. multistaminea* Burt-Utley, *B. nelumbifolia* Schltld. & Cham., *B. ulmifolia* Wild. and *B. varipeltata* D.C.Thomas. were grown in a petri dish and placed in a growth chamber with a temperature of 23.6 °C, humidity of 74.5%, and a light intensity of 3,431 lux. The results showed that the different types of *Begonia* showed significant differences in all the parameters tested. *B. nelumbifolia* required 7.89 ± 0.18 days to germinate and is the fastest time than other types. The highest percentage of seed germination was also obtained by this species, amounting to 63.22%. In addition, *B. nelumbifolia* showed the best response to the germination rate coefficient, germination rate index, and germination index. This study revealed complete information about the germination of six types of *Begonia*. The ability to produced many seeds and the ease of germination made this plant have the potential to be cultivated through seeds. Furthermore, the information can be used as a basis for seed storage as an *ex-situ* conservation method to maintain genetic diversity.

Key words: *Begonia nelumbifolia*; conservation; Eka Karya Bali Botanic Gardens; genetic diversity; seed

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INTRODUCTION

Begonia (Begoniaceae) is one of the fifth or sixth largest taxa with more than 1,800 species. The morphological diversity of *Begonia* is high, and the actual number of these species in nature is estimated to be between 2,000 and 2,500 species (Haba, 2015; Tian et al., 2018). *Begonia* is one type that has been conserved by the Eka Karya Bali Botanic Gardens (BBG). The number of *Begonia* collections that have been conserved at BBG based on the Registration unit catalog as of February 2021 is 310 species.

Begonia is an ornamental plant that has a high level of morphological diversity (Haba, 2015). Most of the *Begonia* is a monoecious plant. That is, male and female flowers are in one plant. The female flowers can be recognized by the presence of an ovary in the form of a winged capsule. Pollinated flowers will form fruit and produce seeds. *Begonia* seeds are small and many. Efendi et al. (2019) stated that the seeds of several begonias were 252.42-451.25 µm long and 160.06-284.83 µm wide. Haba (2015) stated that *Begonia schmidtiana* fruit 'Chauncy' produces about 6,500 ovules per capsule. Not all

of the ovules will become seeds, but this plant has the potential to produce many offspring in one flowering cycle. The problems are, male fertility is not necessarily linked to female fertility as different genes may underlie male and female meiosis, so it cannot produce seed easily (Dewitte et al., 2011).

Wahyuni and Siregar (2020) stated that the genus of Begoniaceae has been able to become one of the top priority taxonomic groups plant conservation in Indonesia because: (1) high endemism; (2) can be reintroduced into nature; (3) potential to be conserved *ex-situ*; and (4) has an economic value as an ornamental plant because of its uniqueness and the beauty of the leaves. *Begonia* is generally included in plants that are easily propagated, both vegetatively and generatively. However, vegetative propagation of *Begonia* is more preferred than generative propagation. Shoot and leaf cuttings are the most common and easy propagation methods in *Begonia*. Information about the propagation of *Begonia* plants through seeds has not been done much. On the other hand, investigating seed storage behavior is needed for seed conservation strategies (Latifah et al., 2020). Efendi et al.

(2019) stated that germplasm management through the seeds presents a challenge to ensure high-quality seeds are worth using and have a long saving power of life.

Based on this phenomenon, the study of *Begonia* propagation by seed is an interesting challenge to study. Measurement of germination can provide valuable information about initial, speed, uniformity, and final germination percentage (Soltani et al., 2015). Hay and Probert (2013) mentioned that research should be more focused on seed development and germination as a reference in implementing *ex-situ* conservation through seed banks. Therefore, this study aimed to examine the germination of the six types of *Begonia* seeds in BBG to be used as a basis for conservation purposes.

METHODS

This research was conducted from October to November 2020 at the Seed Bank Laboratory of the Eka Karya Bali Botanic Gardens - LIPI. The materials used were six types of *Begonia* from the BBG collection, namely *B. x albopicta* W. Bull 'Coral Rubra', *B. gambutensis* Ardi & D.C.Thomas, *B. multistaminea* Burt-Utley, *B. nelumbifolia* Schltld. & Cham., *B. ulmifolia* Wild. and *B. varipeltata* D.C.Thomas.

Seed collection and preparation

The six seeds were harvested from BBG's *Begonia* Garden in October 2020. The seeds were taken from ripe fruit that had been browned, and all parts of the fruit had dried on the tree, or the seeds were ripe for harvest. Furthermore, the seeds were separated from the fruit's skin and cleaned from other fruit parts manually. Cleaned seeds were weighted as much as 0.002 grams and counted for the number of seeds for each type (Table 1). The seed shape of each species was also observed with a binocular microscope Olympus CX-31.

Table 1. Number of seeds of six types of *Begonia* per 0.002 grams

Types of <i>Begonia</i>	Number of seeds
<i>B. x albopicta</i>	488
<i>B. gambutensis</i>	462
<i>B. multistaminea</i>	529
<i>B. nelumbifolia</i>	503
<i>B. ulmifolia</i>	532
<i>B. varipeltata</i>	472

Seed germination

Each type of *Begonia* was germinated in a petri dish that had been laced with tissue paper moistened with water. Then the petri dishes were placed in the growth chamber with a temperature of 23.6°C, the humidity of 74.5%, and a light intensity of 3,431 lux. Watering was done using a micropipette twice a week or depending on needs. Observations were carried out every day for 30 days by taking the germinated seeds and observed under a Dino-Lite AM4515T5 digital portable microscope.

Germination measurement

The experimental parameters of germination in this study were based on Al-ansari and Ksiksi (2016), namely:

- Mean Germination Test (MGT)

$$MGT = \frac{\sum \text{seeds germinate (G) on day } x}{\sum \text{seeds germinate}}$$
- Final Germination Percentage (FGP)

$$FGP = (\text{number of seeds germinated} / \text{number of seeds sown}) \times 100 \%$$
- Coefficient Velocity of Germination (CVG)

$$CVG = \frac{N_1 + N_2 + \dots + N_i}{100 \times N_1 T_1 + \dots + N_i T_i}$$

N = number of seeds germinated
T = germinating day
- Germination Rate Index (GRI)

$$GRI = \frac{G_1}{1} + \frac{G_2}{2} + \dots + \frac{G_i}{i}$$

G = percentage of germination
- Germination Index (GI)

$$GI = (20 \times G_1) + (19 \times G_2) + \dots + (1 \times G_{20})$$

Experimental design and statistical analysis

The experiment was prepared with a one-factor, completely randomized design, namely the type of plant (six types of *Begonia*) repeated three times. The data obtained were analyzed using analysis of variance (ANOVA) using SPSS 16.0. If the variance results had a significant effect, then the data were proceed with the Least Significant Difference test (LSD) with significance level of 5%.

RESULTS AND DISCUSSION

Begonia has specific characteristics, namely in the form of erect, shrub or creeping, with stems that watery, and asymmetrical leaf blade (begoniifolia), so easy to distinguish from other plants (Dewitte et al., 2011). Until 2020, the BBG has succeeded in collecting 310 *Begonia* consisting of native and exotic *Begonia*. Native *Begonia* was obtained from the exploration of plants in Indonesian forests like *B. gambutensis*,

B. multistaminea, *B. nelumbifolia* and *B. varipeltata*. While the exotic *Begonia* were those had been widely cultivated by hobbyists and ornamental plant lovers like a *B. x albopicta*. The

plant used in this study is presented in Figure 1, and the microscopic appearance of seeds is shown in Figure 2.



Figure 1. Six types of *Begonia* collection of Eka Karya Bali Botanic Gardens.

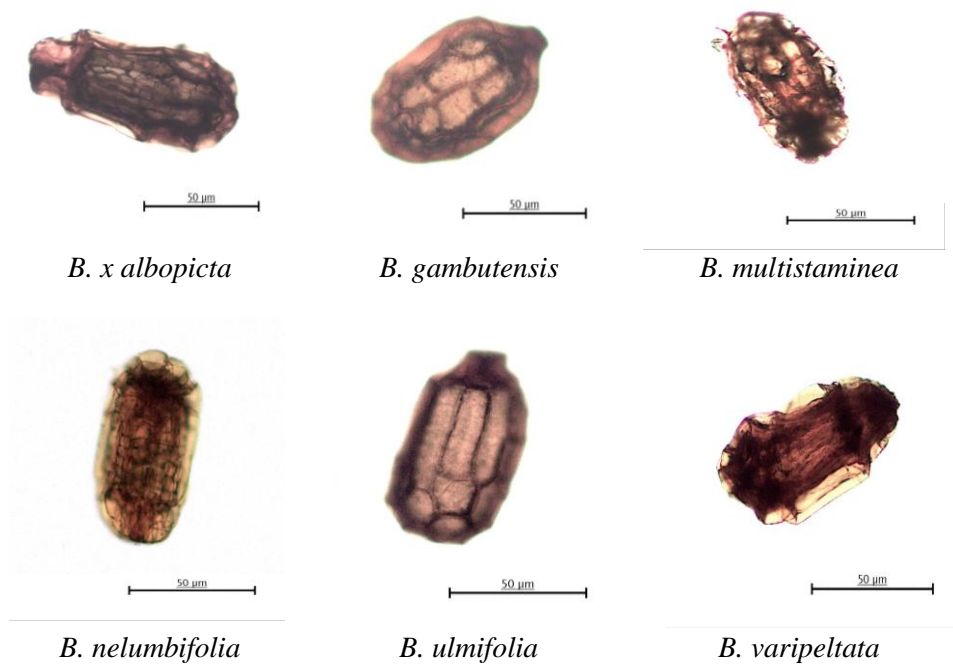


Figure 2. Microscopic appearance of six types of *Begonia* seeds.

In general, the *Begonia* seed consists of four parts (Figure 3), including the hilum micropyle, which is the part that attaches to the fruit wall, operculum or seed cap, the collar cell of the seed that is split when germinating, which is a characteristic of *Begonia* seed, and the common part of the testa cell on the seed coat. Stages of germination start when imbibition seeds, and then the hypocotyl elongates and penetrates the operculum. Furthermore, the hypocotyl that penetrates the operculum develops into collets, which are structures that produce root hairs. At the bottom of the collet, the root meristem will appear. It occurs simultaneously with the development of the cotyledons on the other side of the hypocotyl (Haba, 2015). Based on the observation, all *Begonia* seeds show the same sprout growth. Likewise, *B. fischeri*, *B. muricata* and *B. scottii* had the same stages of germination (Haba, 2015; Efendi et al., 2019).

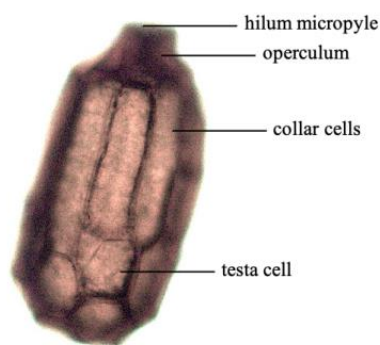


Figure 3. The parts of a *Begonia* seed

The different types of *Begonia* showed significant differences in all the parameters tested (Table 2). *B. nelumbifolia* seed has the best on average germination time, percentage of germination, germination rate coefficient, germination rate index, and germination index compared to the others. The average germination time is defined as the length of time it takes for one seed to germinate (Soltani et al., 2015; Al-ansari & Ksiksi, 2016). The lower the average germination time value, the faster the seeds will germinate. The results of mean germination time are varied, from 7.89 ± 0.18 to 14.77 ± 0.28 days. The seed that germinates fastest was *B. nelumbifolia* and the one that takes the longest was *B. gambutensis*. These differences are not only caused by the species but also due to the maturity of the seeds. Zuhri (2017) mentioned the same results that seed maturity accelerates the germination process on *Cestrum elegans*.

The highest percentage of seed germination was *B. nelumbifolia*, which was 63.22 % and followed by *B. gambutensis* was 52.6 %. Meanwhile, the others were below 50%. Even *B. x albopicta* germination was the lowest, which was 4.17%. Germination of *B. nelumbifolia* began on the fifth day after the seeds were sown. Furthermore, most seeds germinate one day after the first seeds sprout and decreased with increasing observation time (Figure 4). This pattern is the same as that shown in *B. multistaminea* and *B. ulmifolia*. Meanwhile, different germination patterns are shown in *B. x albopicta*, *B. gambutensis* and *B. varipeltata*. The initial period of germination is marked by a line parallel to the axis and then begins to rise and reach its peak on the second and third days after the first seeds germinate. The first and last days of germination showed significant differences between treatments. In this study, the highest germination index value achieved by *B. nelumbifolia* and the lowest was *B. x albopicta*. This indicates that *B. nelumbifolia* undergoes faster initiation and end of germination than other types of *Begonia*.

The results showed that four types of *Begonia* had a germination percentage below 50%, even *B. x albopicta* was only $4.17 \pm 0.31\%$. In general, it is caused by differences in genetic traits. Yamburov et al. (2020) reported different results, the percentage of *B. ludwigii* seeds that germinated was very high, namely 92.8%, even though the sprouts started to appear in the second week. Saefudin and Wardiana (2013) stated that the varieties affect the speed of germination. The low germination rate may be caused by the seeds used have not reached the appropriate level of maturity. Seeds harvested from fruits that have not reached physiological maturity have embryos and other components that are not yet fully formed so that the seed vigor is low (Wulananggraeni et al., 2016). The level of fruit maturity also affected the dry weight of the seeds (Susanti et al., 2019). Darmawan et al., (2014) also mentioned that the dry seed weight affected the endosperm content of the seeds, thus causing differences in germination. Delaying harvest until the fruit is dry and ripe is recommended for *B. areolata* species. *B. hooveriana*, *B. isoptera*, *B. kudoensis*, *B. Muricata*, and *B. sudjanae* (Efendi et al., 2019).

Another factor thought to influence pollination is pollinators. Yamburov et al. (2020) stated that pollinating insects were one of the factors causing the low germination percentage

of *B. ludwigii* grown in greenhouses. Without pollinating insects, plants can only pollinate geitonogamy, which means male flowers pollinate female flowers in the same inflorescence. If the male and female flowers do not emerge simultaneously, the seed productivity

is low. Seed productivity can be increased by cross-pollination or xenogamy. Through this pollination, the seed productivity increased almost three times, up to 1587.8 ± 75.8 seeds per capsule (Yamburov et al., 2020).

Table 2. Value of germination parameters for six types of *Begonia* in Eka Karya Bali Botanic Gardens

Jenis begonia	Parameter				
	MGT	FGP	CVG	GRI	GI
<i>B. x albopicta</i>	10.87±0.65 b	4.17±0.31 a	9.22±0.57 b	1.97±0.07 a	408.67±18.58 a
<i>B. gambutensis</i>	14.77±0.28 d	52.67±3.92 d	6.77±0.13 a	17.03±1.72 c	3951.67±362.15 c
<i>B. multistaminea</i>	10.95±0.82 b	25.14±1.68 b	9.17±0.71 b	12.81±1.29 b	2668.67±246.84 b
<i>B. nelumbifolia</i>	7.89±0.18 a	63.22±2.76 e	12.68±0.30 c	41.26±0.72 d	7347.00±264.76 d
<i>B. ulmifolia</i>	10.74±0.10 b	29.17±4.95 b	9.31±0.09 b	14.68±2.61 bc	3145.80±546.99 b
<i>B. varipeltata</i>	13.88±0.37 c	38.63±7.10 c	7.21±0.19 a	13.61±2.55 b	3122.67±585.19 b

Note: MGT = mean germination time; FGP = final germination percentage; CVG = germination rate coefficient; GRI = germination rate index; GI = germination index; Numbers accompanied by the same letter in the same column do not show a significant difference based on the 5% LSD test ($p = 0.05$).

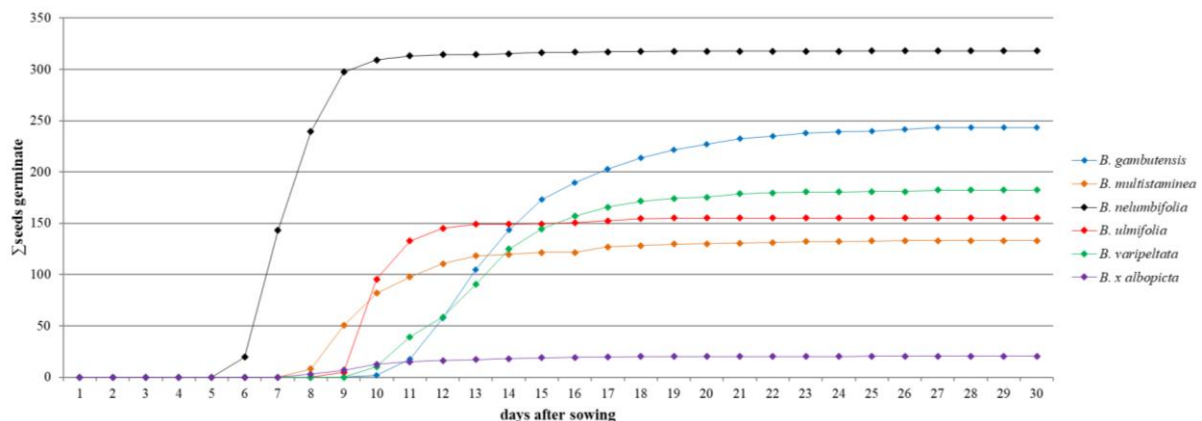


Figure 4. The number of seeds germinated in six types of *Begonia* in Eka Karya Bali Botanic Gardens

Begonia are found in varying habitats and altitudes, ranging from 19 to 2,300 masl (Ardi et al., 2014; Hughes & Takeuchi, 2015; Hughes et al., 2015; Undaharta & Ardi, 2016; Ardaka & Ardi, 2019). Some species come from humid tropics and can only grow in environments with high humidity, while others prefer in dry conditions (Haba, 2015). In their natural habitat, the seeds of *B. grandis* subsp. *evansiana* may be dispersed at the end of the rainy season and will not germinate until after a dry winter (Haba, 2015). The seeds will experience dormancy during this time. Dormancy is a condition that causes seeds not to germinate even though they have been grown in optimal environmental conditions. A specific temperature is required for

the seeds to germinate. Kumar et al. (2011) reported that the germination percentage of *Andrographis paniculata* Wall. ex Nees reached 95% when germinated at 25 °C and decreased at a temperature above or below. The seeds used in this study were collected from different habitats and conserved at BBG. *B. x albopicta*, *B. nelumbifolia* and *B. ulmifolia* were from America, *B. multistaminea* was from Mexico and *B. gambutensis* and *B. varipeltata* were from Sulawesi and all were germinated at the same temperature of 23.6 °C. This can lead to less than optimal seed germination. Haba (2015) stated that the germination of *B. dregei* reached its maximum at a temperature of lower than 12 °C, reflecting the natural environment of the seeds in

their natural habitat.

Several efforts can be made to increase seed germination, including soaking seeds in liquid smoke (Tang et al., 2020), immersion in GA3 (Herlina & Aziz, 2016), giving Plant Growth Promoting Rhizobacteria (PGPR) (Wahdah et al., 2018), and hydropriming (Herlina & Aziz, 2016). Tang et al. (2020) stated that peanut seeds soaked with liquid smoke had more lateral roots. Furthermore, the provision of GA3 had a significant effect on the length of the radicle of black cumin seeds grown in a germinator at a temperature of 25 ± 1 °C (Herlina & Aziz, 2016). Normal root growth will maximize nutrient absorption so that it can accelerate the formation of normal sprouts. Germination can also be stimulated by the biostimulants contained in PGPR. Wahdah et al. (2018) reported that the single PGPR application and the interaction with the variety and duration of seed storage significantly affected the increase in germination, synchronous seed growth, and rice speed seeds after three months of storage. In addition, seed viability can be increased by hydropriming treatment. Herlina and Aziz (2016) stated that hydropriming affected the germination and vigor of black cumin seeds. Hydropriming is a hydration-dehydration process by immersing seeds in water as an effort to increase seed viability. During the priming process, the need for water in the seeds is sufficient to produce radicles, prepare for the metabolic process, and then starting the germination process (Herlina & Aziz, 2016).

The seed is part of a plant that stores high genetic diversity because it combines two genetic information through crossing or mating. To prevent future loss of genetic resources of various species, seed storage for *ex-situ* conservation is recommended. Hay and Probert (2013) stated that seed development studies are needed, for example, to identify germination of species, to predict how to conserve and how seeds develop because each species has specific traits. The data obtained, such as average germination time, percentage of germination, germination rate coefficient, germination rate index, and germination index that indicated the germination pattern of *Begonia* affected by the species. This complete information about the germination of six types of *Begonia* were new data that had not been presented in previous *Begonia* studies, which revealed more about new species and their morphological variations. In addition, *Begonia* could produce many seeds and

had the ease of germination made this plant potential to be cultivated through seeds. Therefore, these results provided an alternative way to propagate this plant other than vegetatively. Further studies on storage time and possible seed deterioration are needed as an effort to conserve *Begonia* seeds *ex-situ*, and the results will be beneficial for BBG staff, as well as for *Begonia* breeders and collectors.

CONCLUSION

The six types of *Begonia* seeds can germinate based on germination time, percentage of germination, germination rate coefficient, germination rate index and germination index. Among all, *B. nelumbifolia* was the highest for all the parameters. Subsequently, information about the germination of six types of *Begonia* can be used as a basis for seed storage as an *ex-situ* conservation method to maintain genetic diversity.

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