Reproductive biology of *Tabebuia pallida* (Lindl.) Miers. (Bignoniaceae) collection of Purwodadi Botanic Garden

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**Abstract.** Botanic garden play a central role in the ex-situ plants conservation. It is important to maintain the survival life of the living plant collections. Knowledge on the details of reproductive biology of the living collections becomes vital to monitor the success or failure of conservation efforts. *Tabebuia pallida* (Lindl.) Miers. (Bignoniaceae) is one of the living plants collection in Purwodadi Botanic Garden that need to be monitored. So far information on the reproductive biology of this species is still limited. Based on that, in relation to the conservation effort, the objectives of this study were to provide knowledge about the reproductive biology of *T. pallida*. The research was conducted at Purwodadi BG, from September 2019 to February 2020. Several important aspects of reproductive biology, including flower biology, pollen viability and stigma receptivity, breeding system (Out-Crossing Index), and pollination biology were investigated. The results showed that *T. pallida* was monocious, has a pinkish tubular corolla with a yellow throat. There was no spatial separation between the anther and stigma. At the time of anthesis, both pollen and stigma have been receptive. Based on flower biology, pollen ornamentation (reticulate type), and the Out-Crossing Index (OCI=3), the flower showed some characters of entomophily, but self-pollination was possible. There are some insects visitor, but that acts as a pollinating agent was Vespula. The availability of effective pollinators in botanic garden was an obvious requirement for successful plants conservation.

Keywords: flower biology, pollination, *Tabebuia pallida* (Lindl.) Miers.

1. Introduction

As the climate change is accelerating, botanic garden holds a vital role in conserving plant species used by humans [1,2]. It is crucial for ex-situ conservation and plant biodiversity exploration [3]. Various botanic gardens all over the world have helped conserving millions of living plant specimens [4]. Hence, an effective conservation management requires assorted strategies to maintain the survival rate of living specimens collection in botanic gardens [2].

*Tabebuia pallida* is one of the living plants collection of Botanic Gardens in Indonesia, including Purwodadi Botanic Garden. This is a species in the family Bignoniaceae. This group is known for its high glands of nectar that attract pollinator [5,6]. It provides nectar as a source of energy used by
pallida for feeding [7]. It becomes important in a service ecosystem such as botanic gardens. Therefore, in managing a botanic garden, it is crucial to preserve the continued life of Bignoniaceae. In such case, detailed knowledge of the flower’s reproductive biology becomes vital for the conservation management [8]. The data will be necessary to monitor the success or failure of conservation efforts [9].

To this date, little information was found on the T. pallida reproductive biology. Under the Bignoniaceae family, studies in relation to reproductive ecology, reproductive biology, pollination, breeding system or the like have been conducted to Tabebuia chrysanth [10], Pachyptera hymenacea [11], Campsis radicans [12,13], Jacaranda caroba [14], Pyrostegia venusta [15], Tabebuia caraiba [16], Tabebuia aurea and Tabebuia ochracea [17]. This research aims to figure out the reproductive biology of T. pallida, especially those in Purwodadi Botanic Garden.

2. Materials and Method

2.1. Study Sites and Plant Materials

The study was conducted in Purwodadi Botanic Garden. Latitude and longitude coordinates are -7°47’55”S and 112°44’42”E and altitude is 300 m a.s.l. Field monitoring and laboratory observation was taken from September 2019 until February 2020. The object of the observation in this study was Tabebuia pallida (Lindl.) Miers. in Purwodadi Botanic Garden.

2.2. Research Method

2.2.1. Biological characteristics of T. pallida flower

a. Observation on flower morphology and flowering stage development

Observation on T. pallida flowers was implemented towards 50 flower samples on anthesis stage, which were taken randomly. A detailed observation was performed on the flower structure, and the form and size of each flower (calyx, corolla, stamens, and pistil). To examine the flowering stage development, observation was done for 3 months (September-November 2019) on 20 flower samples starting from bud stage until pollination. Moreover, a record of the environmental factors such as temperature and moisture during the observation was taken as supporting data.

b. Detection of pollen viability and stigma receptivity

Detection of pollen viability was done towards flowers at the day of anthesis stage and the one day before anthesis stage. Ten samples of flowers were used on each stage. Viability test was conducted by a coloring test using 1% TTC (2,3,5 Triphenyl tetrazolium chloride) [18-20]. This method is regarded as more efficient for it is quick and simple [19,21]. Such pollen viability test using 1% TTC has ever been done on Bignoniaceae family like Campsis grandiflora [22]. Freshly collected pollens were separated from the anthers, and then put on a microscope slide set. Next, 1% TTC was dropped on it. After 1 hour, an observation was performed via Olympus CX31 microscope. Red pollen showed fertility/viability while those that are transparent showed sterility. The percentage of viability was calculated by using the following formula:

\[
\text{Pollen viability (\%) = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains}} \times 100}
\]

Stigma receptivity was detected by using hydrogen peroxide test [23]. Stigma on the day of anthesis stage and the one day before anthesis stage was put on a microscope slide set and then dropped by 6% hydrogen peroxide (H₂O₂). The stigma was categorized as receptive if it reacted positively to the hydrogen peroxide solution, which was characterized by formation of air bubbles on its surface.

c. Observation on pollen morphology
Descriptive analysis was performed to the pollen morphology with reference to several palynology publication [24]. The parameters observed were the dispersal unit of pollen, polarity, length of polar axis (P), length of equatorial axis (E), P/E ratio, shape of pollen, aperture type, and exine ornamentation.

2.2.2. Breeding system of T. pallida flower: OCI (Out-Crossing Index) Estimation
Ten blooming flowers were randomly chosen for OCI estimation by referring to the Cruden standards [25]. The OCI value was taken from a total of 3 parameters that were being measured. The parameters include diameter of flower, temporal separation of anther dehiscence and stigma receptivity, and spatial relationship of stigma and anthers [26].

2.2.3. Pollination mechanism
The experimental design was used Completely Randomized Design (CRD) with three replication. The observation on insect visitors of T. pallida at the flowering season of October 2019 from 7:00 am to 3:00 pm. Identification of insect visitors were done in Laboratory of Plant Pests and Diseases, Indonesian Sweetener and Fiber Crops Research Institute, Agricultural Research and Development Agency, Ministry of Agriculture. Insect visitor identification using Insect Classification. The abundance of the insect visitors were recorded by scoring system using the following pollinator abundance criteria [27]:
+ = < 5 insect visitor
++ = 6-10 insect visitor
+++ = 11-15 insect visitor
++++ = > 15 insect visitor

3. Results and Discussions

3.1. Biological characteristics of T. pallida

a. Flower morphology and flowering stage development
T. pallida was categorized into polycarpic species and monoecious. Table 1 and Figure 1 showed a detailed morphology and the size of T. pallida flower parts. The flowers were trumpet-like, and their corolla part into 5 lobes. They were whitish pink in color, and had a yellow throat (Figure 1.a.b.c). The calyx form a cup-like structure of 3 lobes, green in color. The androecium consisted of 4 epipetalous stamens. Moreover, the stamens were didymous (Table 1, Figure 1.e). Each of the stamens had a basifixed anther, which was supported by 1 filament. A very short staminode was found in T. pallida (Figure 1.e). The staminode was sterile in nature, and on each species such as Jacaranda oxyphylla (Bignoniaceae), it functions to attract pollinator, and determines the success of the reproductive biology [28]. The T. pallida gymnoecium contains of 1 bilabiate stigma (Figure 1.g), 1 style, and 1 superior ovary (hypogynous). The gynoeicum was longer than androecium (Figure 1.d). Such condition could be a barrier occurrence the autogamy, so that it required pollination intermediary.

| Period | Floral parts | Size     |
|--------|--------------|----------|
| D-2 anthesis (Figure 1.h.1) | Bud length | ± 10 mm  |
| D-1 anthesis (Figure 1.h.2) | Bud length | ± 30 mm  |
| D anthesis (Figure 1.h.3)   | Calyx length | ± 10 mm  |
|                          | Corolla length | 60 – 75 mm |
|                          | width (corolla diameter) | 45 – 65 mm |
|                          | throat length | 40 – 50 mm |
|                          | throat diameter | 18 x 8 mm – 22 x 14 mm |
|                          | Androecium | 1 pair of upper stamens length | 14 – 15 mm |
1 pair of lower stamens length 9 – 10 mm  
Staminode length 2.5 – 3 mm  
Gynoecium Length 26 – 30 mm  

D+1 anthesis (Figure 1.h.4) Corolla has fallen  

Note: D-2 anthesis = two day before anthesis stage, D-1 anthesis = one day before anthesis stage, D anthesis = the day of anthesis stage, D+1 anthesis = one day after anthesis stage

Figure 1. Floral morphology and development phase of T. pallida and its generative organs. (a) flower on anthesis, side view; (b) back view; (c) front view; (d) position of generative organs; (e) epipetalous + didynamous stamens and 1 staminode; (f) Gynoecium; (g) bilabiate stigma; (h) flower development phase, 1. D-2 anthesis stage; 2. D-1 anthesis stage; 3. D anthesis stage; 4. D+1 anthesis stage.  
Notes: Gy = Gynoecium, An = Androecium, Sd = Staminode, Sg = Stigma, St = Style, Ov = Ovary

The observation results showed a relatively short flowering stage development on T. pallida. The process starting from bud stage (Figure 1.h.1) until pollination (Figure 1.h.4) only required 3 days. On the D-1 anthesis (Figure 1.h.2), when the corolla had not open, the anthers were still closed. The next stage was anthesis (Figure 1.h.3). On this stage, the corolla had entirely bloomed with whitish pink corolla. The gynoecium was fresh green with opening stigma, while the androecium was yellow with opened anthers. On this phase, the flowers extracted fragrance, attracting the pollinators. The condition of both generative organs along with the fragrance extraction showed that pollination occurred at this phase. On the D+1 anthesis, the corolla and androecium organ attached inside them were fallen, leaving the gynoecium and calyx. The gynoecium turned brownish yellow and the stigma had closed (Figure 1.h.4). Such stage showed that the fertilization has occurred.

3.2. Pollen viability and stigma receptivity
A viable pollen and receptive stigma were determinants in fertilization. The results of pollen viability test in this study demonstrated mostly semi-viable pollens on D-1 anthesis, pink in color (Figure 2.a), with a percentage of 56.68 %. As little as 3.10 % pollens were viable (dark red in color), while the remaining 40.22 % were sterile (not viable) (Table 2). The following day (D anthesis), 100 % of the pollens had already been viable (Table 2), dark red in color (Figure 2.b). The results showed that a small potential of pollination because the pollens were highly viable for only 1 day, i.e. on the D anthesis.
The test using hydrogen peroxide (H$_2$O$_2$) showed that stigma on D-1 anthesis and D anthesis had been receptive (Table 2). After being dropped by H$_2$O$_2$, air bubbles were found on the surface of stigma (Figure 2.c,d). Even though both were receptive, but the stigma showed more air bubbles on the D anthesis compared with those on D-1 anthesis (Figure 2.c,d). Generally, a receptive stigma could be reach maximally just after the anthesis stage [29]. Receptive stigma varies between species based on temperature and moisture. A receptive stigma will have a surface containing extracellular protein. Esterase and peroxidase were critical components of such protein, and they determine the stigma reception [29][30]. The formation of air bubbles on the stigma tested by H$_2$O$_2$, which acts as a substrate for enzyme, showed peroxide enzyme activity on the surface of the stigma [9]. The results of this study were in line with that of Barros (2001), stating that the generative organs (anthers and stigma) of Tabebuia aurea and Tabebuia ochracea had short maturity, which was only for a day [17]. On the next day, the anthers had turned into pale yellow and stigma turned into brown in color.

### 3.3. Pollen morphology

The following dispersal units of *T. pallida* pollens were taken from the observation results of pollen morphology under a microscope with 40 x 10 magnification (Table 3, Figure 3).

### Table 3. Description of *T. pallida* pollen in a dry condition (dry pollen)

| Parameter               | Description                                                                 |
|-------------------------|-----------------------------------------------------------------------------|
| Dispersal unit of pollens| Monad                                                                      |
| Pollen size             | Average of polar axis diameter = 37.96 $\mu$m                      |
|                         | Average of equatorial axis diameter = 22.36 $\mu$m                    |
| P/E Index               | 1.7                                                                      |
| Form                    | Prolate                                                                  |
| Pollen class            | Colpate                                                                  |
| Polarity                | Isopolar                                                                 |
| Apertures               | 3 in number                                                             |
|                         | Colpus                                                                   |
|                         | Tricolpate                                                                |
| Exine ornamentation     | Reticulate                                                               |

Notes: + : receptive stigma, ++ : high receptive stigma
Monad means the dispersal unit of pollens consisting of 1 unit pollen grain. Isopolar means *T. pallida* pollens with similar or symmetrical proximal and distal half. Tricolpate was a pollen with 3 elongated apertures. The exine ornamentation on *T. pallida* was reticulate (Figure 4). Reticulate or echinulate ornamentations on pollen reflected biotic pollination (entomophily) while softer ornamentations on pollen reflected abiotic pollination (anemophily) [30].

![Figure 3. Pollen morphology of *T. pallida* while hydrated](image)

3.4. *T. pallida* pollination system
According to the standard OCI, the first parameter score was 3 since the *T. pallida* diameter was 45-65 mm, which means > 6 mm. On the other hand, on the second parameter, the stigma matures before anthers so that the score was 0. The score was also 0 on the third parameter since the stigma and anthers were still in 1 flower. Thus, the total score of the three parameters was 3, which was their OCI value. The score 3 means that the flower was self-compatible, while dichogamy (anthers and stigma do not mature at the same time), or protogyny (stigma matures before anthers) flowers produce nectar and need pollinators for pollination [31]. Relevant to the results of this research, *T. pallida* was also categorized as dischogamy leading to protogyny so that this species would require a pollinator as supporting agent to reproduce. Some literatures point out that Bignoniaceae produce nectar to attract pollinators. However, based on the OCI score, a self-pollination was possible with a slight chance. It was because the stigma position was higher than the anthers (Figure 1.d).

3.5. Pollination mechanism
The insect visitors on *T. pallida* showed that the *T. pallida* was categorized into entomophily or a plant that requires insects as pollinators. From the total 7 insect visitors, consist of 3 orders and 5 families (Table 4, Figure 4). Among the 7 insect visitors, *Frankliniella sp* (Figure 4) visited most frequently, followed by the *Vespula* sp (Figure 4.a) on the second position.

![Table 4. Insect visitors on *T. pallida* collection in Purwodadi Botanic Garden.](table)

| Order          | Family       | Spesies        | Description                                      |
|----------------|--------------|----------------|--------------------------------------------------|
| Hymenoptera    | Vespidae     | *Vespula* sp.  | - Abundance ++                                   |
|                |              |                | - Visit Time: morning-afternoon                  |
|                |              |                | - Visit Duration of 209-291 second/flower        |
|                |              |                | - Insect visitors entered the inside part of the corolla |
| *Delta companiforme* |            |                | - Abundance +                                   |
|                |              |                | - Visit Time: morning-afternoon                  |
|                |              |                | - Visit Duration of 10-20 second/flower          |
|                |              |                | - Insect visitors only stayed on the outer part of the corolla |
### Polistes metricus
- Abundance +
- Visit Time: morning-afternoon
- Visit Duration of 60 seconds/flower
- Insect visitors only stayed on the outer part of the corolla

### Amegilla cingulata
- Abundance +
- Visit Time: morning-afternoon
- Visit Duration of 8-27 seconds/flower
- Insect visitors only stayed on the outer part of the corolla

### Euphyes vestris
- Abundance +
- Visit Time: morning-afternoon
- Visit Duration of 120 seconds/flower
- Insect visitors only stayed on the outer part of the corolla

### Amauris sp.
- Abundance +
- Visit Time: morning-afternoon
- Visit Duration of 120 seconds/flower
- Insect visitors only stayed on the outer part of the corolla

### Frankliniella sp.
- Abundance ++++
- Visit Time: morning-dusk
- Insect visitors entered the inside part of the corolla

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**Figure 4.** Insect visitors to *T. pallida* collection in Purwodadi Botanic Garden. a. *Vespula* sp. (Vespidae), b. *Delta companiforme* (Vespidae), c. *Polistes metricus* (Vespidae), d. *Amegilla cingulata* (Apidae), e. *Euphyes vestris* (Hesperiidae), f. *Amauris* sp. (Papilionidae), g. *Frankliniella* sp. (Thripidae)

1-2 *Frankliniella* sp. (Thrips) were found in almost every flower. Their tiny size enables them to reach the depth of the corolla. However, a study about Bignoniaceae taken in Central American forests claimed that a high number of *Frankliniella* could cause a massive damage to clusters of flowers leading them to drop off [32]. A high number of *Frankliniella* was found on the plant collection during the field observation, and it was assumed to cause clusters of *T. pallida* to drop off. Thrips act as pollinators for Anacardiaceae, Annonaceae, Dipterocarpaceae, and Moraceae [9]. Thrips like smaller flowers that tend to have pale and white in colors with a little fragrance. Thus, *Frankliniella* did not act as a pollinator on *T. pallida*, even though it can reach the depth of corolla with its tiny size.
In general, insect pollinators to Bignoniaceae were bees and wasps [9,33]. The morphology of *T. pallida* corolla, i.e. long, thin, and forming a narrow tube in the middle (Figure 1.a.b.c), with hidden anthers sacs (Figure 1.e) require a more specific wasp as pollinator. From the observation, it was known that *Vespula* sp. can push their heads through *T. pallida* deep corolla tubes for long duration (209-291 seconds/flower). *Vespula* sp. was relatively small of around 10 mm long while the depth of *T. pallida* corolla tubes was approximately 18 x 8 mm – 22 x 14 mm. With such size variations, *Vespula* sp. could smoothly enter the deep corolla tubes of the flowers, and reach their generative organs (anthers and stigma). Therefore, it could be concluded that *Vespula* sp. was an efficient pollinator for *T. pallida* based on its high frequency of visit, and ability to reach the inside parts of the corolla tubes. *Vespula* was categorized as social wasps. They were also known as yellowjackets based on their yellow and black markings.

A wide range of insects that visited *T. pallida* showed that they were attracted by the color of the corolla. The existence of a highly visited species was necessary for conservation management in botanic gardens since it may attract pollinators for its surrounding collections. A species with such potential was crucial for ecosystem service.

4. Conclusion

According to the several aspect of reproductive biology, the flower showed some characters of entomophily, but self-pollination was possible. There were some insects visitor, but that acts as a pollinating agent was *Vespula*. The availability of effective pollinators in botanic garden was an obvious requirement for successful plants conservation.

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