Effect of Fruit Maturity Level on Morphology, Water Content and Germination of *Eumachia montana* Seeds

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***Abstract-*. *Eumachia montana* is one local plant species from Rubiaceae family that has potential as medicinal plant. Seed storage of *E. montana* depends on seed quality. Seed quality can be determined from fruit harvesting accuracy at physiological maturity through fruit maturity level. The aim of this study was to analyze effect of *E. montana* fruit maturity level to seed morphology, water content and germination. Fruits were harvested based on maturity level which was grouped based on fruit skin color. *E. montana* seeds are morphometric characterized, measured for water content and sown. Data were analyzed by variance analysis, Principal Component Analysis, cluster and correlation analysis using statistical program PAST ver. 3.04. Fruit maturity level of *E. montana* significantly influence to seed morphology and germination, but did not significantly influence to seed water content. Grade III with dark orange (10R 5/10) fruit skin color is recommended as fruit maturity level of *E. montana* for harvesting. Seed length is parameter of seed morphology which influence to fruit maturity level. Correlation between fruit maturity level to seed morphology, water content and germination is low, with seed morphology character is positively correlated to germination of *E. montana* seeds.**

Keywords; Conservation; Germination; Maturity; Seed; Storage

I. INTRODUCTION

Genus *Eumachia* (Rubiaceae) was considered to include a single species from Tonga for 185 years. *Eumachia* has added 94 species since 2015 and is a genus of pantropical shrubs, most of whose members were originally included in *Psychotria.* One species of *Psychotria* from Southeast Asia that has been identified as potentially included in the genus *Eumachia* is *Eumachia montana* (Blume) I.M. Turner [1]. Several plant parts of the genus *Eumachia* (such as leaves, roots and rhizomes) have been widely used as ulcers, stomach aches, coughs, and bronchitis. Plants from this species are also commonly used for treatment where there is an infection in the female reproductive system [2]. An example of a species included in the genus *Eumachia* is *E. montana* which is a local plant native to Indonesia which is spread from Andaman Islands, Bangladesh, Borneo, Cambodia, Southeast China, East Himalaya, India, Java, Lesser Sunda Islands, Malaya, Myanmar, New Guinea, Nicobar Islands, Sumatra, Thailand, Vietnam, to Nusa Tenggara [3,4].

Seeds from Rubiaceae family, especially *Eumachia,* have orthodox seed storage behavior. The success storage of orthodox seeds depends on seed quality, one of which is seed maturity level at harvest. Quality seeds are obtained from seeds that are harvested on time, namely when the seeds are physiologically ripe. Each plant species has a different physiological fruit ripening time [5,6,7]. Physiologically ripe seeds are seeds that have good physiological and genetic quality. Seeds that are harvested before physiological maturity will affect their viability because the seeds do not have sufficient food reserves and the embryos are not yet fully formed [8,9,10]. Fruit maturity level will affect seed germination through germination percentage and seed growth rate parameters [9]. This is because in general, seeds will have high vigor if harvested after fruit reaches physiological maturity. This condition is one of the stages of seed development that can determine seed size, seed weight, and color, resulting in significant differences in seed vigor [11].

Determining the right time for harvesting or collecting fruit to obtain high-quality seeds as indicated by its high seed viability is essential. This is important because the seeds will achieve a high germination percentage and be able to produce new conservation strategies for certain species that require it [12]. Based on [13], *E. montana* seeds collected from conservation areas in Bromo Tengger Semeru National Park (BTSNP) for seed bank purposes did not show any germination. Although the seed quality was good through the cut-test results show that 80% seeds were healthy and fresh seeds. The seed storage character of *E. montana* is also unknown, although most of seeds from genus *Eumachia* are orthodox seeds [14]. *E. montana* fruit collected from BTSNP is fruit with maturity level indicated by red to blackish fruit skin colour. Purwodadi Botanic Garden (PBG) as one of the scientific conservation areas for

dry lowland plants also has a collection of *E. montana* which has not been widely studied in relation to fruit maturity level in order to obtain quality seeds for storage or propagation as an additional collection.

Purpose of this study was to analyze fruit maturity level of *E. montana* on seed morphology, water content and germination of its seeds. Results of this study are expected to be used as a reference in determining the harvest time of *E. montana* seeds in the field, in order to obtain quality seeds as a source of seeds, both for storage, propagation and for other research activities.

II. RESEARCH METHODS

A. Study Site

This research was conducted in August 2022 – June 2023 at seed bank laboratory and Bidara greenhouse, Scientific Conservation Area Purwodadi Botanic Garden, Pasuruan, East Java, Indonesia. Seed materials used in this study came from direct seed collection from *E. montana* plants in block X.A.18, PBG (Fig. 1). This plant collection was obtained from exploration result in Timor, Kupang, Indonesia.

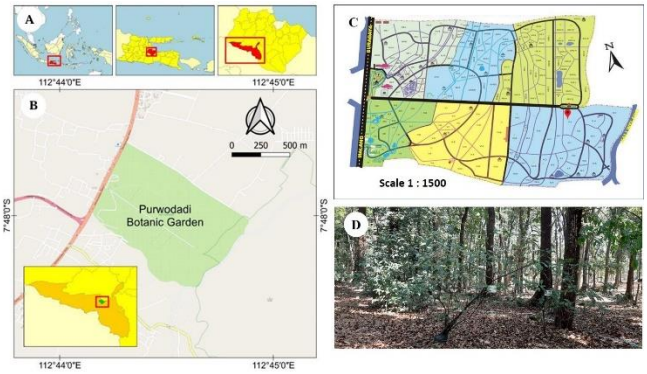

Fig. 1. *Eumachia montana* (Blume) I.M. Turner in block X.A.18, Purwodadi Botanic Garden, Pasuruan, East Java, Indonesia. A. Map of Indonesia, East Java, Pasuruan district, B. Map of Purwodadi Botanic Garden, C. Study site in Purwodadi Botanic Garden (location symbol), and D. Habitus of *Eumachia montana* in block X.A.18

B. Research Procedure

Harvesting Eumachia montana fruit

E. montana fruit is harvested by picking directly based on different maturity levels, consisting of 4 levels (grades). If the grade is higher, the fruit maturity level will be higher, or the fruit skin colour will be darker. The differences in the level of maturity of *E. montana* fruit can be seen in Table 1 and Fig. 2.

Table 1 Differences Skin Fruit Color Of *Eumachia Montana* Based On Maturity Level

Grade	Skin fruit color	Color based on Munsell Color Chart
I	Light orange	5YR 6/10
II	Orange	10R 6/10
III	Dark orange	10R 5/10
IV	Red	10R 4/10

Fig. 2. *Eumachia montana* fruit based on maturity levels

Processing Eumachia montana fruit

The harvested *E. montana* fruit is then processed to obtain ready-to-use seeds by removing the fruit flesh and rinsing it with running water while gently rubbing the surface of the seed skin to ensure that no fruit flesh is left behind. The seeds are then air-dried for 3 days until they are ready to use.

Measuring water content of Eumachia montana seed

The water content of *E. montana* seeds from each grade was measured first before measuring and observing the seed morphological characteristics. There were 10 *E. montana* seeds with 3 replications, each of which was weighed for its wet weight and then oven-dried at a temperature of 108°C for 18 hours [15]. The dry weight of the oven-dried seeds was then measured and the measurement results were entered into (SWC, 1). The oven method is a destructive method.

$$SWC\ (\%) = \frac{\text{weight of seeds before oven} - \text{weight of seeds after oven}}{\text{weight of seeds before oven}} x100\% \quad (1)$$

Measuring and observing morphological characteristics of Eumachia montana seed

Measurement and observation of morphological characteristics of *E. montana* seeds were carried out using morphometric method, where measurements were made on the parts of the seeds that could be measured (such as length, width, thickness, and weight of seeds) and other qualitative characteristics such as shape, colour and texture of seeds. Measurements of length, width, and thickness of *E. montana* seeds using a digital calliper, while measurements of seed weight using a 4-digit analytical balance. Observations of seed shape and texture refer to [16], and observations of seed colour refer to Munsell Colour Chart. Seed samples used for each grade were 10 seeds due to the limited samples obtained. The results of observations of

qualitative morphological characteristics of *E. montana* seeds will be scored in numeric.

Sowing Eumachia montana seed

E. montana seeds were then sown in cocopeat media in seed trays. Each grade consisted of 15 seeds with 3 replications. The sown seeds were watered daily or, if necessary, when the planting medium began to look dry. Parameters observed during the sowing of *E. montana* seeds include germination percentage [17, (GP, 2)], germination rate [18, (Kct, 3)], and initial seed germination.

$$GP\ (\%) = \frac{\text{total seeds were germinate}}{\text{total seeds were sown}}\ x\ 100\% \quad (2)$$

$$Kct = \sum_{i=1}^{n} \frac{(KN)i}{Wi} \quad (3)$$

Whereas: KN is normal seedling and Wi is time of germination

C. Data analysis

Data of seed water content and seed germination of *E. montana* were analysed using analysis of variance (ANOVA) and continued with Least Significant Difference test analysis at 95% confidence level if significant differences were found between fruit maturity levels (grades) using PAST ver.3.04 statistical program. Data of *E. montana* seed morphology observations were analysed descriptively and continued with Principal Component Analysis (PCA) and cluster analysis using PAST ver.3.04 statistical program to determine the seeds morphological characteristics that affect *E. montana* fruit maturity level. Data of seeds morphological characteristics, seed water content, and seed germination will be continued with correlation analysis to determine the relationship betweeen 3 parameters and fruit maturity levels using PAST ver.3.04 statistical program. This analysis aims to determine at what grade *E. montana* fruit can be harvested to produce seeds quality for storage in seed banks or for plant propagation.

III. RESULT AND DISCUSSION

Seed water content of Eumachia montana based on fruit maturity level

The results of seed water content measurements showed that seed water content of *E. montana* decreased drastically along with fruit ripening, from 12.019% (grade II, orange skin fruit colour) to 10.156% (grade III, dark orange skin fruit colour) (Table 2). Seed water content varies in *E. montana* fruit maturity level, where seed water content is high at grade II and seed water content is low at the ageing stage in grade III [19]. Seed water content will decrease with increasing fruit age and reach a minimum water content when fruit is ripe or mature [20].

Table 2 Seed Water Content Of *Eumachia Montana* Based On Fruit Maturity Level

Grade	Seed water content (%)
I	10.756 a
II	12.019 a
III	10.156 a
IV	10.839 a

Seed morphology of Eumachia montana based on fruit maturity level

Seeds that are successfully processed based on fruit maturity level can be seen in Fig. 3. Seed colour will appear darker along with the older maturity level. *E. montana* seeds have a wavy in upper surface, and rough in upper and lower surface. The lower surface of seed is flat and the upper surface of seed is convex with an oval shape and narrows at the end (semi-ellipsoid). The seed characteristics of Rubiaceae family generally include seeds that are flat on one side, convex on the other side, and wavy or have distinctive grooves on their surface [21].

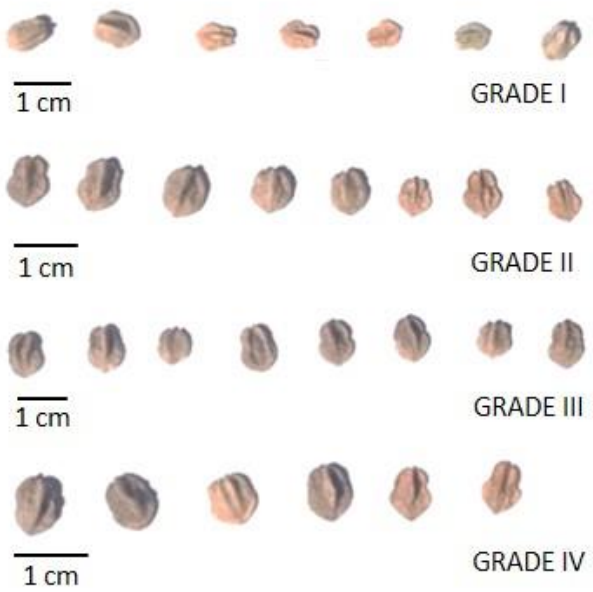

Fig. 3. Differences between seed morphology of *Eumachia montana* based on maturity levels

The highest average of the morphometric measurements of seeds at each fruit maturity level (grade) is seeds at grade III, marked by dark orange skin colour fruit (Fig. 4). Seeds harvested at the right level of maturity can achieve maximum seed viability and vigour. If the harvest is delayed, it will cause seed quality to decrease due to adverse environmental conditions such as high humidity,

rainfall, excessive drying, temperature, pest and disease attacks [22].

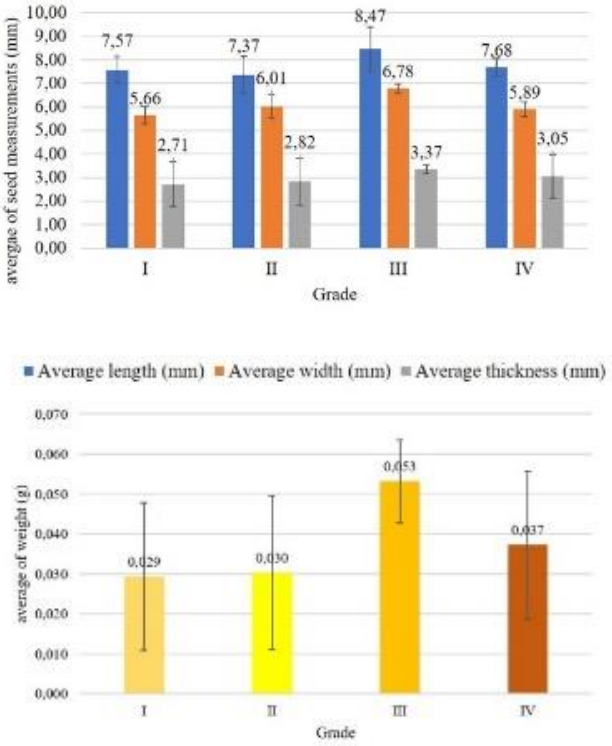

Fig. 4. Graphic of seed morphometric of *Eumachia montana*

Based on Fig. 5, it can be seen that grade III is the recommended fruit maturity level for harvesting and it is influenced by morphological characters with seed length parameters. Other morphological characteristics such as width, thickness, weight, color, texture, and shape of seed do not strongly influence determining fruit maturity level. This is done by grouping seeds based on grade, which shows that grade III is in one cluster while the other grades are scattered (Fig. 6).

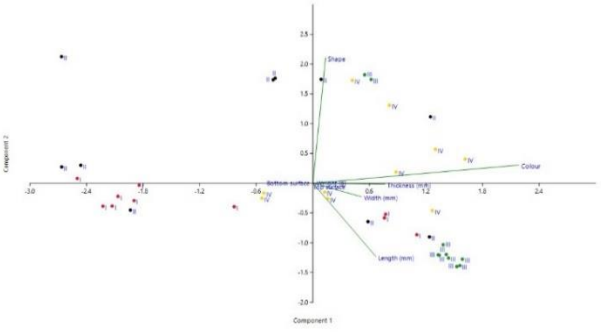

Fig. 5. PCA of seed morphometric *Eumachia montana* based on maturity levels

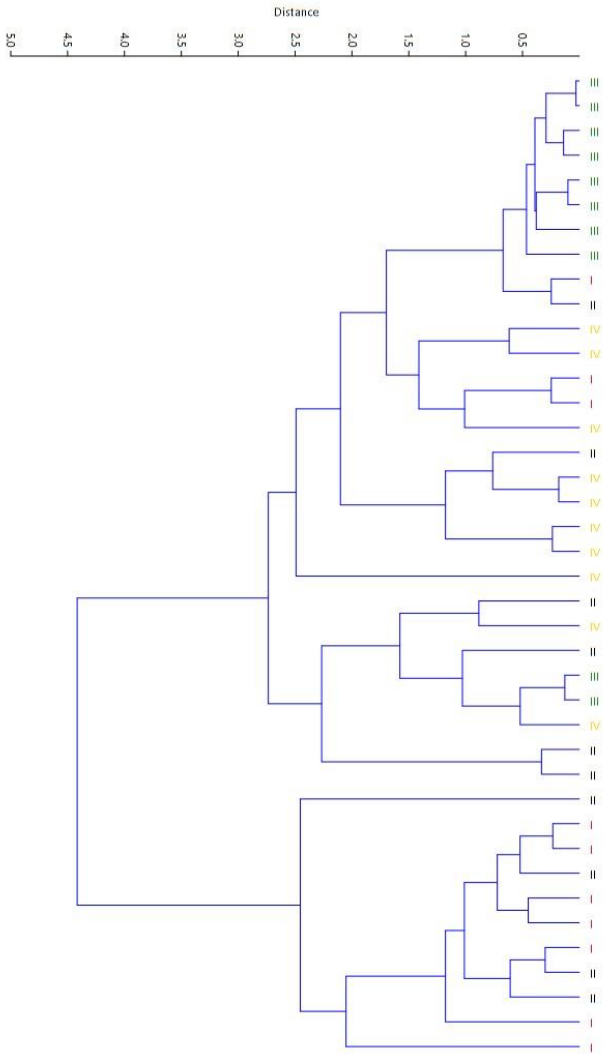

Fig. 6. Cluster of seed morphometric *Eumachia montana* based on maturity levels

Seed maturation is an essential component of seed quality and a prerequisite for successful germination. Study on the physical quality of *E. montana* seeds showed that seeds with maturity in grade III, characterized by dark orange fruits, had the best morphological characteristics (length, width, thickness, and weight of seed) and the lowest seed water content compared to other maturity levels. Seed size and seed water content can be parameters for assessing seed quality. Several studies on seed germination significantly and positively correlated with seed size [23,24]. Seed shelf life is greatly influenced by storage environment, with seed water content being one of the parameters that affect seed viability during storage. Seed germination percentage will decrease as seed water content increases [11]. Therefore, *E. montana* seeds with maturity in grade III, characterized by dark orange skin fruits color, should be harvested and

stored in seed banks because they have the best seed quality in physical and physiological parameters.

Seed germination of Eumachia montana based on fruit maturity level

Based on Table 3, maturity level of *E. montana* fruit has a significant effect on seed germination. Fruit with maturity level of grade III can increase the germination of *E. montana* seeds, although the germination percentage is low (<50%). *E. montana* seeds from grade I are unable to germinate. This is probably because the seed embryo has not been fully formed as a food reserve. Seeds from grades II and IV only germinated nine weeks after sowing, while seeds from grade III were slower to germinate, namely 13 weeks after sowing (Fig. 7). The initial emergence of *E. montana* sprouts and the changes in each phase of the sprouts from the emergence of hypocotyls with an epigeal germination type (Fig. 8) to the cotyledons being fully formed are relatively long.

Table 3 Germination Percentage Of *Eumachia Montana* Based On Fruit Maturity Level

Grade	Germination percentage (%)
I	0 a
II	6.667 a
III	20 b
IV	13.333 a

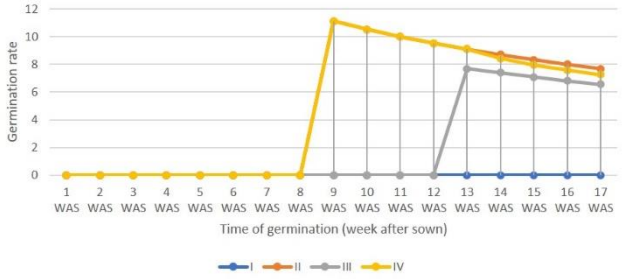

Fig. 7. Germination rate of *Eumachia montana* seed based on maturity levels. WAS = Week After Sown

Fig. 8. Germination of *Eumachia montana* seed from hypocotyl emergence to cotyledon formed

Table 4 shows that fruit maturity level of *E. montana* has a positive correlation with seed germination, whereas the riper fruit, the higher percentage of germination. However, fruit maturity level of *E. montana* negatively correlates with seed water content because the differences in seed water content between grades is not much different. Seed morphological characteristics in seed width and shape positively correlate with seed water content, where seeds are wide and almost round in shape will have high seed water content. Seeds morphological characteristics such as length, width, thickness, weight and seed color positively correlate with seed germination. The best seeds physical or morphological quality, the higher percentage germination of *E. montana* seeds will be. The resulting correlation between fruit maturity level with seed water content, seed germination and morphological characteristics of *E. montana* seeds is categorized as low or weak correlation as indicated by R2 value of <0.4 (Fig. 9, [25]).

Table 4 correlation analysis result between fruit maturity level of *eumachia montana* to seed water content, seed germination, and seed morphological characteristics. %mc=%moisture content, %g=%germination, l=length, w=width, wg=weight, c=color, ts=adaxial surface, bs=abaxial surface, and s=shape

	%MC	%G	L	W	T	WG	C	TS	BS	S
%MC	1									
%G	-0.58	1								
L	-0.31	0.47	1							
W	0.02	0.46	0.36	1						
T	-0.10	0.09	0.38	0.15	1					
WG	-0.34	0.27	0.61	0.38	0.87	1				
C	-0.02	0.27	0.23	0.37	0.76	0.63	1			
TS	0	0	0	0	0	0	0	1		
BS	0	0	0	0	0	0	0	0	1	
S	0.36	-0.19	-0.69	0.21	0.08	-0.07	0	0	0	1

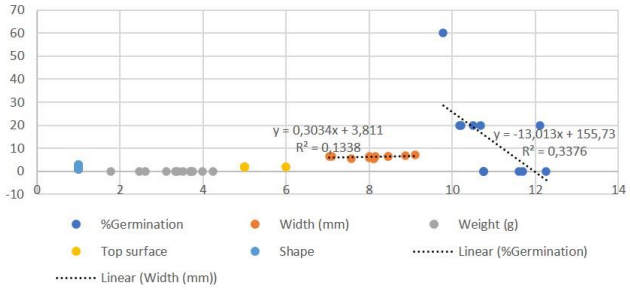

Fig. 9. Graphic correlation between fruit maturity level of *Eumachia montana* to seed water content, seed germination and seed morphological characteristics

Fruit maturity level of *E. montana* does not significantly affect seed water content, but has a significant effect on seed morphology and germination. This condition is likely caused by the seed storage behaviour of *E. montana* seeds is orthodox where seed water content is low (ranging from 10-15%). This is contrast with recalcitrant and intermediate seeds, which have high seed water content when fruit at the physiological ripe stage [26, 27].

The effect of fruit maturity level through fruit skin colour indicator can be used as a reference when fruit harvesting. Fruit harvested when physiologically ripe will affect its seeds viability. However, fruit skin colour as an indicator of physiological maturity in each plant species varies. *E.*

montana fruit can be recommended to be harvested when fruit skin colour is dark orange. [10] stated that coffee fruit maturity indicated by red fruit skin and significantly affects coffee seeds viability. A different thing is shown in harvesting of mango (*Mangifera indica*) fruit, which is recommended to be harvested when fruit is not fully ripe so that seedling growth and vigour index are better than fruit harvested when fully ripe [28]. The difference in fruit maturity level to produce seeds with high viability does not apply to intermediate fruits. Seeds from intermediate fruits do not require maturity based on fruit skin colour to germinate [12]. The low percentage of *E. montana* seed germination (<50%) indicates that *E. montana* seeds likely have dormancy period, requiring specific treatments to encourage seed germination. Further studies on breaking *E. montana* seed dormancy can be conducted, considering that studies on breaking dormancy in orthodox seeds from Rubiaceae family have not been widely studied.

Fruit maturity level also affects seeds size were produced. Fully ripe fruits are produce seed size larger than not fully ripe fruits. Mature seeds will have perfect embryos to be used as food reserves when the seeds are stored or as a source of energy during germination. *E. montana* seeds are longer in size cause better germination percentahe that shorter seeds. This is evidenced by positive correlation between seed morphology and seed germination of *E. montana*, even though the value is low. The correlations produced from seed size to seed germination varies, including height and diameter of seedlings [29], speed of seed germination [30], germination time and seed survival [31]. Based on the results analysis, the recommendation for fruit harvesting at physiological maturiy for *E. montana* to produce high quality seeds from both physical or morphological and physiological characteristics (through seed water content and germination) is fruit with maturity level in grade III, with fruit's skin when ripe is dark orange (10R 5/10). The recommendations can improve seed storage quality in PBG seed bank.

IV. CONCLUSION

Fruit maturity level of *E. montana* has significant effect on seed morphology and germination, but does not have substantial impact on seed water content. The recommended maturity level of *E. montana* fruit for harvesting, which is thought to be physiologically ripe for producing seeds quality, is grade III, with fruit's skin is dark orange when ripe fully fruit. The seed morphology that affects to the fruit maturity level of *E. montana* is seed length. The correlation between fruit maturity level of *E. montana* and water content, germination and morphology of seeds is categorized as low or weak correlation.

ACKNOWLEDGMENT

Sincere thanks dedicated to Purwodadi Botanic Gardens technicians in Rubiaceae block and Slamet Mulyadi who helped author for seeds collecting. Authors would also thanks to the Independent Learning Independent Campus (MBKM) period 2022/2023 for their assistance through research student assistance.

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