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Influence of Plant Growth-Promoting Rhizobacteria (PGPR) Concentration and Soaking Duration on the Vegetative Growth of Premna oblongifolia Merr

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Abstract: Indonesia is a country with abundant natural wealth, including a wide variety of flora and fauna that grow and thrive within its territory. Among them are plants that can be utilized as medicinal resources and food ingredients, one of which is green grass jelly (*cincau hijau*). This study aims to determine the effect and interaction between soaking time and PGPR concentration on the growth of green grass jelly (*Premna oblongifolia* Merr.) stem cuttings. The research was conducted from June to September 2024 in Sambirejo Village, Gabus District, Pati Regency. This study used a randomized complete block design (RCBD). The first factor was the length of soaking (L), namely L1 (20 minutes), L2 (40 minutes), and L3 (60 minutes). The second factor is concentration (K), namely K0 (control), K1 (2 ml/L), K2 (4 ml/L), and K3 (6 ml/L). The results showed that soaking duration had no significant effect on the growth of green grass jelly (*Premna oblongifolia* Merr.) stem cuttings. In contrast, PGPR concentration significantly influenced the growth of the cuttings, particularly root length and root dry weight. A PGPR concentration of 6 mL L^{-1} resulted in the highest growth performance of green grass jelly stem cuttings. An interaction between soaking duration and PGPR concentration was observed for root length, with the best response obtained from a soaking duration of 40 minutes combined with a PGPR concentration of 6 mL L^{-1}.

Keywords: Soaking Time, PGPR Concentration, Cuttings, Green grass jelly (*Premna oblongifolia* Merr.)

1. Introduction

Indonesia is rich in natural resources, particularly its diverse flora and fauna. Many of these plants can be used as food and medicinal resources, including green grass jelly (cincau hijau). Green grass jelly shrubs contain various beneficial nutrients, such as vitamins A, B, and C, minerals, and bioactive compounds such as saponins, tannins, and flavonoids (Purniawati et al., 2021).

Based on data from the Central Statistics Agency of Indonesia (Djafar et al., 2021), the production of green grass jelly reached 2,897.79 tons in 2020 but slightly decreased to 2,883.71 tons in 2021. This decline was mainly caused by the COVID-19 pandemic, which reduced consumer demand. As a result, current production levels have not yet fully met market demand. Although green grass jelly has traditionally been produced in Java, its use as a beverage ingredient has recently begun to spread to South Kalimantan. However, cultivation of green grass jelly shrubs in this region remains limited and needs further development (Natalia, 2023).

Green grass jelly plants are generally propagated vegetatively through stem cuttings (Preece, 2003). According to Nahar (2025) one advantage of stem-cutting propagation is that the resulting plants have characteristics identical to those of the parent plant. However, this method is still considered relatively slow and often shows low success rates, particularly due to difficulties in root formation. One approach to stimulate root development is the application of Plant Growth Promoting Rhizobacteria (PGPR).

Plant Growth Promoting Rhizobacteria (PGPR) are soil microorganisms that live around plant roots (Egamberdieva, 2015). These bacteria promote plant growth by releasing essential plant hormones such as auxins, cytokinins, and gibberellins. In addition, PGPR produce enzymes that help plants absorb important nutrients, including phosphorus and iron, from the soil. Indirectly, PGPR also support plant growth by producing antimicrobial compounds that suppress the growth of plant pathogenic fungi (phytopathogens) and by producing siderophores (Elshahat et al., 2016).

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PGPR offers economic advantages as they are relatively inexpensive and easy to obtain, making them a potential alternative to chemical fertilizers and pesticides (Hasan et al., 2024). The application of PGPR to green grass jelly stem cuttings is expected to enhance root and shoot growth. Higher concentrations of PGPR may result in better plant growth, as the bacteria can produce phytohormones such as auxins, cytokinins, and gibberellins, which play an important role in plant growth. Previous studies have shown that rhizobacteria used as PGPR significantly influenced the growth of leaf cuttings in ornamental Peperomia plants (Alves et al., 2022). Based on the above considerations, this study aims to evaluate the effects of soaking duration and PGPR concentration on the growth of green grass jelly (Premna oblongifolia Merr.) stem cuttings.

2.0 Material and Methods

This study was conducted on agricultural land in Sambirejo Village, Gabus District, Pati Regency, at an altitude of approximately 4 meters above sea level. The research was carried out from June to September 2024. The materials used in this study included green grass jelly stems, PGPR FloraOne, planting media consisting of soil, manure, and rice husk charcoal, polybags (15 cm × 20 cm), 70% shade net, bamboo/wood, raffia string, Lannate insecticide, and newspapers. The equipment used comprised hoes, rulers, knives, pruning scissors, buckets, trays, measuring tapes, graduated cylinders, electronic scales, labels, mobile phone cameras, stationery, and an oven.

The experiment was arranged in a factorial Randomized Complete Block Design (RCBD) with two treatment factors and six replications (Raza & Masood, 2009). The first factor was soaking duration, consisting of three levels: 20 minutes (L1), 40 minutes (L2), and 60 minutes (L3). The second factor was PGPR concentration, consisting of four levels: 0 ppm (K0), 2 mL L^{-1} (K1), 4 mL L^{-1} (K2), and 6 mL L^{-1} (K3). Observational data were statistically analyzed using Analysis of Variance (ANOVA) (Armstrong et al., 2000). When significant or highly significant effects were detected, further analysis was conducted using the Least Significant Difference (LSD) test at the 5% significance level with Minitab Statistical Software.

The plant material used in this study was shrub-type green grass jelly (Premna oblongifolia Merr.). Stem cuttings were selected based on good quality criteria, including brown-colored stems, free from pest and disease infestation, and neither too young nor too old. The selected stems were then separated from the mother plants using pruning scissors and cut into 20 cm lengths. The basal end of each cutting was shaped into a V-cut to increase water-absorbing surface area and promote balanced root development.

3.0 Results

3.1 Number of Shoots and Shoot Length

The analysis of variance for the number of shoots and shoot length at 12 weeks after planting showed that neither PGPR concentration nor soaking duration had a significant effect. No interaction was observed between soaking duration and PGPR concentration. The effects of soaking duration and PGPR concentration on the number of shoots are presented in Table 1.

Table 1: Effects of soaking duration and PGPR concentration on the number of shoots and shoot length of green grass jelly (*Premna oblongifolia* Merr.) stem cuttings at 12 weeks after planting (WAP)

Treatments	Number of Shoots	Shoot length (cm)
Soaking Duration		
20 minutes (L1)	1.61 a	1.61 a
40 minutes (L2)	1.56 a	1.56 a
60 minutes (L3)	1.55 a	1.55 a
Concentration		
Control (K0)	1.62 d	1.62 d
2 ml/l (K1)	1.50 d	1.50 d
4 ml/l (K2)	1.59 d	1.59 d
6 ml/l (K3)	1.59 d	1.59 d
Interaction	(-)	(-)

Note: Values followed by the same letter within the same column are not significantly different based on the LSD test at the 5% significance level. The symbol (–) indicates no interaction between treatments.

3.2 Root Length

The results showed that soaking duration and PGPR concentration had significant effects on root length. An interaction was observed between soaking duration and PGPR concentration on root length.

Table 2: Effects of soaking duration and PGPR concentration on root length of green grass jelly (*Premna oblongifolia* Merr.) stem cuttings at 12 weeks after planting (WAP)

Soaking Duration	Concentration			
	Control (K0)	2 ml/L (K1)	4 ml/L (K2)	6 ml/L (K3)
20 minutes (L1)	16.21 de	22.80 abc	20.72 cd	22.26 abc
40 minutes (L2)	25.70 ab	13.96 e	21.18 bc	21.29 bc
60 minutes (L3)	21.88 abc	19.44 ab	21.89 abc	26.00 a

Note: Values followed by the same letter within the same column are not significantly different based on the LSD test at the 5% significance leve

The LSD test at the 5% significance level presented in Table 2 shows that a soaking duration of 20 minutes (L1) resulted in the shortest root length, at 16.21 cm, and was significantly different from soaking durations of 40 minutes (L2) and 60 minutes (L3) under the control PGPR treatment (K0). For the 40-minute soaking treatment (L2), the shortest root length was observed at 13.96 cm, which was significantly different from the 20-minute (L1) and 60-minute (L3) soaking treatments when combined with a PGPR concentration of 2 mL L^{-1} (K1).

At a PGPR concentration of 4 mL L^{-1} (K2), the 20-minute soaking treatment (L1) produced the shortest root length of 20.72 cm; however, this value was not significantly different from the other soaking duration treatments. Meanwhile, the most extended root length was recorded under the 60-minute soaking treatment (L3), reaching 26.00 cm, which was significantly different from the 40-minute soaking treatment (L2) but not significantly different from the 20-minute soaking treatment (L1) when combined with a PGPR concentration of 6 mL L^{-1} (K3).

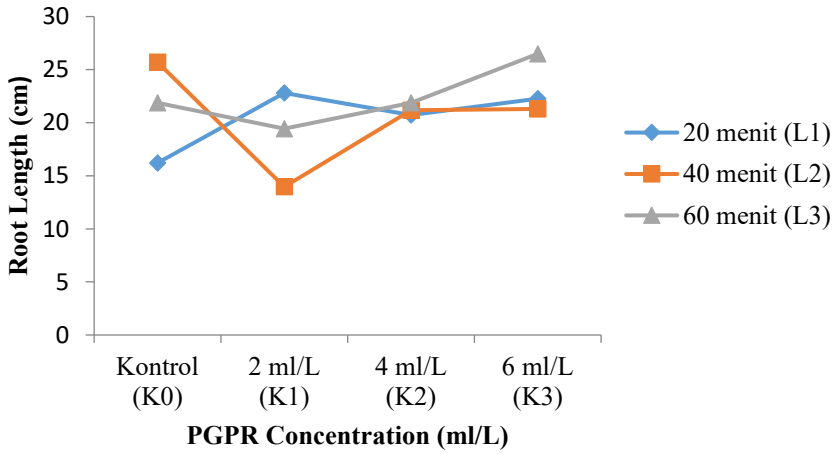

Figure 1: Interaction effects of soaking duration and PGPR concentration on root length

Figure 1 shows an interaction between treatments on root length. The graph indicates that a soaking duration of 20 minutes (L1) combined with the control PGPR concentration (K0) resulted in an increase in root length at 2 mL L^{-1}, followed by a decrease at 4 mL L^{-1}, and then an increase again at 6 mL L^{-1}.

For the 40-minute soaking treatment (L2) combined with the control concentration (K0), root length decreased at 2 mL L^{-1} and then gradually increased up to 6 mL L^{-1}, although the increase was relatively moderate. In contrast, the 60-minute soaking treatment (L3) combined with the control concentration (K0) showed a decrease in root length at 2 mL L^{-1}, followed by a marked increase up to 6 mL L^{-1}, with a relatively higher rate of improvement.

3.3 Fresh and Dry Root Weight

The results showed that soaking duration in PGPR had no significant effect on either fresh root weight or dry root weight. PGPR concentration did not significantly affect the fresh root weight of the stem cuttings, but it did significantly affect root dry weight. No interaction was observed between soaking duration and PGPR concentration on either fresh or dry root weight.

Table 3: Effects of soaking duration and PGPR concentration on fresh root weight and dry root weight of green grass jelly (*Premna oblongifolia* Merr.) stem cuttings

Treatments	Fresh Root Weight (g)	Dry Root Weight (g)
Soaking Duration		
20 minutes (L1)	1.09	0.40 a
40 minutes (L2)	1.33	0.43 a
60 minutes (L3)	1.08	0.44 a
Concentration		
Control (K0)	1.13	0.44 de
2 ml/l (K1)	0.96	0.28 e
4 ml/l (K2)	1.26	0.47 d
6 ml/l (K3)	1.32	0.51 d
Interaction	(-)	(-)

Note: Values followed by the same letter within the same column are not significantly different based on the LSD test at the 5% significance level. The symbol (–) indicates that there was no interaction between treatments.

The LSD test at the 5% significance level showed that a soaking duration of 60 minutes (L3) resulted in a root dry weight of 0.44 g, which was not significantly different from soaking durations of 20 minutes (L1) and 40 minutes (L2). At a PGPR concentration of 2 mL L^{-1} (K1), the root dry weight was 0.28 g, which was significantly different from that at 6 mL L^{-1} (K3), which reached 0.51 g. However, the value at 2 mL L^{-1} (K1) was not significantly different from the control treatment (K0) at 0.44 g or from the 4 mL L^{-1} treatment (K2) at 0.47 g.

4.0 Discussion

The faster shoot emergence observed in green grass jelly cuttings treated with PGPR concentrations is attributed to the presence of Pseudomonas fluorescens, which is known to accelerate shoot development by producing indole-3-acetic acid (IAA). IAA is a naturally occurring plant hormone that promotes rapid growth and root elongation (Tanimoto, 2005). IAA functions by stimulating root growth through cell elongation, while gibberellins enhance the growth of lateral meristems in leaves, and cytokinins promote growth through cell division (Aloni et al., 2006).

In general, short soaking durations did not show an apparent effect on the number of shoots because the amount of auxin absorbed by the cuttings was still insufficient. Conversely, longer soaking durations may cause plant tissues to become saturated with auxin, resulting in no additional effect on shoot growth (Went, 1938). This indicates that soaking duration influences water absorption in green grass jelly cuttings, which may lead to early tissue decay and subsequently affect plant survival.

A PGPR concentration of 6 mL L^{-1} produced longer roots compared to concentrations of 0 mL L^{-1} (control), 2 mL L^{-1}, and 4 mL L^{-1}. Similarly, the 6 mL L^{-1} PGPR treatment resulted in higher root dry weight than the lower concentrations. According to Patten and Glick (2002), bacterial IAA can enhance plant growth by stimulating root differentiation, particularly during root hair formation. At low IAA concentrations, the hormone mainly promotes elongation of the primary root, whereas at higher concentrations, it can induce the formation of lateral and adventitious roots. It is strongly suggested that bacteria present in the PGPR solution, such as Aspergillus niger and Pseudomonas fluorescens, play a role in solubilizing soil phosphorus, thereby improving nutrient availability and plant nutrient uptake. The application of PGPR at a concentration of 6 mL L^{-1} effectively enhanced root length and root dry weight, indicating that an appropriate PGPR concentration can support optimal growth of green grass jelly plants. Furthermore, Egamberdieva et al. (2015) reported that PGPR can stimulate plant growth and root physiology while also reducing damage caused by pests and diseases. PGPR application contributes to soil fertility by improving soil structure, making it more friable, and enhancing soil chemical properties through the stimulation of phytohormone production, cation exchange capacity, and soil biological activity. These findings are closely related to the role of PGPR as a biostimulant, particularly through the production of phytohormones such as auxins, cytokinins, and gibberellins.

5.0 Conclusions

Based on the results of this study, soaking duration had no significant effect on the growth of green grass jelly (*Premna oblongifolia* Merr.) stem cuttings. In contrast, PGPR concentration significantly influenced the growth of the cuttings, particularly root length and root dry weight. A PGPR concentration of 6 mL L^{-1} resulted in the highest growth performance of green grass jelly stem cuttings. An interaction between soaking duration and PGPR concentration was observed for root length, with the best response obtained at a soaking duration of 40 minutes and a PGPR concentration of 6 mL L^{-1}.

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Conflict of Interest

The authors declare no conflicts of interest.

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