

GROWTH OF SLIPPER ORCHID *Paphiopedilum javanicum* (Reinw. ex Lindl.) Pfitzer DURING ACCLIMATIZATION STAGE

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Abstract. *Paphiopedilum javanicum* (Reinw. ex Lindl.) Pfitzer known as slipper orchid is an endemic terrestrial orchid from Indonesia. This orchid is threatened with extinction and listed under CITES Appendix I. In-vitro propagation of *P. javanicum* has been done in Bali Botanic Garden (BBG) for last six years but acclimatization stage has not given satisfied result. The purpose of this study was to know the effect of liquid fertilizer on vegetative growth of *P. javanicum* planlet during acclimatization stage. Beyonic StarTmik liquid fertilizer applied by five different doses considered as treatment of 0, 10, 20, 30 and 40 mL. They were 10 planlets for each treatment considered as replication. In this research we observe vegetative growth of *P. javanicum* by quantitative and qualitative parameter. Quantitative data were analysis by ANOVA. Result showed, high percentage of planlet survival was observed of 98% and liquid fertilizer dose for 30 mL gave the optimal vegetative growth of *P. javanicum* planlet with average height of 2.2 cm and leaves number of 13 pieces. There were no differences on qualitative parameter in all treatment that all leaves color was dark green and leaves tessellation appearance was obvious.

Keywords: acclimatization, growth, *Paphiopedilum javanicum* (Reinw. ex Lindl.) Pfitzer, planlet, survival rate.

Citation

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INTRODUCTION

Paphiopedilum javanicum (Reinw. ex Lindl.) Pfitzer also known as slipper orchid is a terrestrial endemic orchid from Indonesia (Cribb, 2000). Pouch on its flower resulted from modified labellum (Figure 1). This special character that makes the orchid highly demanded by the orchid collectors. Uncontrolled *P. javanicum* exploitation lead this species to extinction and listed on CITES Appendix I. This will be the first publication about *P. javanicum* acclimatization and the information will be useful for conserva-

tion on this endangered and endemic orchid.

In vitro culture is one of the techniques applied for many orchid propagations. Because conventional propagation considered inefficient for planlet propagation. In vitro culture characterized by high humidity, relatively constant temperature, low Photosynthetic Photon Flux Density, high diurnal CO₂ difference, high sugar content, accumulated toxic substrate resulted from explant and lack of microorganism (Xiao et al., 2011; Shin et al., 2014). Hazarika (2006) mentioned that planlet from in vitro culture has low photosynthetic efficiency, stomatal malfunction and

physiological function disorder. Consequently planlet from in vitro culture cannot survive when planted directly in field. Acclimatization is important stage in in vitro culture in order to make healthy planlet before planted in field (Shin et al., 2014).

Studies have been conducted on propagation by in vitro technique on *Paphiopedilum* spp. i.e. *P. delenatii* (Nhut et al., 2005); *Paphiopedilum* Alma Gavaert (Hong et al., 2008); *P. javanicum* (Hendriyani 2010); *Paphiopedilum* Deperle & *Paphiopedilum* Armeni White (Liao et al., 2011); *P. rothschildianum* (Ng & Saleh, 2011); *P. callosum* (Wattanawikkit et al., 2011); *Paphiopedilum* Hsinying Rubyweb (Udomdee et al., 2012); *Paphiopedilum wardii* (Zeng et al., 2012); *P. hangianum* (Zeng et al., 2013); *P. armeniacum* (Zhang et al., 2015); and *P. spicerianum* (Chen et al. 2015). However information related to their acclimatization is still limited.

Since 2007, *P. javanicum* propagation by in vitro culture has been conducted in Bali Botanic Garden. In 2012 the planlet of

P. javanicum is entering the acclimatization stage but the process was not given very good results (unpublished data). Main factor that affect low success on the acclimatization of *P. javanicum* is their slow growth. Success in acclimatization stage will give planlet with high survival rate and optimal growth (Hazarika et al., 2006). One way to increase plant growth and productivity is by using fertilizer (Rychter et al., 2016). Jose da silva et al. (2017) mentioned that liquid fertilizer application can increase nutrition absorption and will increase the plants growth.

Biofertilizers are the formulations of living microorganisms, which are able to fix atmospheric nitrogen become the available form to plants, either by living freely in the soil or being associated symbiotically with plants (Chandrasekar et al., 2005).

Consequently, the purpose of this research was to study the effect of liquid fertilizer application on *P. javanicum* vegetative growth in vitro culture during acclimatization stage.



Figure 1. *Paphiopedilum javanicum* (Reinw. ex Lindl.) Pfitzer flower

MATERIALS AND METHODS

Acclimatization stage was conducted in Bali Botanic Garden Researcher Greenhouse. Research location was established on elevation of 1350 m asl, air temperature of 17-25°C during day and 10-15°C in the night.

This research used *P. javanicum* planlet from in vitro germinated seed (Figure 2A). Planlet characteristic selected were at least had three leaves, healthy rooting and height of 2 cm. Planlet removed from bottle culture and media gently by tap water to avoid root damage. *P. javanicum* planlet soaked in 2 gr/L fungicide Benlate® and dipped in 2.8 ml/L Liquinox Start B1. Planlet put in single pot diameter of 10 cm with fern bark cutting and carchoal ratio of 3:1 v/v media (Figure 2B).

To reduce evaporation by the planlet, the pot covered by plastic.

Beyonic StarTmik liquid fertilizer treatment was given in early research by dose 0, 10, 20, 30 and 40 mL with 10 replications. Parameter observation conducted every two weeks for five times observation (10 weeks observation time). Growth parameter observed including planlet height and number leaves. Plant survival percentage also counted as one of the quantitative indicator for acclimatization successful. The average quantitative data were being counted and analyzed by the statistical tool using ANOVA to know liquid fertilizer treatment effect for planlet height and leaves number. For qualitative parameters we observed leaves color and leaves tessellation appearance.

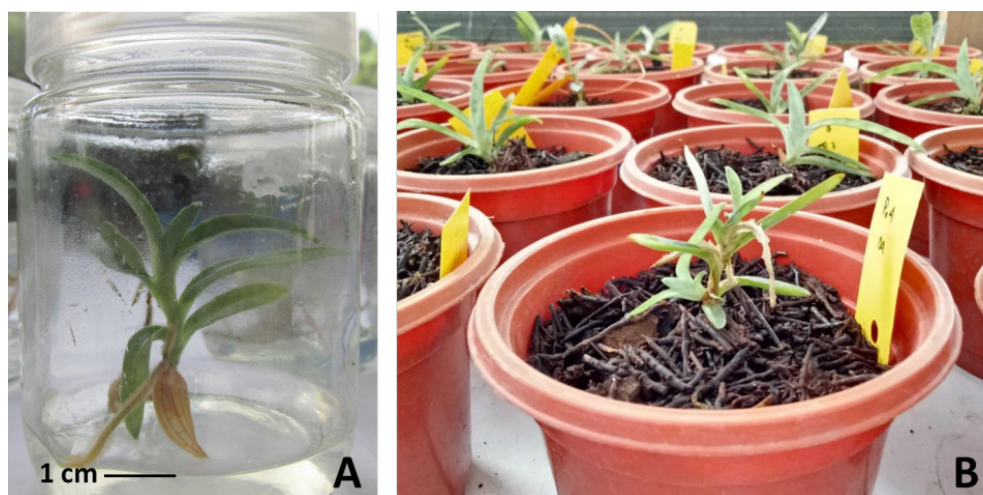


Figure 2. (A) Cultured *P. javanicum* ready to acclimatized, (B) Acclimatized *P. javanicum* planlet

RESULTS AND DISCUSSION

Plant survival percentage during observation on *P. javanicum* planlet is presented in Figure 3. Plant survival percentage was 100% on the first and second observations, and then decreased to 98% on third observation or six weeks after acclimatization (planlet removed

from culture bottle). The leaves were withered and then dry and died, this was because of the planlet incapable to adapt to environmental change during acclimatization stage (fluctuated temperature and humidity). One of in vitro planlet character is an incomplete function of stomata and leaf cuticle (Hazarika et al., 2006).

Based on visual observation, the size of planlet which was removed from in vitro bottle influenced their survival ability in ex vitro environment. Big-sized planlets survived, whereas small-sized planlets tended not to survive (Warseno et al., 2015). So that homogenous big-sized of planlets has to consider.

Plastic cover provision and the placement of planlets (kept away from pest attack) contributed to improving planlet survival rate.

The addition of planlet height and number of leaves were observed. The lowest of average planlet height was 1.69 cm in control and the highest was 2.2 cm in the treatment of 30 mL liquid fertilizer dose (Figure 4).

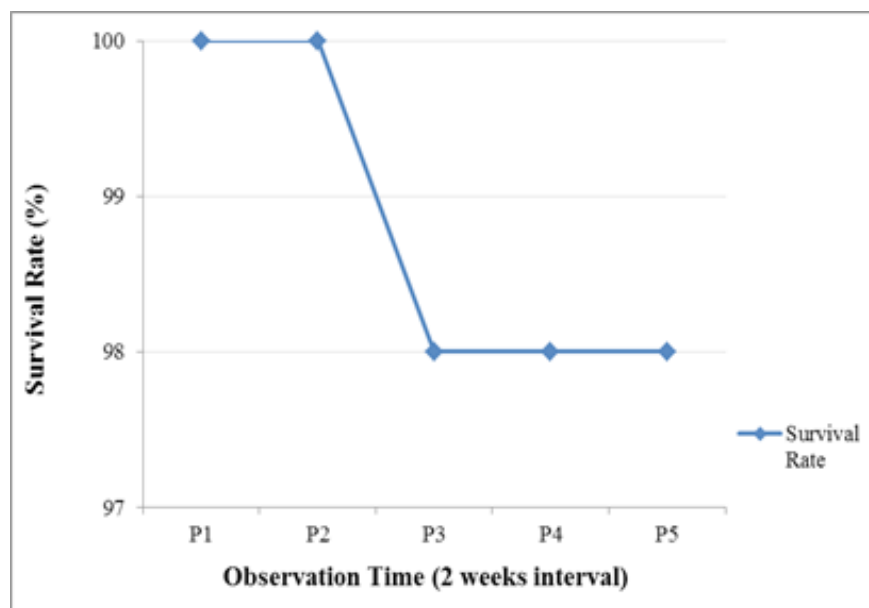


Figure 3. Plant survival percentage of *P. javanicum* during observation period

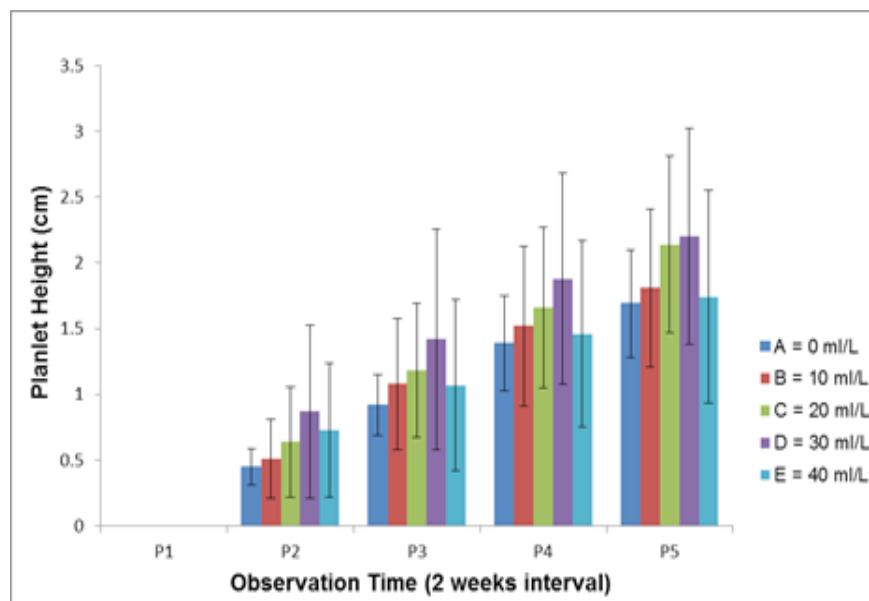


Figure 4. *P. javanicum* planlet height accretion graph during observation period

While on leaf number parameter, the highest leaves addition was found in the treatment of 30 mL liquid fertilizer dose and the lowest was in the treatment of 10 and 20 mL liquid fertilizer dose (Figure 5). The result of One Way ANOVA analysis on *P. javanicum* planlet height and addition of leaves (Table 1) was not significantly different between control and treatments, although the treatment of 30 mL liquid fertilizer dose gave the best result of height and addition of leaves. This is because 30 mL liquid fertilizer contained more microbial activator for phosphate sol-

vent, nitrogen fixation, plant growth regulator production than the other doses (10 and 20 mL). The liquid fertilizer application was done only once in the beginning of this study, so the planlets did not optimally grow. According to Hazarika (2006), one of the physiological abnormalities of in vitro cultures is low photosynthetic efficiency because plants are accustomed to using nutrient sources of sugar from the culture medium. Therefore, to optimize the growth of planlet during acclimatization stage, the application of liquid fertilizer should be applied periodically.

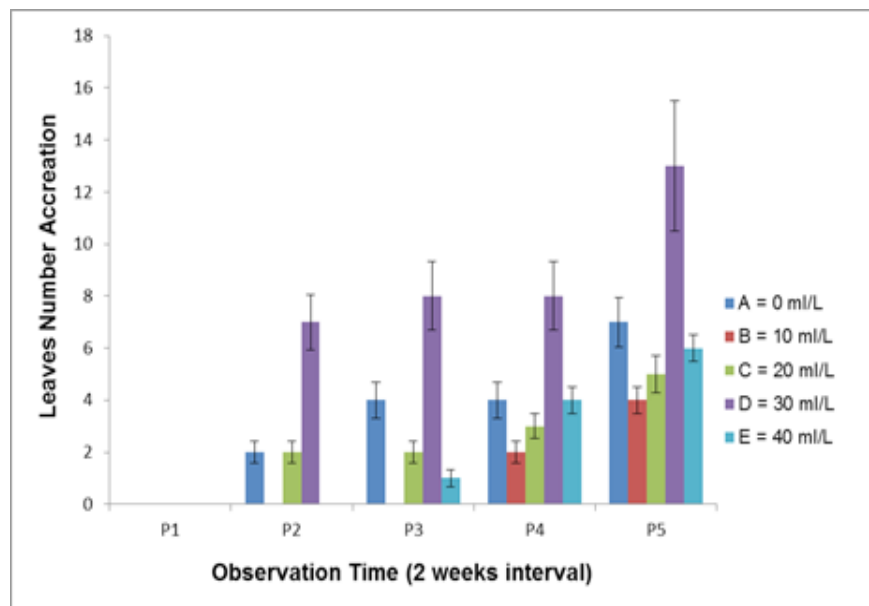


Figure 5. *P. javanicum* planlet leaves number accretion graph during observation period

Table 1. Liquid fertilizer dose effect on height and leaves number accretion on *P. javanicum* planlet

Liquid Fertilizer Dose	Planlet Height	Leaves Number
A (0 mL)	1.69 ^a	9 ^{ab}
B (10 mL)	1.81 ^b	4 ^a
C (20 mL)	2.14 ^{ab}	4 ^a
D (30 mL)	2.2 ^{ab}	13 ^b
E (40 mL)	1.74 ^{ab}	6 ^{ab}

Number followed by the same letter are not statistically different by Duncan test at $\alpha = 5\%$.

Qualitative parameters in this study were observation of the color and tessellation of the leaves plantlets on each treatment (Table 2). The obvious tessellation of *P. Javanicum* leaves were seen on mature leaves. Plantlet propagated by in vitro technique did not showed obvious tessellation on their leaves. According to Table 2, all the treatment of liquid fertilizer dose gave the same effect for the color and tessellation of the leaves.

P. javanicum plantlet survival percentage was 98% during acclimatization observation. In this research 30 mL liquid fertilizer dose was the best treatment for plant height and number of leaves. All liquid fertilizer doses had the same effect for the leaves color and leaves tessellation appearance.

Table 2. Liquid fertilizer dose effect on leaves color and tessellation

Liquid Fertilizer Dose	Leaves Color	Leaves Tessellation
A (0 mL)	Dark green	Tessellation obvious
B (10 mL)	Dark green	Tessellation obvious
C (20 mL)	Dark green	Tessellation obvious
D (30 mL)	Dark green	Tessellation obvious
E (40 mL)	Dark green	Tessellation obvious

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