NUSANTARA BIOSCIENCE
Vol. 17, No. 1, pp. 1-10
May 2025

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n170101

Morphological and flowering phenology characterization of wild *Begonia* (*B. hooveriana* and *B. hijauvenia*) collected at Bogor Botanic Gardens, Indonesia

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Manuscript received: 23 July 2024. Revision accepted: 13 December 2024.

Abstract. *Agustiani A, Garvita RV, Siregar HM, Windarsih G. 2025. Morphological and flowering phenology characterization of wild* Begonia *(*B. hooveriana *and* B. hijauvenia*) collected at Bogor Botanic Gardens, Indonesia. Nusantara Bioscience 17: 1-10. Begonia hooveriana* and *B. hijauvenia* are known for their varied morphology. This study aimed to describe the morphological characteristics of three *Begonia* accessions and determine their flowering phenology and pollen viability *B. hooveriana* with green and red color and *B. hijauvenia*. The *Begonia* were compared to characterize the plants, stomata on the underside of the leaves were examined, flowering phenology was observed daily during the flowering phase, and pollen viability was observed at the anthesis and post-anthesis phases. Data were recorded and analyzed descriptively. Based on morphological characterization data, the only morphology that distinguishes the two accessions of *B. hooveriana* is the color. The most prominent difference between *B. hijauvenia* and the previous two comparators is the type of plant. Accession *B. hooveriana* has anisocytic stomata, while *B. hijauvenia* has parasitic stomata. Based on the results of flowering phenology, the two accessions of *B. hooveriana* have a flowering period of 10-21 days, and *B. hijauvenia* has a flowering period of 17 days. The highest percentage of pollen viability in both flowering phases was found in *B. hooveriana* red (anthesis: 96.60±4.08%, post-anthesis:96.30±4.46%). The varied morphological characteristics of these three plants can be used as a basis for plant breeding programs.

Keywords: *Begonia hijauvenia, B. hooveriana*, Bogor Botanic Gardens, flowering phenology, morphology, pollen viability

INTRODUCTION

Begonia is one of the genera of Angiosperms with the highest number of species, comprising more than 3.000 plant species (Wahyu and Lucyanti 2019). Most members of the genus *Begonia* have the potential to become ornamental plants. In addition, some *Begonia* species can also be used as medicines, and the parts used are the leaves and stems. The efficacy of this plant is as a medicine for ulcers, to cure dizziness and fever, and to remove phlegm (Hartutiningsih 2017, et al. 2018; Wahyu and Lucyanti 2019). *Begonia* has a varied habitat type; this plant can thrive in forest areas, humid areas, mossy soils, and rather shady areas at altitudes up to 2.400 m asl. (Munawaroh and Hartutiningsih 2018). Its wide distribution causes *Begonia* to have variations and a wide range of morphological characteristics and results in complex species boundaries. Indonesia has a variety of habitats suitable for the growth of wild *Begonia* and almost all parts of Indonesia can be found wild *Begonia*. Sulawesi and Sumatra are rich in *Begonia* diversity (Thomas et al. 2013).

Begonia hooveriana Wiriad. is discovered in Sulawesi, Indonesia in 1998. The name was given in honor of Mr. Walter Scott Hoover of the Tropical Conservatory of New England, USA, who sponsored an expedition to search for a new species of *Begonia* in Indonesia (Wiriadinata 2013). Meanwhile, *Begonia hijauvenia* Girm., Ardi & M.Hughes. is found in West Sumatra, Indonesia, especially in lowland forest areas. The name hijauvenia comes from the Indonesian word meaning green, referring to the green veins found on its leaves (Girmansyah et al. 2022).

Research about those two species of *Begonia* is poorly reported. Wiriadinata (2013) reported that the characteristics of *B. hooveriana* are its flat-shaped leaves and shiny dark green color, while Alvitasari (2022) reported that *B. hooveriana* has morphological variations in the form of size, shape, and color in its morphology. Then Girmansyah et al. (2022) also reported that *B. hijauvenia* is unique in male flowers, which have pink color in the adaxial part and white in the abaxial part.

Bogor Botanic Gardens, Indonesia, managed 134 species of *Begonia*, consisting of 97 species of wild *Begonia* and 37 species of exotic *Begonia* in 2019. *Begonia* cultivation and breeding at Bogor Botanic Gardens has produced 17 new varieties (Riswati 2019). The data is certainly subject to change as research on the genus increases. Efforts to explore the potential utilization of *Begonia* as ornamental plants have been carried out at Bogor Botanic Gardens, including characterization activities (Hartutiningsih et al. 2018). Taxonomic approaches are useful in uncovering the diversity of plant species based on morphological characteristics. Therefore, other approaches are needed to complement existing morphological data, such as anatomical characterization of stomata and flowering phenology that can also be important characters in *Begonia* characterization (Chuine and Régnière 2017). To support breeding programs in flowering plants and create new cultivars, information on flowering phases is crucial, particularly regarding stigma receptivity duration and pollen viability.

Data on morphology, flowering phenology, and pollen viability are very important for plant breeding programs. However, studies on the phenology of those species of *Begonia* are still limited. Therefore, it is necessary to conduct a more in-depth study of the morphological characterization, flowering phenology, and pollen viability of *B. hooveriana* and *B. hijauvenia* so that it is hoped that this research can provide new data on morphological diversity and flowering phenology and pollen viability so that it can help in the plant breeding process.

MATERIALS AND METHODS

Plant materials

The study was conducted from September to December 2023 at the nursery greenhouse and laboratory of Bogor Botanic Gardens, National Research and Innovation Agency (BRIN), Bogor, Indonesia. The samples consisted of 3 wild *Begonia* accessions maintained at the Bogor Botanic Gardens (Table 1), and each accession consisted of 3 individuals.

Procedures

Morphological characterization

Morphological characters were characterized to compare the three wild *Begonia* accessions used. Secondary data was obtained through literature studies referencing Tjitrosoepomo (2020) and the General Guide for Describing Ornamental Plants (PVTPP 2021). Meanwhile, quantitative data were obtained by measuring morphological characteristics in the three *Begonia* accessions. The characteristics observed were leaf size, stem size, stalk size, male flower size, and rhizome size. The method used involved direct observation of all samples used.

Stomatal preparation was conducted by creating a mold using dental resin on the lower surface of the leaf (Weyers and Johansen 1985). Once the mold dried, it was coated with transparent nail polish. After drying, the nail polish was carefully peeled off so that the stomata adhered to the layer of nail polish. It comprised an objective magnification of 40x and an ocular magnification of 10x to observe the types and sizes of stomata using a light microscope. The observed stomatal characteristics include stomatal type as well as the length and width of the guard cells. Stomata were observed on the abaxial leaf surface in all three *Begonia* accessions observed. The stomata were collected on the abaxial surface of the leaf because the surface has more stomata compared to the adaxial surface. This is suspected due to the adaxial surface being directly exposed to sunlight, which can lead to damage to the stomata (Papuangan et al. 2014).

Flowering phenology

Observations of flowering phenology were made by following the method of Arteca (2013). The flowering phenological observation was conducted daily from the onset of flower bud emergence until flower blooming and wilting. The length and width of petals were measured each day. Each stage was photographed and documented. Data on flower development phases observed included the number of days of flower development, the duration from flower bud to blooming, and the time from blooming to wilting.

Pollen viability

Pollen viability testing of the three *Begonia* accessions was conducted using staining with 1% acetocarmine solution following the method of Warid and Palupi (2009). Pollen viability was assessed at two different flower maturation times, including: (i) On the first day during flower blooming and (ii) On the 4^{th} day after blooming. The pollen was placed on a microscope slide using a brush, then stained with acetocarmine, and incubated at room temperature for 15 minutes. Subsequently, the preparation was observed under a light microscope with a total magnification of 100x, comprising an objective magnification of 10x and an ocular magnification of 10x. Pollen grains were deemed viable based on the color formed as an indicator. Partial staining of pollen grains after acetocarmine staining indicated non-viable pollen grains, whereas complete staining was categorized as viable pollen grains (Shivakumar et al. 2014). Pollen grains were considered normal if they absorbed at least 70% of the stain, resulting in a dark red color (Dewi et al. 2015).

Table 1. *Begonia* samples observed

Species	Location origin	Collection origin	Code number
Begonia hooveriana Wiriad. with green color	Indonesia, South Sulawesi, Tana Toraja, Makale, 590-1109 m asl,	Bogor Botanic Gardens	B2013070053
Begonia hooveriana Wiriad. with red color	Indonesia, South Sulawesi, Tana Toraja, Makale, 590-1109 m asl,	Bogor Botanic Gardens	MHS20
Begonia hijauvenia Girm., Ardi & M.Hughes	Indonesia, West Sumatra	Bogor Botanic Gardens	MHS15

Data analysis

Data analysis was carried out using a comparative descriptive method with qualitative and quantitative approaches. Morphological analysis was carried out directly by comparing data from observations and measurements; then, a score was made. Data on pore size and stomatal protective cells were taken from images taken using a microscope, then measured using ImageJ software, and analyzed using standard deviation using SPSS 23.00. The determination of mean and standard deviation was performed using Microsoft Office Excel 2010.

Data on flowering phenology were taken from the results of measurements and documentation carried out directly and analyzed for size and the length of the flowering phase from budding to wilting and falling flowers (Arteca 2013). Data on pollen viability were taken from microscope images, then measured the length and width of pollen visible in the field of view with imageJ software. Determination of pollen viability is based on the formula:

$$\text{Pollen viability} = \frac{\text{TOTAL POLLEN STAINED}}{\text{TOTAL POLLEN IN THE FLIED OF VIEW}} \text{x } 100\%$$

RESULTS AND DISCUSSION

Morphological characterization

The environment can affect the morphological and anatomical structures of plants. One of the methods to identify morphological characteristics in plants is by directly observing particular organs, such as roots, stems, leaves, flowers, and fruit (Susetyarini et al. 2020). The wild *Begonia* collection at Bogor Botanic Gardens, namely *B. hooveriana* with green color, *B. hooverianaa* with red color, and *B. hijauvenia*, were suspected to have varying characteristics. This hypothesis is determined based on the diverse morphological variations observed among those three *Begonia* accessions. The most notable morphological variations are the shape, size, and color of the plants and their parts, such as leaves and flowers. A comparison of these variations among the three *Begonia* accessions can be seen in Table 2.

Begonia hooveriana *with green color*

The plant is shrubby, and the upper surface of the leaves is shiny. The petiole is positioned at the edge of the leaf blades. It has a type of inflorescence, protandrous, where male flowers mature earlier than female flowers, and male flowers grow clustered in one peduncle (Figure 1).

Begonia hooveriana *with red color*

The morphological characteristics of *B. hooveriana* with red color are nearly identical to the *B. hooveriana* with green color. The plant is shrubby, the upper leaf surface is shiny, with two color variations, i.e., green and red as spots; has a type of inflorescence, protandrous where male flowers appear earlier than female flowers; male flowers have two color variations, with the petals being light pink when in the bud, then turning white when fully bloomed, leaving pink spots at the tips of the petals; growing clustered in one peduncle (Figure 2).

Begonia hijauvenia

Begonia hijauvenia is an endemic species in Sumatra, with has interesting leaf patterns, brownish with green veins, stiff leaf surfaces, long-haired petioles, grow directly from rhizomes, have an inflorescence type, male flowers have four petals, two larger petals reddish on the outside of the petals and white on the inside of the petals, the outer surface of the petals is hairy with moderate intensity. At the time of observation, female flowers could not be identified because they had not yet appeared. After all, this plant has protandus flowers, where male flowers appear first before female flowers (Figure 3).

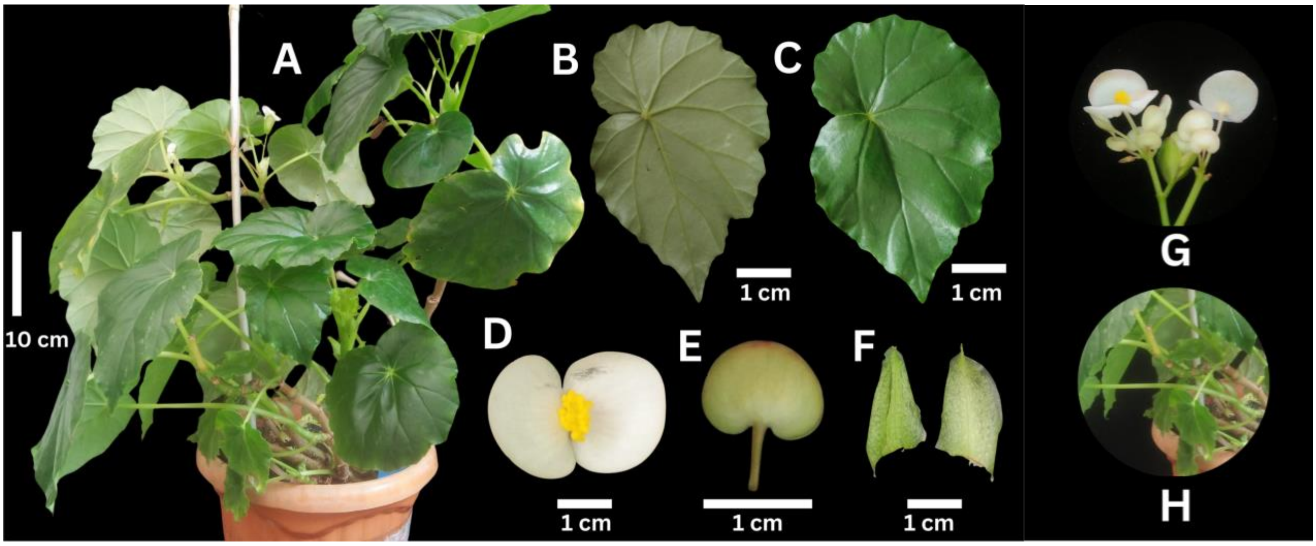

Figure 1. Morphology of *Begonia hooveriana* with green color: A. Habitus; B. Abaxial leaf surface; C. Adaxial leaf surface; D. Male flower in bloom; E. Male flower bud; F. Stipule; G. Inflorescence; H. Petiole. Bar = 10 cm and 1 cm

Table 2. Comparison of morphological characters of 3 accessions of *Begonia* collected at Bogor Botanic Gardens, Bogor, Indonesia

Character	*B. hooveriana*		*B. hijauvenia*
	Green	Red	
Plant type	having a stem	having a stem	rhizome
Plant height (cm)	13.1-80.3	55.0-78.8	14.9-19.7
Leaf type	single	single	single
Leaf shape	ovate	ovate	orbicular
Leaf wrinkles	none	none	finely creased
Leaf base	cordate	cordate	emarginate
Leaf tip	tapered (acuminate)	tapered (acuminate)	tapered (acuminate)
Leaf incision	shallow	shallow	none
Leaf margin	undulate	undulate	entire
Color of leaf blade surface	green	green, red	green, red
Primary color of upper surface of leaf blade	Dark Yellow Green A Green Group 139	Moderate Olive Green A Yellow Green Group 147	Strong Yellow Green A Green Group 143
Secondary color of upper surface of leaf blade	none	Strong Yellow A Green Group 143	Greyish Red A Greyed-Red Group 178
Base color of lower surface of leaf blade	Moderate Olive Brown A Gray Brown Group 199	Greyish Red A Greyed Red Group 178	Strong Yellow Green D Green Group 143
Secondary color of lower surface of leaf blade	none	Strong Yellow Green C Green Group 143	Greyish Brown A Greyed-Orange Group 166
Hair distribution on leaf blade	none	none	entire leaf surface
Intensity of hairs on leaf blade surface	none	none	medium
Petiole length (cm)	7.0-20.5	6.0-16.3	3.5-9.5
Petiole color	Strong Yellow Green A Yellow Green Group 145	Light Yellow Green A Yellow Green Group 145	Moderate Reddish Brown B Greyed-Orange Group 166
Petiole shape	cylinder	cylinder	cylinder
Intensity of hairs on petiole surface	none	none	dense
Flower type	compound	compound	compound
Color of petals	white	white, pink	white, red
Base color of the outer petals of male flowers	White C White Group NN15	White C White Group NN155	Moderate Red B Greyed Red Group 180
Male petal inner base color	White C White Group NN15	White C White Group NN155	Moderate Purplish Pink D Red Purple Group 170
External secondary color male flower petals	none	Vivid Purplish Pink A Red Purple Group N66	none
Secondary color of the inside of male petals	none	Vivid Purplish Pink A Red Purple Group N66	White B White Group NN155
Intensity of hairs on the outer surface of male flowers	none	none	medium
Stem length (cm)	21.6-76.7	20.4-106.4	none
Rhizome length	none	none	4.0-37.5 cm
Color of stem	Strong Yellow Green A Yellow Green Group 145	Strong Yellow Green A Yellow Green Group 145	none
Rhizome color	none	none	Strong Yellow Green C Yellow Green Group 145

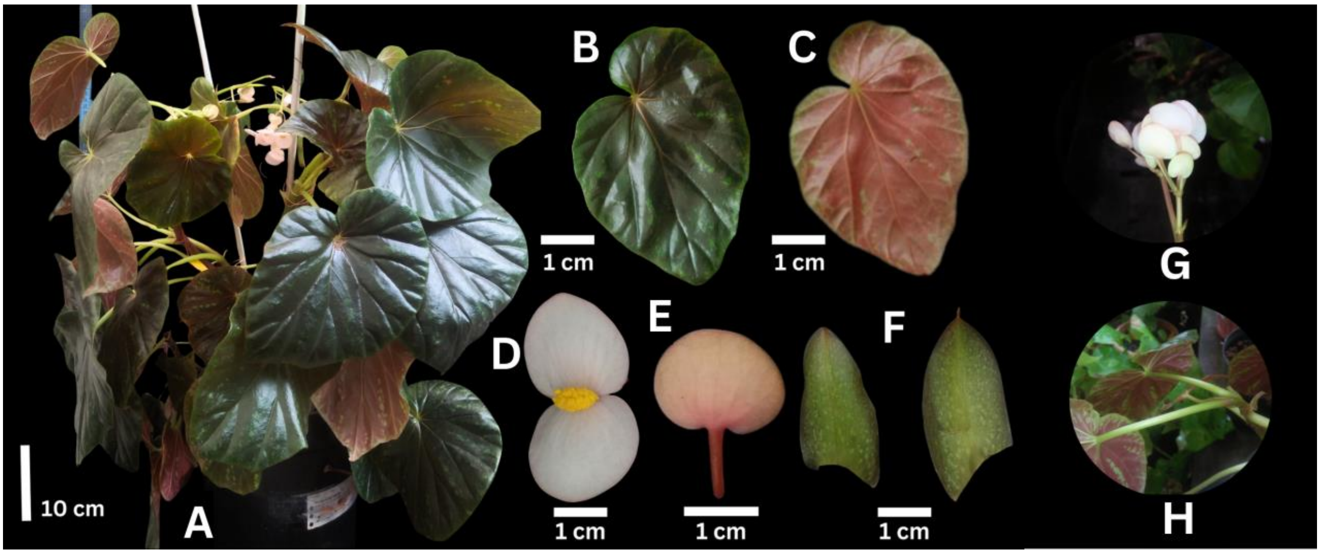

Figure 2. Morphology of *Begonia hooveriana* with red color: A. Habitus; B. Adaxial leaf surface; C. Abaxial leaf surface, D. Male flower in bloom; E. Male flower bud; F. Stipule; G. Inflorescence; H. Petiole. Bar = 10 cm and 1 cm

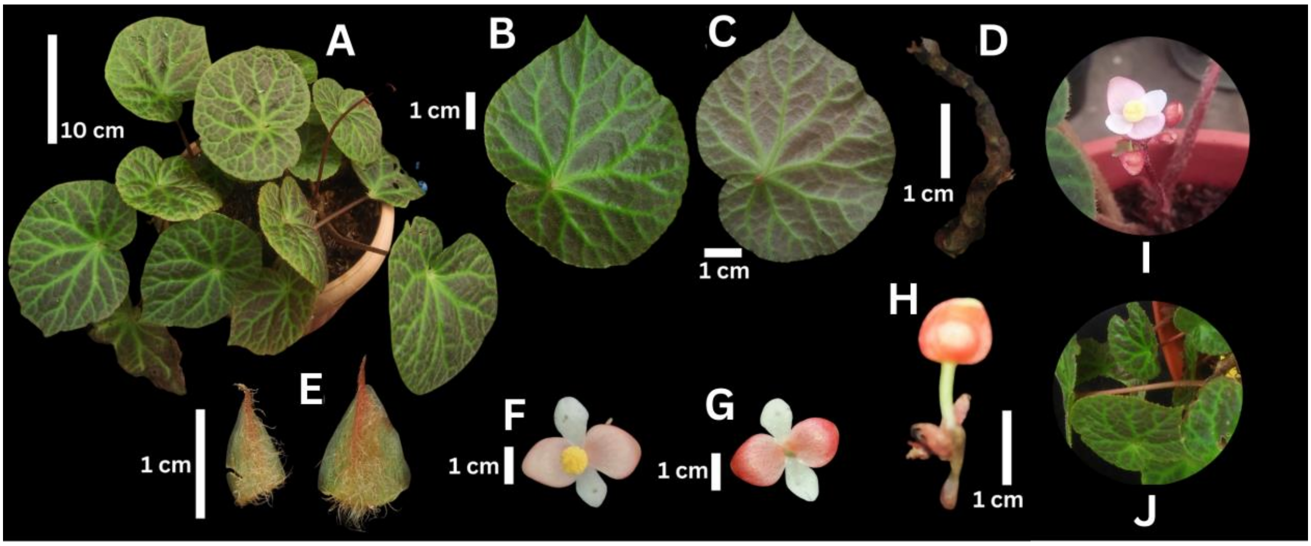

Figure 3. Morphology of *Begonia hijauvenia* with green color: A. Habitus; B. Abaxial leaf surface; C. Adaxial leaf surface; D. Rhizome; E. Stipule; F. Upper surface of male flower when blooming; G. Lower surface of male flower when blooming; H. Male flower bud; I. Inflorescence; J. Petiole. Bar = 10 cm and 1 cm

The morphological characters in plants can change because it is controlled by genetic traits under the influence of environmental factors, such as climate, temperature, soil type, soil conditions, altitude, and air humidity. Red color variations on the stem can occur due to plant responses to abiotic factors, such as low temperature (Hadiyanti et al. 2018). In *B. hijauvenia*, there is a rhizome that groups up to 30 cm long (Girmansyah et al. 2022). Serrated leaf margin variation was found in *B. hooveriana*; this serrated leaf margin variation can show the link between species and their habitat (Nicotra et al. 2011). However, the function of serrations on the edge of *Begonia* leaves is not yet known with certainty because more serrated leaf edges are also found in lowland locations and vice versa; at high altitudes, individuals found have leaf edges with wider serrations (Ayu et al. 2019). While *B. hijauvenia* has flat leaf edges and there are hairs with moderate intensity.

There is no difference in the structure of male flowers in *B. hooveriana* with green color and *B. hooveriana* with red color. Male flowers of both accessions have two petals. However, the male flower petals on *B. hooveriana* with green color are larger than male flower petals on *B. hooveriana* with red color. The male flowers of *B. hijauvenia* have 4 petals with 2 petals that have a larger size, and there are hairs with medium intensity on the outside of the petals. The color of the petals on the three *Begonia* accessions varied from white to white with a pink tinge and red.

Stomatal characteristics

Stomatal type

The results showed that *B. hooveriana*, both with green and red colors, have anisocytic stomatal type. Anisocytic stomata are characterized by three neighboring cells of different sizes (Figure 4). This result supported Efendi (2019), which indicates that *B. hooveriana* has anisocytic stomatal type. Meanwhile, *B. hijauvenia* has a parasitic stomatal type, characterized by each guard cell joining with one or more neighboring cells and the long axis of neighboring cells parallel to the guard cell without a gap (Efendi 2019).

Stomatal size

The stomata of the three *Begonia* accessions observed have different sizes, with the longest guard cells found in the green-colored *B. hooveriana* (5.09±0.27 µm), which is not significantly different from the red-colored *B. hooveriana*, but significantly different from *B. hijauvenia*; followed by *B. hijauvenia* (4.38±1.04 µm), which is significantly different from the other two accessions, and the red-colored *B. hooveriana* (3.73±0.17 µm), respectively (Table 3). As reported by Efendi (2019), the stomatal size characteristics in *Begonia* can not be used to differentiate between species. According to Hong et al. (2018), stomatal size can directly affect transpiration and photosynthesis rates. High stomatal density and size can increase conductance and photosynthetic gas exchange before the leaves undergo final senescence (Sabina and Sameena 2022). Plants with small stomatal density and size will have the ability to maintain high gas exchange rates under high-temperature stress by opening the stomatal pores (Caine et al. 2019).

Flowering phenology

Phenology is one of the plant physiological processes related to biological event timing and environment (Lestari et al. 2023). Observation of flowering phenology was conducted to determine the flowering period in one period, from bud initiation to flower withering and subsequent shedding. The development of flowers in a plant can also be used to determine whether the plant exhibits self-pollination or cross-pollination characteristics (Rahayu et al. 2015). Information about flowering and fruiting phenology is essential to increase productivity and understanding plant physiology and environmental response (Lestari 2019).

When the male flower buds first appear, the petals of *B. hooveriana* have a greenish-white color, while the petals of *B. hijauvenia* are red. On the 2nd day, the color of the petals on red *B. hooveriana* undergoes a color change where a pink color appears at the tip of the petals. When the flowers begin to bloom fully, green *B. hooveriana* petals have a white color until the flowers fall, red *B. hooveriana* petals have a red, white, and pink color at the tip of the petals until the flowers fall, while *B. hijauvenia* petals have two color variations on the 4 petals; the outside and inside of the two large petals have a red color, while the small petals have a white color (Figure 5).

Table 3. Stomatal characteristics observation of the three accessions of *Begonia* collected at Bogor Botanic Gardens, Indonesia

Species	Stomatal type	Length of guard cell (µm)	Width of guard cell (µm)
B. hooveriana with green color	Anisocytic	5.09±0.27^{a}	1.32±0.08^{b}
B. hooveriana with red color	Anisocytic	3.73±0.17^{a}	1.31±0.03^{b}
B. hijauvenia	Parasitic	4.38±1.04^{ab}	1.14±0.08^{a}

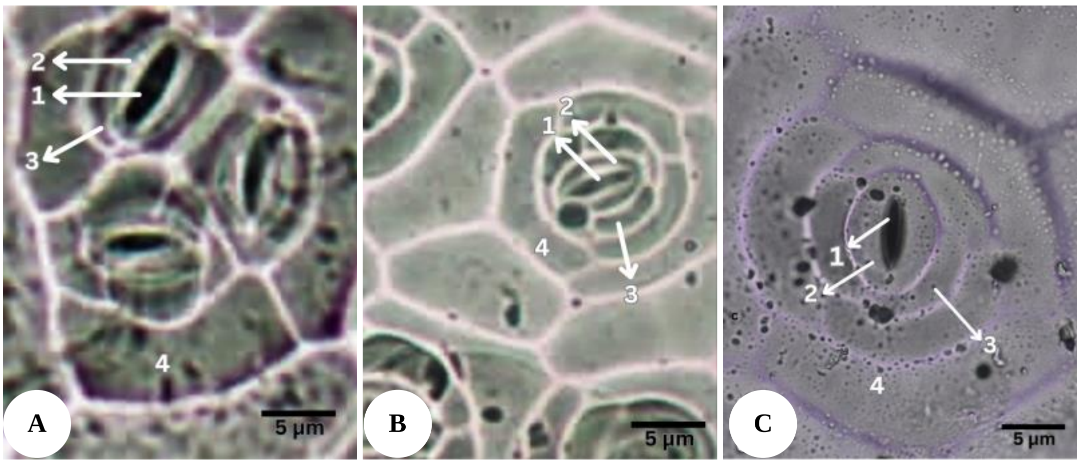

Figure 4. Stomatal anatomy with anisocytic stomatal type in: A. *Begonia hooveriana* with green color; B. *Begonia hooveriana* with red color, and parasitic stomatal type in: C. *Begonia hijauvenia*: 1. Stomatal pore; 2. Guard cell; 3. Neighbor cell; 4. Epidermal cell. Bar = 5 µm

Figure 5. Growth stages of: A. *Begonia hooveriana* with green color; B. *Begonia hooveriana* with red color; and C. *Begonia hijauvenia*: 1. First appearance of flower bud; 2. Flower bud on the 2nd day; 3. Flower bud on the 5th day; 4. Flower bud on the 7th day; 5. Fully open flower on the 1st day; 6. Fully open flower on the 2nd day; 7. Fully open flower on the 4th day; 8. Fully open flower on the 5th day; a. Flower bud; b. Sepals; c. Flower bud changing color; d. Stamen. Bar = 1 cm

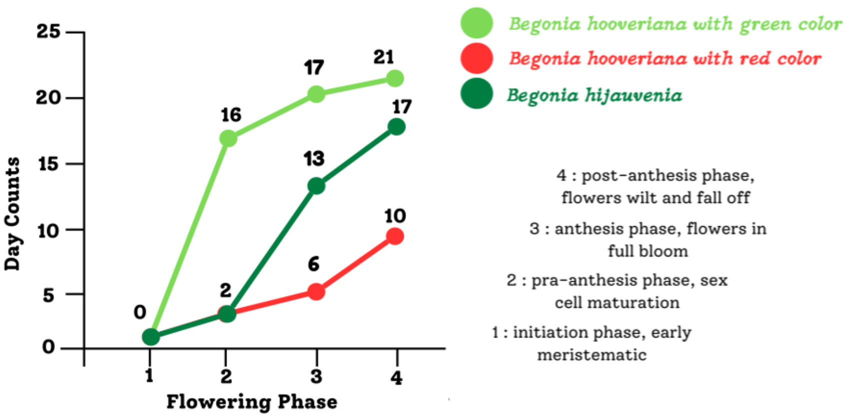

Figure 6. The flowering phase of *Begonia hooveriana* with green color, *Begonia hooveriana* with red color, and *Begonia hijauvenia*

Begonia hooveriana *with green color*

The initiation phase is marked by the appearance of a peduncle with bracts, which are small leaf-like structures that are green in color. This is followed by the emergence of oval-shaped, green organs in the center of the bract, which represent the flower buds. Sepals are the first organs to appear after the bracts. Initially, these are small spheres measuring 9.06-17.15 mm in length and 7.40-14.80 mm in width. The pre-anthesis phase lasts from day 2nd to day 16th, during which the corolla remains in bud form, measuring 10.08-22.50 mm in length and 8.96-14.96 mm in width, with a whiter coloration. The anthesis phase occurs from day 17th to day 20th, during which the flower is fully open, the corolla is white, and the pollen is matured. The final phase, post-anthesis, occurs on day 21st when the flower begins to wither and eventually falls off (Figure 6).

Begonia hooveriana *with red color*

The initiation phase is marked by the emergence of a peduncle with bracts, which are small leaf-like structures that are green in color. This is followed by the appearance of oval-shaped, green organs in the center of the bract, representing the flower bud. Sepals are the first organs to appear after the bracts, initially forming small spheres measuring 10.22-11.80 mm in length and 8.07-9.53 mm in width. The pre-anthesis phase lasts from day 2nd to day 5th, during which the corolla remains in bud form, measuring 12.26-15.44 mm in length and 9.59-12.25 mm in width. The corolla exhibits a whiter and pinkish coloration on the edges. The anthesis phase occurs from day 6th to day 9th, during which the flower is fully open, the corolla is white, and the pollen is matured. The post-anthesis phase occurs on day 10th when the flower begins to wither and eventually falls off (Figure 6).

Begonia hijauvenia

The initiation phase is characterized by the emergence of a red-colored peduncle with fine hairs on its surface. This is followed by the appearance of oval-shaped, red organs at the tip of the stalk, which represent the flower bud. Sepals are the first organs to appear, initially forming small spheres measuring 2.64-4.10 mm in length and 3.26-5.44 mm in width. The pre-anthesis phase lasts from day 2nd to day 12th, during which the corolla remains in bud form, measuring 4.90-6.12 mm in length and 5.56-6.10 mm in width. The corolla is red with some whitish areas. The anthesis phase occurs from day 13th to day 16th when the flower is fully open, the corolla is white, and the pollen is matured. The post-anthesis phase occurs on day 17th when the flower begins to wither and eventually falls off (Figure 6). Based on the results of observation, each flower cluster can consist of 4-10 flower buds, with the flowers blooming asynchronously, alternating between each other from top to bottom. In cross-pollinating plants, this increases the chance of genetic diversity in their offspring, thus beneficial for maintaining their species to prevent extinction (Reed and Frankham 2003). The process of flowering is fundamentally influenced by the interaction of external factors, such as temperature, light, humidity, rainfall, and nutrient availability, with internal factors, such as genetic factors and hormones (Yang et al. 2016; Singh et al. 2018; Navas-Lopez et al. 2019). When plants grow in an environment with optimal conditions, these conditions will induce the plant's flowers to bloom and undergo fertilization.

Pollen viability

Stamens and pistils ripen at different times; the stamens ripen first, and then after a few days, the pistils will ripen. This implies bidirectional movement of the two organs as the pistil elongates and the stigma enlarges (Middleton 2016). The temporal difference in the maturity of male and female organs is termed dichogamy (Cardoso et al. 2018). The quality and quantity of pollen produced by flowers are crucial components for the sustainability of a plant species. This relates to the function of pollen as male gametes are responsible for fertilizing the egg cells, enabling pollination and fertilization to produce fruit and seeds (Hasrianda et al. 2020). Moreover, preservation of the viability and longevity of pollen is very important for plant breeding to overcome the obstacles of hybridization of species with different flowering times (Baninasab et al. 2017). Observations of viable pollen are conducted by observing color changes that serve as indicators of pollen viability, assuming the nutritional content within the pollen is sufficient. Pollen is considered viable if its nutritional content meets certain criteria. Viable pollen typically exhibits a deep red color when stained with 1% acetocarmine, while non-viable pollen remains transparent. The red color observed in viable pollen test results is due to acetocarmine reacting with the exine structure and nucleus. The pollen viability test results indicate varying percentages of pollen viability among the three observed *Begonia* accessions.

The observation of colored pollen showed that the pollen decreased in the post-anthesis phase compared to the colored pollen in the anthesis phase. The highest viability value resulted from red *B. hooveriana* pollen, and the lowest viability value resulted from *B. hijauvenia* pollen (Table 4). This result shows that pollen can experience damage and quality decline along with the length of storage (Samudra and Herawati 2020).

The viability of pollen tested based on coloring also gives diverse results in each *Begonia* with different storage times. In the anthesis phase, the highest percentage of pollen viability is owned by *B. hooveriana* with red color as well as in the post-anthesis phase *B. hooveriana* with red color has the highest percentage of pollen viability (Table 4). This level of fertility is determined by the pollen staining reaction that produces a dark red color after being treated with 1% acetocarmin solution. In comparison, the unstained pollen remains transparent and does not have any reaction (Figures 7-8).

The appearance of red color in pollen is caused by the reaction between acetocarmine and starch content in pollen (Ernawiati et al. 2018). Based on the average percentage of pollen stained with acetocarmine, pollen viability decreases in the post-anthesis phase. This is suspected due to the influence of flower maturation time; the longer the pollen is stored, the more the growth ability decreases. This is related to the water and nutrient content in the pollen; the longer it is stored, the more the water and nutrients deteriorate (Ulfa et al. 2016).

Table 4. Pollen viability in the three *Begonia* accessions from the Bogor Botanic Gardens, Bogor, Indonesia collection

Species	Pollen viability (%)*	
	Anthesis	Post-anthesis
Begonia hooveriana with green color	92.15 ± 6.34^{ab}	91.31 ± 6.53^{b}
Begonia hooveriana with red color	96.60 ± 4.08^{b}	96.30 ± 4.46^{b}
Begonia hijauvenia	84.00 ± 10.20^{a}	83.30 ± 7.84^{a}

Note: *The anthesis phase is observed when the flowers blossom; the post-anthesis phase is observed on the 4th day after the flowers blossom

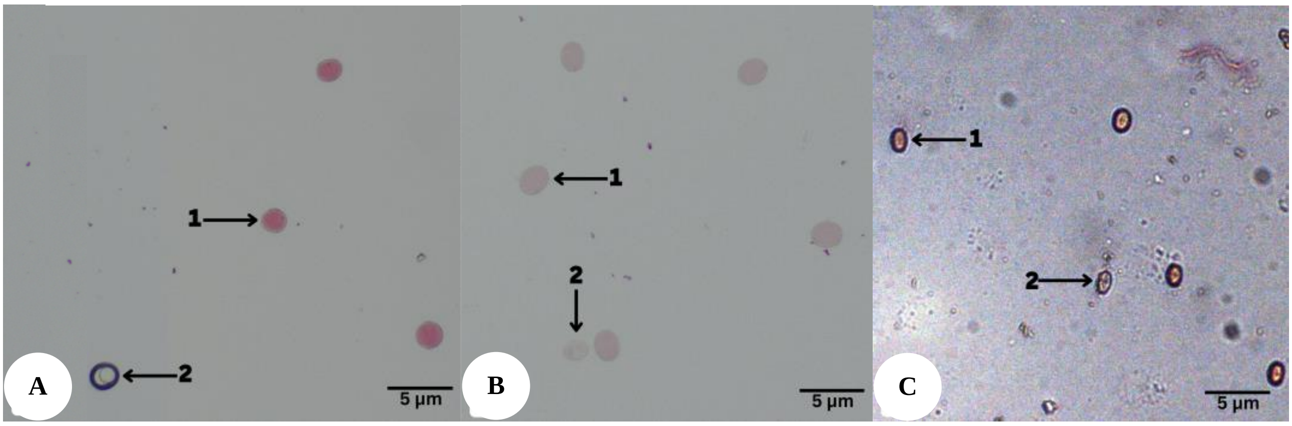

Figure 7. Pollen viability at the anthesis stage: A. *Begonia hooveriana* with green color; B. *Begonia hooveriana* with red color; and C. *Begonia hijauvenia*; 1. Viable pollen; 2. Non-viable pollen. Bar = 5 µm

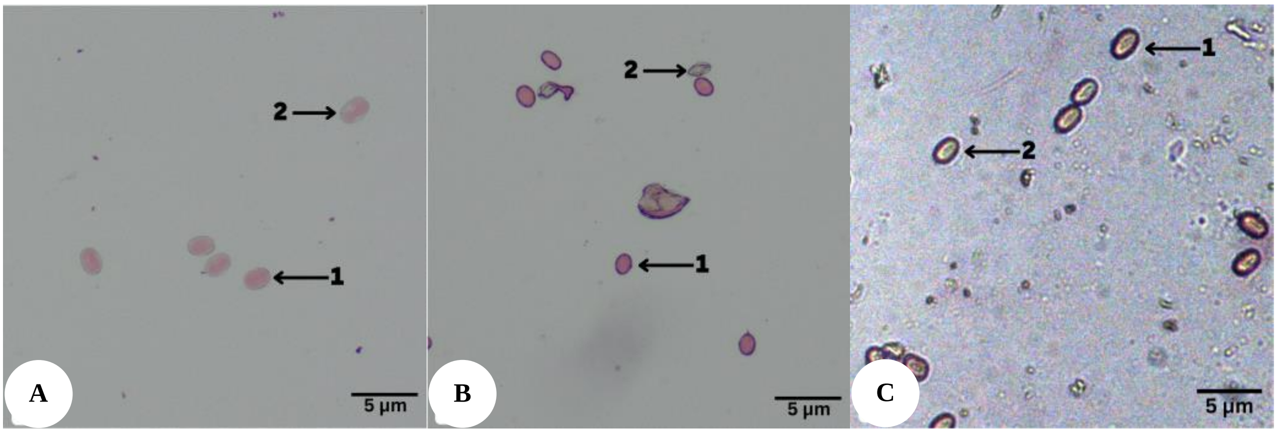

Figure 8. Pollen viability at the post-anthesis stage: A. *Begonia hooveriana* with green color; B. *Begonia hooveriana* with red color; C. *Begonia hijauvenia*; 1. Viable pollen; 2. Non-viable pollen. Bar = 5 µm

The decrease in pollen viability is suspected to occur because the pollen dries out, caused by prolonged storage, leading to a further decrease in viability and loss of germination capacity. Additionally, this is indicated by the wilting of the stamen and the drying of the pollen. In an open environmental condition during flower blooming, pollen is in optimal viable condition when the flower has just started to bloom. These findings are significant as they provide a deeper understanding of the factors influencing pollen viability, which is crucial for plant reproduction and conservation in the field of botany.

In conclusion, three accessions of wild *Begonia* collected at the Bogor Botanic Gardens have morphological variations in stems, leaves, and flowers. Stomata characters reveal differences in size and kind, with *B. hooveriana* possessing the biggest protective cell size and green hue. Anisocytic and parasitic stomata, which are present in all three *Begonia* species, may play a supporting role in the section-level grouping of Begonias. The flowering period of *B. hooveriana* with green color and *B. hooveriana* with red color has a longer flowering period from flower buds to fall compared to *B. hijauvenia*, which is about 10-21 days. At the same time, *B. hijauvenia* flowers have a blooming period of about 17 days. Acetocarmin dye gave high viability values to the three plants. Especially in *B. hooveriana* with red color, in both phases used, namely the anthesis and post-anthesis phases, this plant has the highest viability value among the other two comparisons. *B. hooveriana* has the potential to be used as an ornamental plant because it can flower throughout the year, and the length of bloom is relatively long. This research is an initial study of the diversity of *Begonia*. The data presented can be used as basic information in plant breeding efforts and Begonia conservation. Furthermore, other approaches, such as molecular, are needed to add complete diversity information about *Begonia*.

ACKNOWLEDGEMENTS

We thank the Head of the Research Center for Applied Botany, National Research and Innovation Agency (BRIN), Indonesia, Andes Hamuraby Rozak, for permission to conduct this research. The authors also extend gratitude to the workers at the Greenhouse Nursery, Bogor Botanic Gardens, Indonesia, who helped care for the plants during the research, and Arif Gozali Warso Saputro and Layla Fatimah for their significant assistance during the writing process.

REFERENCES

Alvitasari D. 2022. Morphological and Cytological Diversity of *Begonia hooveriana* Wiriad. in Sulawesi Island. [Thesis]. Institut Pertanian Bogor, Bogor. [Indonesian]

Arteca RN. 2013. Plant Growth Substances: Principles and Aplications. Springer Science and Business Media, Berlin. DOI: 10.1007/978-1-4757-2451-6.

Ayu SAD, Putrika A, Girmansyah D. 2019. Morphological analysis of *Begonia isoptera* Dryand. ex Sm. (Begoniaceaea) complex in Java. Floribunda 6: 53-63. DOI: 10.32556/floribunda.v6i2.2019.263. [Indonesian]

Baninasab B, Tabori M, Yu J, Zhang Y, Wang X, Deschiffart I, Khanizadeh S. 2017. Low-temperature storage and in vitro pollen germination of selected spring wheat accessions. J Agric Sci 9: 1-6. DOI: 10.5539/jas.v9n9p1.

Caine RS, Yin X, Sloan J, Harrison EL, Mohammed U, Fulton T, Biswal AK, Dionora J, Chater CC, Coe RA, Bandyopadhyay A, Murchie EH, Swarup R, Quick WP, Gray JE. 2019. Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. New Phytol 221: 371-384. DOI: 10.1111/nph.15344.

Cardoso JCF, Viana ML, Matias R, Furtado MT, de Souza Caetano AP, Consolaro H, de Brito VLG. 2018. Towards a unified terminology for Angiosperm reproductive systems. Acta Bot Bras 32: 329-348. DOI: 10.1590/0102-33062018abb0124.

Chuine I, Régnière J. 2017. Process-based models of phenology for plants and animals. Ann Rev Ecol Evol Syst 48: 159-182. DOI: 10.1146/annurev-ecolsys-110316-022706.

Dewi SP, Rahayu A, Rochman N. 2015. Flower morphology and pollen viability of various pamelo (*Citrus maxima* (Burm.) Merr.) accessions. J Agronida 1 (1): 37-45. DOI: 10.30997/jag.v1i1.130. [Indonesian]

Efendi M. 2019. Stomatal types of thirty-two Indonesian natural *Begonia* species in the Cibodas Botanical Garden collection. Berita Biologi 18: 175-183. DOI: 10.14203/beritabiologi.v18i2.3571. [Indonesian]

Ernawiati E, Agustrina R, Irawan B, Nurhasanah E, Kanedi M. 2018. Germplasm diversity of banana (*Musa* spp.) in the city of Bandar Lampung, Indonesia by type of genome and number of chromosome. Sch J Agric Vet Sci 5: 251-254. DOI: 10.21276/sjavs.2018.5.4.10.

Girmansyah D, Hughes M, Sulistijorini, Rugayah, Ardi WH, Chikmawati T. 2022. Six new species of *Begonia* (sect. Jackia, Begoniaceae) from Sumatra, Indonesia. Taiwania 67: 97-109. DOI: 10.6165/tai.2022.67.97.

Hadiyanti N, Supriyadi, Pardono. 2018. Diversity of some ciplukan plants (*Phisalis* spp.) on the slopes of Mount Kelud, East Java. Berita Biologi 17: 91-22. DOI: 10.14203/beritabiologi.v17i2.3238. [Indonesian]

Hartutiningsih MS, Wahyuni S, Ardaka IM. 2018. Morphological characterization of natural *Begonia* leaves (Begoniaceae): Prospects for the development of leaf ornamental plant collection in Indonesian Botanical Gardens. Berita Biologi 14(2): 201-211. DOI: 10.47349/jbi/14022018/201. [Indonesian]

Hartutiningsih MS. 2017. The conservation of native, lowland Indonesia *Begonia* species (Begoniaceae) in Bogor Botanic Gardenss. Biodiversitas 18: 326-333. DOI: 10.13057/biodiv/d180143.

Hasrianda EF, Zaelani A, Poerba YS. 2020. Pollen amounts, assessment of viability and germination of 31 banana (*Musa* sp.) accessions from LIPI germplasm collection. Berita Biologi 19: 197-206. DOI: 10.14203/beritabiologi.v19i2.3859. [Indonesian]

Hong T, Lin H, He D. 2018. Characteristics and correlations of leaf stomata in different *Aleurites montana* provenances. PLoS ONE 13 (12): e0208899. DOI: 10.1371/journal.pone.0208899.

Lestari DA, Ningrum LW, Nada MH, Pradipta NN, Harsono DR. 2023. Flowering and fruiting phenology of *Anaxagorea luzonensis* A. Gray (Annonaceae). Biodiversitas 24: 784-792. DOI: 10.13057/biodiv/d240214.

Lestari DA. 2019. Evaluation of flowering and fruiting periods of selected Annonaceae species in Purwodadi Botanic Garden. AIP Conf Proc 2120: 030023. DOI: 10.1063/1.5115627.

Middleton DJ. 2016. A revision of *Aeschynanthus* (Gesneriaceae) in Singapore and Peninsular Malaysia. Gard Bull Singap 68: 1-63. DOI: 10.3850/S2382581216000016.

Munawaroh E, Hartutiningsih MS. 2018. Ex-situ conservation efforts of the Begoniaceae from Bukit Barisan Selatan National Park in Liwa Botanical Garden, West Lampung Regency, Lampung Province. Florea 5: 44-52. DOI: 10.25273/florea.v5i1.2504. [Indonesian]

Navas-Lopez JF, León L, Rapoport HF, Moreno Alías I, Lorite IJ, de la Rosa R. 2019. Genotype, environment, and their interaction effects on olive tree flowering phenology and flower quality. Euphytica 215: 184. DOI: 10.1007/s10681-019-2503-5.

Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H. 2011. The evolution and functional significance of leaf shape in the Angiosperms. Funct Plant Biol 38: 535-552. DOI: 10.1071/fp11057.

Papuangan N, Nurhasanah, Djurumudi M. 2014. Number and distribution of stomata in greening plants in Ternate City. Bioedukasi 3: 287-292. DOI: 10.333.87/bioedu.v2i.62. [Indonesian]

Pusat Perlindungan Varietas Tanaman dan Perizinan Pertanian (PVTPP). 2021. General Guide for Describing Ornamental Plants. Plant Variety Protection and Agricultural Licensing Center, Ministry of Agriculture of Republic of Indonesia, Jakarta. [Indonesian]

Rahayu S, Wawangningrum H, Garvita RV. 2015. Morphological characteristics and flower development of *Aeschynanthus tricolor* Hook. (Gesneriaceae). Berita Biologi 14: 203-211. DOI: 10.14203/beritabiologi.v14i3.1822. [Indonesian]

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. Conserv Biol 17: 230-237. DOI: 10.1046/j/.1523-1739.2003.01236.x.

Riswati MKR. 2019. Bogor Botanic Gardens's Begonia Flora, Beyond Research and Collection. https://kumparan.com/melani-kurnia-riswati-riswati/flora-begonia-kebun-raya-bogor-tak-sekedar-riset-dan-koleksi-1zPDx3JCIId/1. [Indonesian]

Sabina A, Sameena C. 2022. Plant growth and stomatal responses of potato cultivars under high temperature stress. Indian J Agric Res 56: 18-21. DOI: 10.18805/IJARe.AF-683.

Samudra WCP, Herawati MM. 2020. The effect of temoerature and long storage period of *Petunia* pollen viability (*Petuna inflata*). J Appl Agric Res 20: 135-141. DOI: 10.25181/jppt.v20i1.1626.

Shivakumar CU, Gupta R, Thyagaraju MPC, Vishwanath K, Chakrabarty SK, Dadlani M. 2014. Pollen-pistil interaction in protogyny and self-incompability system of Indian mustard (*Brassica juncea* (L.) Coss.). Grana 53: 103-110. DOI: 10.1080/00173134.2014.897750.

Singh G, Virk HK, Aggarwal N, Khanna V, Gill KK. 2018. Growth environment effect on phenology, agroclimatic indices, symbiotic parameters and yield of kharif mungbean (*Vigna radiata* [L.] Wilczek) genotypes. J Food Legumes 31: 205-208. DOI: 10.54386/jam.v26i2.2577.

Susetyarini E, Wahyono P, Latifa R, Nurrohman E. 2020. The identification of morphological and anatomical structures of *Pluchea indica*. J Phys Conf Ser 1539: 012001. DOI: 10.1088/1742-6596/1539/1/012001.

Thomas DC, Ardi WH, Girmansyah D, Hughes M. 2013. Sulawesi *Begonia* data portal. http://portal.cybertaxonomy.org/floramalesiana-prospective/.

Tjitrosoepomo G. 2020. Plant Morphology. Universitas Gadjah Mada Press, Yogyakarta. [Indonesian]

Ulfa SM, Dorly, Rahayu S. 2016. Flower development and pollen viability test of lipstick flower *Aechynanthus radicans* var. 'Monalisa' at Bogor Botanic Gardens. Bulletin Kebun Raya 19: 21-32. DOI: 10.14203/BKR.V19I1.104. [Indonesian]

Wahyu A, Lucyanti S. 2019. *Begonias*, Friends of Survivors. Directorate General of Nature Resources and Ecosystem Conservation, Ministry of Environment and Forestry, Republic of Indonesia. https://ksdae.menlhk.go.id/berita/5301/Begonia-Kawan-Para-Survivor.html. [Indonesian]

Warid, Palupi ER. 2009. Correlation of in vitro germination and staining method in pollen viability testing. Paper presented in the Seminar of Agronomy and Horticulture Department, Faculty of Agriculture, IPB, Bogor.

Weyers JDB, Johansen LG. 1985. Accurate estimation of stomatal aperture from silicone rubber impressions. New Phytol 101: 109-115. DOI: 10.1111/j.1469-8137.1985.tb02820.x.

Wiriadinata H. 2013. A new spesies of *Begonia* (Begoniaceae) from South Sulawesi Indonesia. Reinwardtia 13: 445-448. DOI: 10.55981/reinwardtia.2013.428.

Yang C, Ye Y, Song C, Chen D, Jiang B, Wang Y. 2016. Cloning and functional identification of the AcLFY gene in *Allium cepa*. Biochem Biophys Res Comm 473: 1100-1105. DOI: 10.1016/j.bbrc.2016.04.022.