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Effect of Seed Priming on Growth of *Andrographis paniculata* and Production of Andrographolide Compound

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ABSTRACT

Andrographis paniculata (Burm. f.) Nees is one of the high-demand medicinal plants and can mostly be found in Asian countries. The plant consists of a few active compounds, mainly andrographolide. This compound has various medicinal properties, including anti-cancer, anti-viral, anti-inflammatory, and anti-malaria. It has been used to treat fever, diabetes, and influenza. The latest research identified an anti-SARS-CoV-2 activity that can be used to treat Covid-19. However, this plant has a low germination rate, which affects its production yield. Thus, seed priming improved germination rate, seedling and plant growth. Osmopriming with polyethylene glycol (PEG) at -0.4 MPa, hormopriming with gibberellic acid at 100 ppm, and control were used in this study and evaluated their effect on plant growth and production of andrographolide compound. PEG treatment significantly produced the highest plant height, number of leaves and branches, leaf area, stem girth, fresh weight of shoot and root, and dry weight of shoot and root compared to the control. This study revealed that seed priming, especially osmopriming, has a high potential to enhance plant growth.

Keywords: *Andrographis paniculata*, *Hempedu Bumi*, seed germination, seed priming, seed treatment

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INTRODUCTION

The application of medicinal plants as an alternative medicine and natural health products is becoming popular. It increases the demand for medicinal plant-based raw materials. *Andrographis paniculata* (Burm. f.) Nees is a high-demand plant originating in South India and Sri Lanka. It is growing in Malaysia, China, Thailand, India, Pakistan

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and Indonesia (Anju et al., 2012; Kumar et al., 2012; Mishra et al., 2007). The plant is from a family of Acanthaceae. It is locally known as 'Hempedu' Bumi' in Malaysia, 'King of Bitter' in English, 'Kalmegh' in India and Bengali, 'Chuan Xin Lian' in China, and 'Quasabhuva' in Arabic (Hossain et al., 2014; Jarukamjorn & Nemoto, 2008). *A. paniculata* consists of a few active compounds, including andrographolide, 12-didehydroandrographolide (DDAG), 14-deoxyandrographolide (DAG), and 14-deoxy-11 that have various medicinal properties, including anti-inflammatory, antidiarrheal, anti-viral, anti-malaria, hepatoprotective, cardiovascular, and anti-cancer (Hossain et al., 2014; Kataky & Handique, 2010; Valdiani et al., 2012). The plant has been used to treat fever, respiratory infection, diabetes, high blood pressure, ulcers, leprosy, bronchitis, skin diseases and influenza (Joseph, 2014; Okhuarobo et al., 2014). Furthermore, the latest study showed an anti-SARS-CoV-2 activity in *the A. paniculata* plant, which has the potential to be used to treat COVID-19 (Sangiamsuntorn et al., 2021). Andrographolide is the main compound in *A. paniculata* with colourless and crystalline labdane diterpenoid lactone and has a very bitter taste (Tang & Eisenbrand, 1992). This compound shows an immunological activity against cancer and human immunodeficiency virus (HIV) (Mishra et al., 2015).

According to Valdiani et al. (2012), China, Thailand, Indonesia, Mauritius, and Malaysia have commercialised and intensively cultivated *A. paniculata* to fulfil the high demand. The plant can be grown between 30 to 100 cm (Figure 1). The stem is dark green, 2 to 6 mm in diameter, with several long divaricates branches (Hossain et al., 2014). The leaves are glossy, with dark green on the upper side and light green on the under (Krishnaswamy & Kushalappa, 2017). The plant can be grown on a variety of soil types but preferably on rich, loamy soil (Shalini & Narayanan, 2015). *A. paniculata* begins to flower 2 to 3 months after transplanting. An optimum time for harvesting is around 3 to 5 months after transplant or when 50% of the plant is flowering, which indicates a high concentration of andrographolide content (Ariffin et al., 2006).

However, this high value of *A. paniculata* has a low seed germination rate and thus can reduce raw material production (Saraswathy et al., 2004). Previously, plasma-treated seed showed a 50% germination percentage compared to the untreated seed with 37.3% on *A. paniculata* (Tong et al., 2020). Meanwhile, seed of *A. paniculata* treated with sodium hypochlorite resulted in 57.33% of seed germination and 22.67% in control (Promwee et al., 2023). Seed priming is an alternative method for reducing emergence time, increasing germination rate, promoting plant growth, and increasing crop yield. It is a simple process that partially hydrates the seed in a controlled environment before drying it without allowing the emergence of radicle (Paparella et al., 2015). Osmopriming is a seed priming technique that soaks the seeds in an osmotic solution with low water potentials, such as polyethene glycol (PEG), glycerol, or mannitol solutions (Kareem & Ismail, 2013). PEG is a water potential-reducing agent and did not have a toxicity effect on the embryo due to its large molecular size

(Thomas et al., 2000). At the same time, hormopriming treats the seeds in hormone solution, including gibberellins, ethylene, and abscisic acid (Ohri et al., 2015; Pirasteh-Anosheh & Hashemi, 2020). This study aims to evaluate the effect of seed priming on plant growth and development and production of andrographolide compound in *A. paniculata* plant.

MATERIALS AND METHODS

Seed Priming

A hundred healthy seeds were used in each treatment. The seeds were surface sterilised with 5% sodium hypochlorite ($NaHClO_3$) for 5 min and then washed with sterile distilled water for 5 m. The sterilised seed was osmoprimed in an aerated solution of PEG-8000 with an osmotic potential at -0.4 MPa. The osmotic potentials of PEG-8000 solutions were calculated using Michel's (1983) formula. Another treatment is hormopriming with 100 ppm of GA_3. All seeds were primed in the dark at 25°C for 24 hours. After priming, the seeds were dried for 24 hours in the dark at 25°C to reach their original moisture content prior to germination.

Seed Germination and Plant Growth

Both primed and non-primed seeds (control) were germinated on a petri dish containing wet Whatman filter paper No.1. The seeds were germinated in between the filter paper. The experiment was conducted in a growth chamber (25 ± 2°C, 65% relative humidity) for a month. Then, the germination seeds of -0.4 MPa of PEG-8000 (64%), 100 ppm of GA_3 (61%), and control (26.5%) were transferred to Jiffy-7 and grown under a rain shelter at the Department of crop science FPSM, UMT for approximately four weeks or until 3 to 4 true leaves emerged. Twenty-seven replicates of each treatment and control with true leaves were transplanted into a polybag (35.56 x 35.56 cm) containing soil mixture (3 topsoil: 2 organic matters: 1 sand) growing media. The seedlings were grown in a greenhouse with a 30 cm × 30 cm distance between polybags at the UMT campus in Bukit Kor, Marang. The plant was watered using a drip irrigation system for 10 minutes in the morning and evening, and 150 kg of compound fertiliser (15:15:15) was applied at a rate of 150 kg per hectare once a month (Abdallah, 2005).

Determination of Plant Growth Parameters

Plant growth parameters, including plant height, number of branches and leaves, and stem girth measurement, were measured at two-week intervals for ten weeks after transplanting (WAT). Plant height was obtained using a ruler from the base of the plant until the end of the plant shoot tip. Stem girt was measured using a Vernier calliper above the plant root collar region.

Plant Harvesting

The plants were harvested once they started flowering (approximately 10 WAT). They were uprooted, washed, and separated into shoots (leaves and stems) and roots. The root was washed carefully using tap water to remove soil and debris, followed by air drying at room temperature to remove access water. The fresh weight of the shoot and root was measured using an electronic weighing scale. The shoot and root were then placed in an oven at 50°C for 48 hours to dry until three times consistent weight was achieved. The dry weight of the shoot and root was measured using an electronic weighing scale.

Andrographolide Analysis

Plant Preparation and Extraction

Fresh leaves of *A. paniculata* were harvested from the UMT campus Bukit Kor Marang after they started flowering. They were carefully washed prior to drying for 24 hours in the dehydrator. The dried sample was ground into powder liquid nitrogen in the mortar and pestle. 10 g of powder leaf samples from each treatment were soaked in denatured absolute ethanol for two days before being filtered. After two days, the extracts were filtered, and the ethanol solvent in the samples was removed using a rotary evaporator. The crude samples were left in the oven at 40°C until all the solvents evaporated. The extracts were sealed and kept in a cold room at 4°C until further analysis.

Sample Preparation

Two (2) mg of ethanol extract was dissolved in 1 ml of 50% methanol. The sample was vortex until all dissolved in the solvent. Then, 1 ml of the sample was filtered into the vial using a syringe and polyvinylidene fluoride (PVDF) (0.25 µm) filter.

Standard Preparation

Two (2) mg of andrographolide standard were dissolved in 1 ml of 50% methanol to make a stock concentration of 2000 ppm. The stock solution was diluted into six concentrations: 1000, 500, 200, 100, 50 and 20 ppm. All the standard solutions were vortex-filtered until all the samples were dissolved in the solvent. Then, 1 ml of standard solution was filtered into the vial using a syringe and PVDF (0.25 µm) filter.

High-performance Liquid Chromatography-UV/VIS Analysis

The identification and quantification of the andrographolide compound were obtained using UFLC from Shidmadzu, Japan (CTO-10AS VP) with a column size of 4.6 × 150 mm, 5 µm (Agilent Zorbax Eclipse XDB-C18). Gradient flow for andrographolide was methanol: water (60:40). The chromatogram was monitored at 223 mm wavelength (andrographolide). The results were expressed in mg per g of dry weight.

Statistical Analysis

The data was analysed using the Statistical Analysis System (SAS) software (version 8.1). A one-way repeated analysis of variance (ANOVA) approach was used to discover significant differences in the means at the $p \leq 0.05$ level, and the means were subjected to the Turkey HSD All-Pairwise Comparisons test.

RESULTS

Seeds primed with -0.4 MPa of PEG-8000, 100 ppm of GA_3 and unprimed (control) were germinated and grown into matured plants for ten weeks (Figure 1). After ten weeks, treatment of -0.4 MPa PEG showed the highest plant height (65.3 cm), number of branches (78) and leaves (155) and a significant difference ($p \leq 0.05$) than GA_3 treatment and control (Figure 2). Meanwhile, no significant difference was observed in the size of stem girth between PEG and GA_3 treatment. On the other hand, all unprimed seeds significantly showed the lowest plant growth analysis at 10 WAT.

The plant height of primed seeds is larger than that of the control, so shoot and root weights are also significantly higher than the control's (Figure 3). Meanwhile, the main active compound, the andrographolide content, showed no significant difference between all treatments and the control (Figure 4).

Figure 1. *Andrographis paniculata* plant at 10 weeks after transplanting (WAT). (A) -0.4 MPa of PEG-8000 with 68 cm height; (B) control with 60 cm; (C) 100 ppm of GA_3 with 63 cm

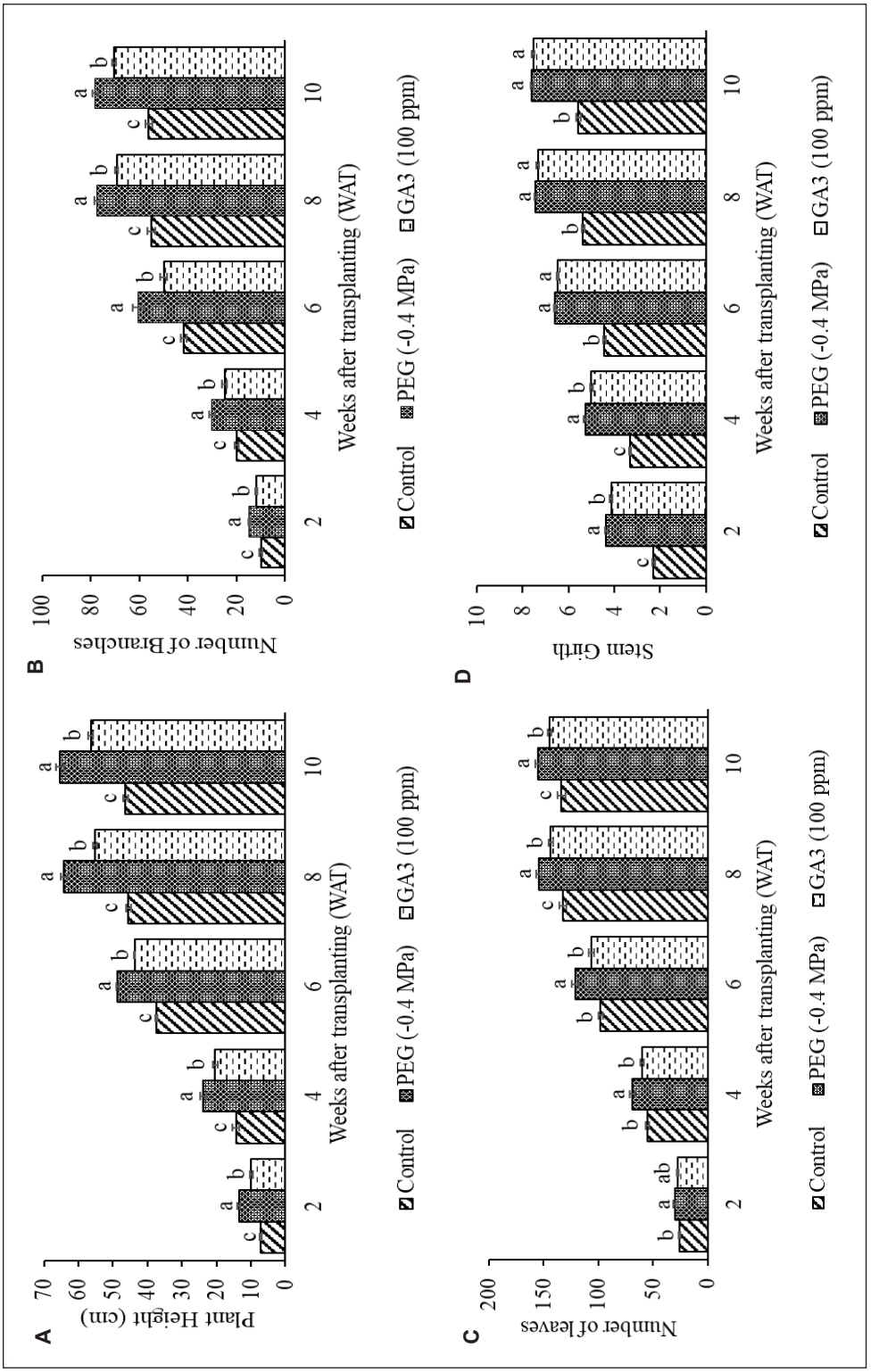

Figure 2. The effect of seed priming on (A) plant height; (B) number of branches; (C) number of leaves; and (D) stem girth of *Andrographis* within 10 weeks of plant growth. Different letters indicate statistically significant differences ($p \leq 0.05$)

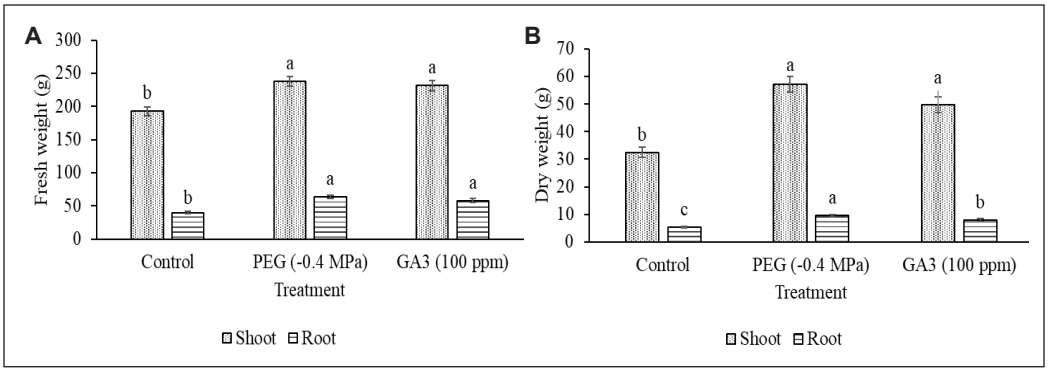

Figure 3. The effect of seed priming on (A) fresh weight of shoot and root and (B) dry weight of shoot and root of *Andrographis paniculata* at 10 weeks after transplanting (WAT). Different letters indicate statistically significant differences ($p \leq 0.05$)

DISCUSSION

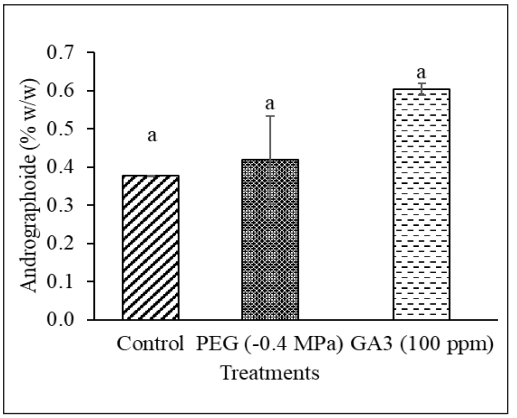

Figure 4. Percentage of andrographolide content (w/w) in *Andrographis paniculata* plant at 10 WAT in control, PEG, and GA_3

Seed priming is a low-cost and effective technique to enhance seed germination, plant growth, and yield. In this study, seeds primed with PEG-8000 at -0.4 MPa and GA_3 at 100 ppm were selected as an optimum treatment with high germination and seedling growth (high seedling vigour index, fresh weight, and length) based on the previous study (Abdullahi et al., 2021). High seedling growth might be because of PEG, which improves sugar accumulation and transpiration rate (Ahmad et al., 2020). As the plant grows, the plant size and length also increase due to the elongation of stem and vascular tissue (Falster & Westoby, 2003). The significant effect of PEG on plant growth may be due to the growing plant's seed structure, biochemistry, enzyme activities, and organic substances (Pradhan et al., 2014). PEG is a non-toxic, inert molecule that aids in enhancing the metabolic activities of seeds, resulting in faster plant growth and yield (Figoli et al., 2014).

As the plant height increased, the number of branches, leaves, and leaf area of *A. paniculata* increased as well, and this showed that the plant grows uniformly based on its age. A similar result was obtained by (Arif et al., 2014; Basra et al., 2003; Pradhan et al., 2014). Hence, results from this study confirmed that plants raised from osmopriming with PEG exhibited better plant height and increased the number of branches, leaves and leaf area compared to plants raised from non-primed seeds. Meanwhile, stem girth measurement

assesses the growth and width of the plant. Stem acts as the main reservoir of stored starch during plant growth. An increase in stem girth might result from initiating metabolic events in primed seeds (Scofield et al., 2009).

As the plant grows, the cell division within the apical meristem of the plant shoot and root increases and enhances the growth of the shoot and root (Farooq et al., 2006). Polyethylene glycol has low water potential that can enhance the hydrolysis of food reserves and thus enhance plant growth (Pradhan et al., 2014). When compared to other priming treatments, PEG has been shown to boost amylase activity for starch hydrolysis, which produces sugar, resulting in faster development, which might lead to heavier root fresh weight and root dry weight in the shoot and root (Zheng et al., 2015). This study is in accordance with the findings of Neamatollahi and Souhani (2010) on canola and Abbas et al. (2018) on wheat. Meanwhile, GA_3 hormone treatment is less effective compared to the PEG treatment on the seed of *A. paniculata.* It might be that most hormone treatments in seed priming technique are commonly used to improve seed germination in stress conditions (Jisha et al., 2013; Masood et al., 2012).

Meanwhile, Andrographolide is a main secondary metabolite in the *A. paniculata* plant. It is responsible for various medicinal properties, including antipyretic, anti-bacterial, anti-virus, anti-inflammatory, anti-angiogenic, and hepatoprotective, and it shows immunological benefits in cancer and HIV (Joselin & Jeeva, 2014). Generally, PEG is a non-toxic agent that can induce drought stress in plants, which later influences plant growth and development and the formation of secondary metabolites (Martinez-Santo et al., 2021; Turkan et al., 2005; Wu et al., 2005). Previously, PEG treatment in cell culture of *Scrophularia striata* significantly increased the total phenol content (Ahmadi-Sakha et al., 2022). Although in this study, no significant difference in andrographolide content was observed in the treated and control plants, PEG treatment significantly enhanced plant growth, including the size of root and shoot and the number of leaves and branches compared to the untreated seed.

CONCLUSION

PEG seed priming at -0.4 MPa significantly increased plant size, including height, number of leaves and branches, and stem girth. Higher plant growth and number of leaves indirectly increased the quantity of andrographolide compound.

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