

Mangrove Management *Rhizophora mucronata* Lamk. in Mangunharjo, Semarang City Based on Pollen Quality

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ABSTRACT

Rhizophora mucronata is a type of mangrove that plays an important role in coastal life. Reproductivity of *R. mucronata* is influenced by environmental factors. This study aims to examine the effect of environmental conditions on pollen quality in terms of viability and germination. The sampling method used was purposive sampling from three stations and two different flowering phases (pre-anthesis and anthesis). Pollen quality was carried out by staining test using acetocarmine dye and germination test using Brewbaker and Kwack (BK) media. The data were then analyzed using analysis of variance. Based on differences in sampling stations, the highest pollen viability was recorded at Station II (91.25%), and the highest was in the pre-anthesis flowering phase (92.88%). The highest pollen germination was recorded at Station III (95.36%), and the lowest was in the pre-anthesis phase from Station I (87.41%). The results showed that the three different location two different flowering phase were still suitable for the growth of *R. mucronata* mangroves.

Key words: *Rhizophora mucronata*, Reproduction, Viability, Pollen germination

Introduction

Rhizophora mucronata, a species of the *Rhizophoraceae* family, is one of the most widespread mangrove species in Southeast Asia and tropical areas such as South Asia, East Asia, East Africa, Northern Australia, the western Pacific Islands, until it was introduced to Hawaii (Cañizares and Seronay, 2016; Kusmana, 2014; Priyashantha and Taufikurahman, 2020; Setyawan and Ulumuddin, 2012). This species can be found from sea to land (Basyuni *et al.*, 2018; Tin-Zar-Ni-Win and Soe-Win, 2020). *Rhizophora* species are widely planted because this species can function as a barrier to coastal areas from natural disasters, besides, this species is tolerant of salinity,

fluctuations in waterlogging, and sediment accumulation. Its root system in the form of taproot helps in adapting to water level fluctuations (Ibrahim-Bathis, 2021; Nugroho *et al.*, 2019; Phandee *et al.*, 2019). Among the various species, *R. mucronata* is widely used for coastal rehabilitation programs and planted in silvicultural ponds (Hastuti and Budihastuti, 2016).

Rhizophora reproduction generally occurs sexually through flowers and the formation of viviparous propagules (Jamili *et al.*, 2009; Proffitt *et al.*, 2006). One of the factors that influences the success of plant reproduction is pollen. Pollen is a male gamete-carrying microspore cell. The pollen that successfully lands on the stigma will then continue with

the fertilization process, which will later result in the formation of seeds or propagules (Agashe and Caulton, 2009; Gupta *et al.*, 2008; Pacini, 2015). Pollen is very susceptible to environmental changes, extreme conditions such as high temperature and low humidity, can cause the development and formation of pollen to be disrupted, resulting in reduced seed sets and can cause male gamete sterility (Luria *et al.*, 2019). Pollen quality can be determined from the percentage of pollen viability. Viability is the ability of pollen to survive and function normally, the ability to germinate and fertilize (Willmer, 2011). Viable pollen is important for species dispersal, as well as subsequent plant survival (Impe *et al.*, 2020). Measurement of pollen viability can be determined by staining and pollen germination methods *in vitro* in sucrose solution (Gupta *et al.*, 2008). Pollen quality can also be determined by *in vitro* and *in vivo* germination tests, seed formation, and semi-*in-situ* germination of flower stigmas (Impe *et al.*, 2020). Pollen viability and germination are important to study for plant breeding programs (Thangaraja and Ganesan, 2008). The low viability of pollen causes a decrease in the percentage of germination, the growth of the pollen tube becomes slower and affects the formation of seeds (Saragih *et al.*, 2013), this can later affect the reproduction and health of *Rhizophora mucronata* mangroves. In addition, pollen can provide information about environmental impacts, so this information can be used to detect environmental conditions are still suitable or not for the growth of a species (Malayeri *et al.*, 2012). This study aims to determine the health condition of the mangrove *R. mucronata* through the quality of the pollen it produces. We hope that the information from this study can be used as a reference in maintaining and cultivating *Rhizophora mucronata*.

Materials and Method

Rhizophora mucronata flowers from two stages of flowering in the form of buds (pre-anthesis) and blooms (anthesis) were collected from three stations in Wanamina Mangunharjo, northern part of Semarang City, Indonesia (Figure 1). The flower samples were then stored in a cooler box to prevent dehydration and maintain flower freshness until they were tested in the laboratory (Agustin *et al.*, 2014; Vizintin and Bohanec, 2004).

The materials used in this study were *Rhizophora mucronata* flowers, distilled water, glycerin, 1%

acetocarmine, sucrose, boric acid (H_3BO_3), calcium nitrate $Ca(NO_3)2 \cdot 4H_2O$, magnesium sulfate ($MgSO_4 \cdot 7H_2O$), potassium nitrate (KNO_3), filter paper, cotton, and ice gel. The tools used in this study include a water checker (Horiba U-52 Multiprobe), cutter, camera, jar, scissors, cooler box, ruler, GPS, pipette, petri dish, erlenmeyer, measuring cup, object glass, cover glass, microscope, and otilab camera.

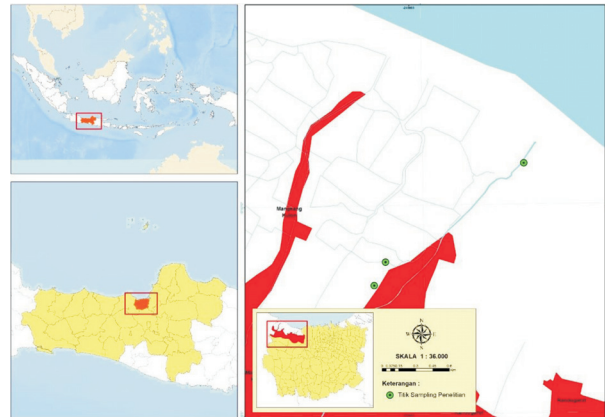


Fig. 1. *Rhizophora mucronata* sampling station in Mangunharjo, Semarang City, Indonesia

Pollen Viability Test

Pollen viability test by staining using 1% acetocarmine (Mantiquilla *et al.*, 2018). The pollen was placed in a glass object and then dripped with acetocarmine and then given the addition of glycerin, after 10 minutes, the pollen was observed using a microscope to observe the population of colored pollen samples. Stained pollen grains are indicated as viable pollen while uncolored or less colored pollen grains are counted as non-viable pollen. Pollen viability calculations were determined using three replicates of approximately 200 pollen grains from each sample. The percentage of pollen viability was determined from the calculation of viable pollen grains divided by the total pollen count.

Germination Test

Pollen germination was carried out on 1 ml of Brewbaker and Kwack or BK media (Brewbaker and Kwack, 1964). The composition of the media consisted of 10% sucrose, 100 ppm H_3BO_3 , 300 ppm $Ca(NO_3)2 \cdot 4H_2O$, 200 ppm $MgSO_4 \cdot 7H_2O$, and 100 ppm KNO_3 dissolved in 1000 ml of distilled water. Pollen was taken using a needle and then placed on a concave object glass that had been given BK me-

dia. The glass preparations were placed in a petri dish containing wet cotton, then incubated for 24 hours, then observed using a microscope. Germinated pollen is characterized by the size of the pollen tube which is approximately equal to or larger than the pollen diameter (Tyagi, 2002). The percentage of pollen germination was determined from the calculation of pollen grains per petri that germinated divided by the total pollen count, seen from the appearance of pollen tubes that were the same size or larger than the diameter of the pollen.

Data Analysis

For statistical analysis, data were analyzed using 5% analysis of variance (ANOVA) followed by further testing using the IBM SPSS program.

Results and Discussion

Environment Parameters

Measurements of the physical and environmental parameters of mangroves from the study area are shown in Table 1. The average temperature of stations 1, 2, and 3 ranges from 24-27 °C, this temperature range is good for mangrove growth, where according to Alongi, (2009) the optimal temperature of mangroves ranges from between 25-30 °C and will experience a decrease in productivity at temperatures above 35 °C. The average pH is 5.34-6.92, where this pH is still in a good range for Mangrove vegetation (Poedjirahajoe *et al.*, 2017). Dissolved oxygen levels were at the third station (6,297 mg/l) and the lowest was at station 1 (4,937 mg/l). Low oxygen can be influenced by phytoplankton activity that is not optimal (Poedjirahajoe *et al.*, 2017). Station 3 has the lowest salinity (21,725 ppt), due to water intrusion from land.

Table 1. Measurement results of mangrove environmental parameters at three locations

Parameter	Station		
	1	2	3
Temperature	24,417b	27,43a	24,517b
pH	5,34b	6,92a	6,28a
ORP	249,00c	306,667b	417,667a
DO	4,937a	4,997a	6,297a
TDS	31,067a	27,767b	21,700c
Salinity	34,063a	29,490b	21,725c
SSG	22,933a	18,567b	13,800a
Depth	0,35a	0,35a	0,40a

Pollen Viability

Analysis using ANOVA with 95% level showed that the research station and flowering phase affected the viability of *Rhizophora mucronata* pollen ($p < 0.05$), but there was no interaction between the research station and the flowering phase on pollen viability ($p > 0.05$). The average pollen viability of *R. mucronata* from Station I was significantly different from Station II and Station III, while the average pollen viability at Station II was not significantly different from Station III (Figure 2). The average pollen viability in the pre-anthesis phase flowers was significantly different from the anthesis phase (Figure 3).

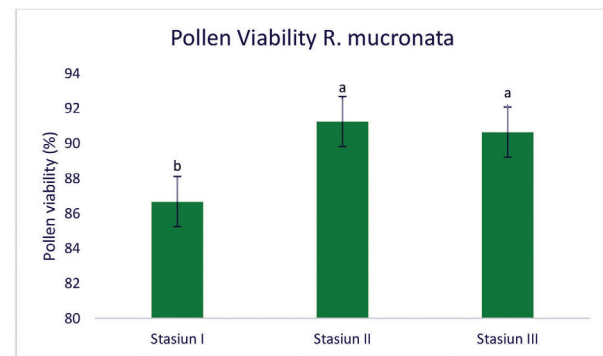


Fig. 2. Graph of the influence of the location of the growing location or research station on the viability of *R. mucronata* pollen. Note: the mean followed by different letters indicates a significant difference ($\alpha = 0.05$). The vertical bar shows the mean \pm standard error

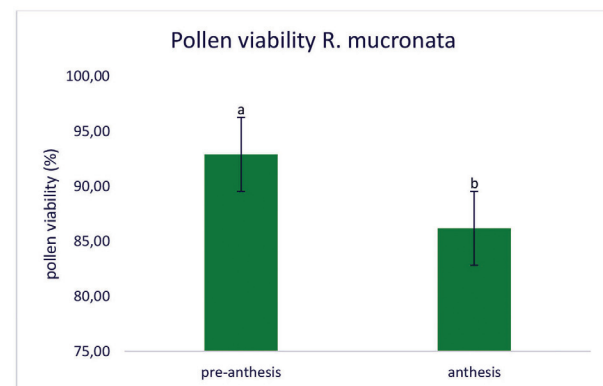


Fig. 3. Graph of the average viability of the pollen viability of *R. mucronata* at different flowering phases. Note: the mean followed by different letters indicates a significant difference ($\alpha = 0.05$). The vertical bar shows the mean \pm standard error.

Acetocarmine dye stains the cytoplasm and chromatin material in viable pollen nuclei (Pascual *et al.*, 2022). Viable pollen grains are indicated by dark red colored pollen grains, while non-viable pollen grains do not absorb color, and appear transparent or clear (Figure 4). The average viability of *Rhizophora mucronata* pollen from three stations and two different flowering phases was high with an average of >70% (Moura *et al.*, 2015; Souza *et al.*, 2002), this indicates that the research environment is still supportive. growth and development of *Rhizophora mucronata* mangroves. The research results of Gupta *et al.* (2008) showed that the viability of *R. mucronata* pollen was 97.66%, this indicates that the pollen will later produce good propagules. Pollen viability can also be influenced by the phase of flower development, where when the anther dehiscence, the pollen grains which apart from the anther act as an independent functional unit and are exposed to the environment, such as temperature, humidity (Kakani *et al.*, 2005). The viability of a pollen is very important for plants because the success in plant reproduction depends on the spread and effectiveness of the pollen for pollination. The lower the viability of pollen, the success of fertilization also decreases, so that determining pollen viability is important in cultivation to determine productivity and production quality (Araújo *et al.*, 2021; Mendez and Acma, 2018).

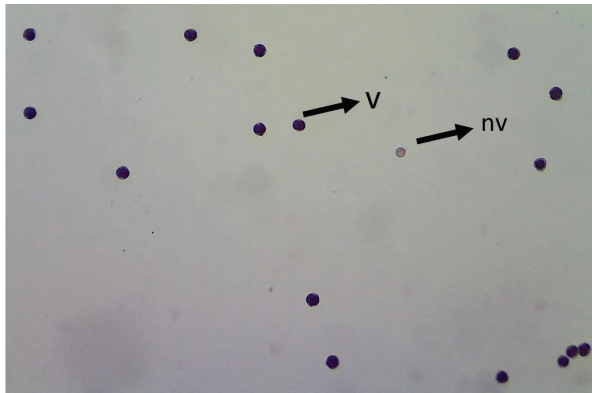


Fig. 4. Pollen viability test with acetocarmine staining under a light microscope with 100x magnification. (v = viable, nv = non viable)

Pollen Germination

Analysis by ANOVA showed that the research station had no effect on pollen germination ($p > 0.05$), while the flowering phase had no effect on pollen germination ($p < 0.05$). There was an interaction between the research station and the flowering phase

on pollen germination ($p > 0.05$) (Figure 5).

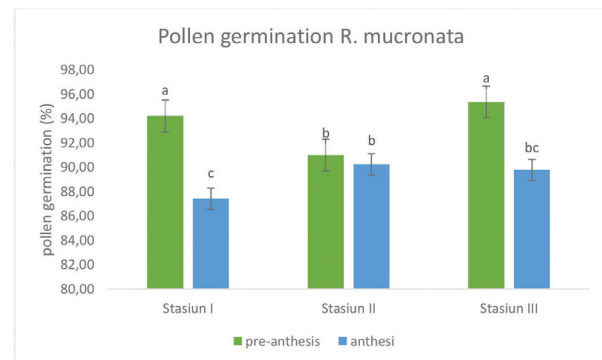


Fig. 5. Graph of *Rhizophora mucronata* Flower Pollen Germination Measurement Results from three stations and different flowering phases in the Mangunharjo area

The average pollen germination from Station I in the pre-anthesis phase was significantly different from the anthesis phase. The average pollen germination from Station II in the pre-anthesis phase was not significantly different from the anthesis phase. The average pollen germination from Station III in the pre-anthesis phase was not significantly different from the anthesis phase. The average pollen germination in the pre-anthesis phase. The average pollen germination in the pre-anthesis flowering phase from the three stations was 90.996-95.537%, while the average pollen germination in the anthesis flower phase ranged from 87.412-90.23%. The low percentage of germination in the anthesis phase can be caused by pollen age, pollen population density, pollen storage process, and the developmental stage of the male gametophyte (Zhang *et al.*, 2010). Germination and pollen tube growth are influenced by various factors, namely, humidity, temperature,

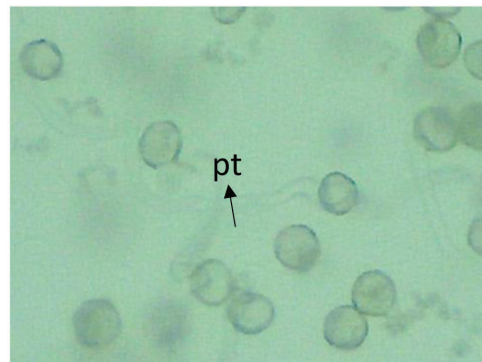


Fig. 6. Germination of *R. mucronata* pollen using Brewbaker and Kwack media at 400X magnification (pt = pollen tube)

genotype differences, vigor, plant physiology, flower age, and materials used in pollen germination media (Youmbi *et al.*, 2015).

The success of the breeding of mangrove *Rhizophora mucronata* can be influenced by the quality of the pollen of mangrove flowers, both in terms of viability and ability to germinate. Pollen quality can be influenced by pollen physiology (age, flower phase) and environmental conditions of mangrove habitat. High viability and pollen germination from each location in the Mangunharjo Mangrove Area indicated successful pollination, and indicated that the natural habitat conditions were still suitable for the growth of *R. mucronata*.

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